Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane; metaldehyde

EC Number: 203-600-2
CAS Number: 108-62-3

CLH-O-0000001412-86-171/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

22 September 2017

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Metaldehyde

EC Number: **203-600-2**

CAS Number: **108-62-3**

Index Number: **605-005-00-7**

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Version number: 06 **Date: 26.02.2016**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Metaldehyde***
EC number:	***203-600-2***
CAS number:	***108-62-3***
Annex VI Index number:	***605-005-00-7***
Degree of purity:	***985 g/kg***
Impurities:	***Relevant Impurity:*** ***Acetaldehyde max. 1.5 g/kg***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Flam. Sol. 2, H228 Acute Tox. 4, H302
Current proposal for consideration by RAC	Flam. Sol. 2, H228 Acute Tox. 3, H301 STOT RE 2, H373
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	The MSDS submitted indicates under Dust explosion class: St(H)2: strong dust explosion, indicator 2. Classification for dust explosiveness is neither foreseen according to CLP Regulation nor according to Directive 67/548/EEC	-	-	Conclusive, but not sufficient for classification
2.2.	Flammable gases	-	-	-	Conclusive, but not sufficient for classification
2.3.	Flammable aerosols	-	-	-	Conclusive, but not sufficient for classification
2.4.	Oxidising gases	-	-	-	Conclusive, but not sufficient for classification
2.5.	Gases under pressure	-	-	-	Conclusive, but not sufficient for classification
2.6.	Flammable liquids	-	-	-	Conclusive, but not sufficient for classification
2.7.	Flammable solids	H228	-	H228	
2.8.	Self-reactive substances and mixtures	-	-	-	Conclusive, but not sufficient for classification
2.9.	Pyrophoric liquids	-	-	-	Conclusive, but not sufficient for classification
2.10.	Pyrophoric solids	-	-	-	Conclusive, but not sufficient for classification
2.11.	Self-heating substances and mixtures	-	-	-	Data inconclusive

	Substances and mixtures which in contact with water emit flammable gases				Conclusive, but not sufficient for classification
	Oxidising liquids				Conclusive, but not sufficient for classification
2.14.	Oxidising solids				Conclusive, but not sufficient for classification
2.15.	Organic peroxides				Conclusive, but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals				Conclusive, but not sufficient for classification
3.1.	Acute toxicity - oral	H301	-	H302	-
	Acute toxicity - dermal	-	-	-	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	-	-	-	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	-	-	-	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	-	-	-	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	-	-	-	Data lacking
3.4.	Skin sensitisation	-	-	-	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	-	-	-	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	-	-	-	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	-	-	-	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	-	-	-	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	H373	-	-	-
3.10.	Aspiration hazard	-	-	-	Data lacking

4.1.	Hazardous to the aquatic environment	-	-	-	Conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	-	-	-	Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word:
Danger
Pictogram:
GHS02, GHS06, GHS08
Hazard statements:
Flam. Sol. 2, **H228**: Flammable Solid
Acute Tox. 3, **H301**: Toxic if swallowed
STOT RE 2, **H373**: May cause damage to organs through prolonged or repeated exposure if swallowed

Proposed notes assigned to an entry: -

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Metaldehyde is a molluscicide for the control of slugs and snails, and was approved in 2008 for Annex I listing as a 3A Review compound under Council Directive 91/414/EEC, with Austria as Rapporteur Member State. In accordance with Article 36(2) of the CLP Regulation, metaldehyde should now be considered for harmonised classification and labelling. Therefore, this proposal considers all physico-chemical, human health and environmental end points. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of metaldehyde under Directive 91/414/EEC. The assessment made under that Directive is attached to the IUCLID 5 dossier.

Metaldehyde is already listed in Annex VI of the CLP Regulation (it was inserted into Annex I of Directive 67/548/EEC in the 19th ATP and updated with the 31st ATP [Tox data were not discussed for update with 31st ATP]) with the classifications as F; R11 and Xn; R22.

This proposal seeks to update these classification and additionally, to include classification for repeated dose toxicity. During the peer review for Annex I Inclusion of metaldehyde Member States and EFSA agreed that Austria should flag the new proposal for classification and labelling to ECHA, including repeated dose toxicity.

2.2 Short summary of the scientific justification for the CLH proposal

Current classification according to Annex VI, Table 3.1 in the CLP Regulation for Metaldehyde is Flam. Sol. 2, H228.

The lowest LD_{50} value of 283 mg/kg for acute oral toxicity was found in rats. According to Regulation (EC) No. 1272/2008 metaldehyde belongs to acute toxicity category 3 ($50 < ATE \leq 300$ mg/kg bw) and requires classification and labelling with **H301 "Toxic if swallowed"**.

Metaldehyde requires classification with **H373 "May cause damage to organs through prolonged or repeated exposure (if swallowed)"** based on the findings of mortality at 30 mg/kg bw/d and testicular findings (moderate to marked diffuse atrophy and/or degeneration of the germinative epithelium) at 90 mg/kg bw/d in a 52-week dog study. Criteria as specified in the Regulation (EC) No. 1272/2008: For the oral route the guidance values to assist in Category 2 classification are $10 <$ dose ≤ 100 mg/kg bodyweight/day. These guidance values refer to effects seen in a standard 90-day toxicity study conducted in rats.

Regarding environment (considering 2nd ATP criteria) following classification will be proposed:

CLP: no classification

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Classification:
Flam. Sol. 2, H228
Acute Tox. 4, H302

Labelling:
pictograms: GHS02, GHS07
signal word: Dgr
hazard statement codes: H228, H302

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Classification:
F; R11
Xn; R22

Labelling:
F; Xn
R: 11-22
S: (2-)13-16-25-46

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

No self-classification and labelling based on CLP Regulation criteria was proposed by the notifier. However, on the ECHA website the following notified classification and labelling according to CLP criteria are given.

Classification		Labelling		
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)
Flam. Sol. 2	H228	H228		GHS07 GHS02 Dgr
Acute Tox. 4	H302	H302		
Flam. Sol. 2	H228	H228		GHS02 Wng
Acute Tox. 4	H312	H312		
Flam. Sol. 2	H228	H228		GHS06 GHS02 Dgr
Acute Tox. 3	H301	H301		
Flam. Sol. 2	H228	H228		GHS02 GHS06 Dgr
Acute Tox. 3	H301	H301		
Aquatic Chronic 3	H412	H412		
Flam. Sol. 2	H228	H228		GHS02 GHS06 GHS09 GHS08 Dgr
Acute Tox. 3	H301	H301		
STOT RE 2	H373 (Testes, liver) (Oral)	H373		
Aquatic Chronic 2	H411	H411		
Flam. Sol. 2	H228	H228		GHS07 GHS02 Wng
Acute Tox. 4	H302	H302		
Flam. Sol. 2	H228	H228		GHS02 GHS06 Dgr
Acute Tox. 3	H301	H301		
Flam. Sol. 2	H228	H228		GHS07 GHS02 GHS08 Dgr
Acute Tox. 4	H302	H302		
STOT RE 2	H373 (Testes) (Oral)	H373		
Aquatic Chronic 3	H412	H412		
Flam. Sol. 2	H228	H228		GHS06 GHS02 Wng
Acute Tox. 3	H301	H301		
Acute Tox. 2	H330	H330		

RAC general comment

Metaldehyde is a molluscicide for the control of slugs and snails. It was approved in 2008 for Annex I listing as a 3A Review compound under Council Directive 91/414/EEC, with Austria as Rapporteur Member State.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Metaldehyde is used as a pesticide. For pesticides there is no need for justification (cf. Article 36(3) CLP Regulation).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

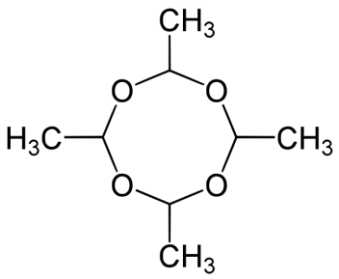
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	203-600-2
EC name:	Metaldehyde 2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane
CAS number (EC inventory):	203-600-2
CAS number:	108-62-3
CAS name:	2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane 1,3,5,7-Tetroxocane, 2,4,6,8-tetramethyl-
IUPAC name:	r-2, c-4, c-6, c-8-tetramethyl-1,3,5,7-tetroxane
CLP Annex VI Index number:	605-005-00-7
Molecular formula:	$C_8H_{16}O_4$
Molecular weight range:	176.2 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Metaldehyde	Min. purity: 985 g/kg	983 – 998 g/kg	-

Current Annex VI entry:

Table 3.1

List of harmonised classification and labelling of hazardous substances

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
605-005-00-7	2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane; metaldehyde	203-600-2	108-62-3	Flam. Sol. 2 Acute Tox. 4 *	H228 H302	GHS02 GHS07 Wng	H228 H302			

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Acetaldehyde (relevant impurity)	Max. content: 1.5 g/kg	-	-

Current Annex VI entry:

Table 3.1

List of harmonised classification and labelling of hazardous substances

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
605-003-00-6	acetaldehyde; ethanal	200-836-8	75-07-0	Flam. Liq. 1 Carc. 2 Eye Irrit. 2 STOT SE 3	H224 H351 H319 H335	GHS02 GHS08 GHS07 Dgr	H224 H351 H319 H335			

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
Confidential	Stabilizer	confidential	-	-

Current Annex VI entry: no entry

1.2.1 Composition of test material

Physico-chemical properties: purity of tested technical material in the range from 99.1% to 99.5%)

Human health hazard assessment: purity of tested technical material in the range from 90.14% to 94.8%

Environmental hazard assessment: purity of tested technical material in the range from 90.2% to 98.7%

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.1 Melting point, freezing point or solidification point (IIA 2.1.1)	EEC/A1 and OECD 102 Metal block and Differential scanning calorimetry method (DSC)) GLP	TGAI purity: 99.5 % (w/w) Two methods according OECD 102 were used in this testing: Metal block method and DSC. Metal block method determination: Heating rate: 1 °C/min -sealed tube In the metal block method the samples (in duplicate) was placed in a sealed tube. The samples went straight to stage B (i.e. a clearance between the sample and the wall due to shrinkage of the melt) as described in method OECD 102 (figure 2) at a temperature of 191 °C. The remaining stages of melting were not observed. Complete sublimation was achieved at 201 °C and at 197 °C respectively. Differential scanning calorimetry determination: Heating rate: 10 °C/min range: 25 °C to 400 °C in atmosphere of air. Tests were performed in open and in sealed crucibles. In each case a sharp endotherm peak with a maximum between 200 and 210 °C was detected. The extrapolated onset temperatures for the sharp endotherm peak were 182 °C for the open and 185 °C for the sealed crucible tests. The endotherms started with broad features with onsets at approx. 100 °C which indicates the initiation of changes in the test substance at this temperature. After each test the crucible was empty. Metaldehyde starts to sublime at 191 °C.	Acceptable The use of technical material is acceptable since purity is > 98 %(w/w)	Comb, A. L. (2007) (Doc. No. 112-002)

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.2 Boiling point (IIA 2.1.2)	Statement Tier 2	Not applicable, as the test item is a solid	Not applicable as **sublimation** is more realistic	
B.2.1.3 Temperature of decomposition or sublimation (IIA 2.1.3)	EEC/A1 and OECD 102 Metal block and Differential scanning calorimetry method (DSC)) GLP	TGAI purity: 99.5 % (w/w) Metaldehyde starts to sublime at 191 °C.	Acceptable The use of technical material is acceptable since purity is > 98 %(w/w)	Comb, A. L. (2007) (Doc. No. 112-002)
B.2.1.4 Relative density (IIA 2.2)	OECD 109 (Gas comparison pycnometer) GLP	Purified product purity: 99.1 % (w/w) Density: 1.27 x 10^3 kg/m^3 (20 ± 0.5 °C)	Acceptable Method is equivalent to EEC/A3 Relative density is not reported	Hogg, A.S.; (1998) (Doc. No. 112-001)
B.2.1.5 Vapour pressure (IIA 2.3.1)	OECD 104 (Static method) not GLP*	Purified product purity: 99.3 % (w/w) Vapour pressure: 6.6 ± 0.3 Pa (25 °C) 4.4 ± 0.2 Pa (20 °C)	Acceptable	Cardinaals, J.M. (1988) (Doc. No.115-001)

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.6 Volatility, Henry's law constant (IIA 2.3.2)	Calculation	3.5 $Pa.m^3.mol^{-1}$ (20 °C) values used for calculation: water solubility: 0.222 g/L at 20 °C vapour pressure: 4.4 Pa at 20 °C	Acceptable	Cardinaals, J.M. (1988) (Doc. No.115-002)
B.2.1.7 B.2.1.8 Appearance: physical state (IIA 2.4.1)	Visual examination	TGAI purity: 99.5 % (w/w) White crystalline powder	Acceptable The use of technical material is acceptable since the purity is > 98%.	Comb, A. L. (2007) (Doc. No. 112-002)
		Technical product purity: 99.3% (w/w) White powder	Acceptable	O'Connor, B. J., Mullee, D. M. (2000) (Doc. No. 172-001)

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.10 Spectra of the active substance (IIA 2.5.1)	UV/VIS - Spectroscopy OECD guideline No.101 GLP	Purified product purity: 99.1 % (w/w) $c = 1.02 \times 10^{-3}$ mol/L (0.18 g/L) Solvent: MeOH/HCl [90/10 (0.1 N) v/v] — no significant absorption occurs at any wavelength. Solvent: MeOH pH 7 buffer [90/10 v/v] — no significant absorption occurs at any wavelength. Solvent: MeOH/NaOH [90/10 (0.1 N) v/v] — no significant absorption occurs at any wavelength.	Acceptable	O'Connor, B.J., Mullee, D.M., (2001) (Doc. No. 119-001)
	^{1}H and ^{13}C-NMR FTIR (KBr, 4000 - 600 cm^{-1}) MS (EI 70eV)	UV/VIS, IR, NMR and MS spectra including interpretation data were submitted. The spectra confirm the molecular structure. Optical purity: not relevant as Metaldehyde has no optical isomers.	Acceptable	

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.11 Spectra of relevant impurities (IIA 2.5.2)	OECD 101 GLP except NMR spectrum	Purified product purity: 99.5% (w/w) Acetaldehyde is considered to be a relevant impurity. Ultraviolet/visible, infrared, nuclear magnetic resonance and mass spectra were recorded and found to be consistent with the assigned structure of the molecule.	Acceptable Although the NMR spectrum was not performed according to GLP because the laboratory (London Metropolitan University) is not a member of the UK GLP compliance programme the structure of acetaldehyde is confirmed by IR, MS and UV.	Comb, A. L. (2009) (Doc. No. 157-001)
B.2.1.12 Solubility in water (IIA 2.6)	EEC A.6 OECD 105 (Flask shaking method) not GLP*	Purified product purity: 99.3 % (w/w) at 20.0 ± 0.2 °C 0.188 g/L at pH 7.2; buffer based on Milli-Q-water (Millipore) 0.196 g/L at pH 5; buffer based on Milli-Q-water (Millipore) 0.186 g/L at pH 9; buffer based on Milli-Q-water (Millipore)	Acceptable	Bohle, J.F., (1989) (Doc. No. 114-001)
	OECD 105 (Flask shaking method) not GLP*	Purified product purity: 99.3 % (w/w) 19.9 - 23.0 °C 0.222 g/L; pH 6.5; Milli-Q-water (Millipore)	Acceptable OECD 105 is comparable to EEC/A6	Cardinaals, J.M. (1988b) (Doc. No. 114-003)
B.2.1.13 Solubility in organic	EEC A.6 OECD 105	Purified product purity: >99.5% (w/w) solvent | solubility at 20.3-22.4 °C [g/L]	Acceptable	Bohle, J.F., (1989)

Study	Method	Results		Conclusion/Comment	Reference
solvents (IIA 2.7)	(Flask shaking method) not GLP*	hexane methanol toluene tetrahydrofurane 1,2-dichloroethane acetone	52.1 x 10^{-3} 1.73 0.53 1.56 3.08 1.46		(Doc. No. 114-004) Comb, A. L. (2007) (Doc. No. 114-006)
	EEC A.6 (Flask shaking method) GLP	Purified product purity: 99.5% (w/w)		Acceptable	O'Connor, B.J., Mullee, D.M., (2001a) (Doc. No. 114-005)
		solvent	solubility at 20.0 ± 0.5 °C [g/L]		
		ethyl aceto acetate	0.754		
B.2.1.14 Partition coefficient n-octanol/water (IIA 2.8)	OECD 107 (Shake flask method) not GLP*	Purified product purity: 99.3 % (w/w) at 19.9-20.1 °C log P_{ow} = 0.12 P_{ow} = 1.33 ± 0.04 at pH 6.7 Effect of pH (4 to 10) is not required, because Metaldehyde is neither an acid nor a base. Because metaldehyde is not an ionisable compound the water phase is not buffered		Acceptable The method is comparable to the EEC/A8 shake flask method	Cardinaals, J.M. (1988b) (Doc. No. 114-002)

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.15 Hydrolysis rate (IIA 2.9.1)	US EPA-FIFRA N-161-1 40 CFR 158.130 not GLP*	^{14}C-Metaldehyde radiochemical purity: > 99% Study performed at 25 ± 1 °C for 30 days in the dark (in pH 5, 7 and 9 buffers) Metaldehyde is hydrolytically stable, no accurate half-life could be determined due to insignificant degradation	Acceptable For details see B 8.4 Fate and behaviour in water	Carpenter, M., (1989) (Doc. No. 711-001)
B.2.1.16 Direct phototrans-formation (IIA 2.9.2)	US EPA-FIFRA N-161-1 40 CFR 158.130 not GLP*	^{14}C-Metaldehyde radiochemical purity: > 97.7% Study performed at 25 ± 1°C for 30 days (in pH 7 buffer) On day 30, 97.5% of the initially administered parent compound was still found. Metaldehyde is photolytically stable, no accurate half-life could be determined due to insignificant degradation	Acceptable For details see B 8.4 Fate and behaviour in water	Carpenter, M., (1989a) (Doc. No. 712-001)
B.2.1.17 Quantum yield (IIA 2.9.3)	FAO revised guidelines on Environmental Criteria for the Registra-tion of Pesticides SETAC 1995	^{14}C-Metaldehyde radiochemical purity: > 97.7% Quantum yield $\Phi \approx 0$ Test substance is stable in the absence of a photo-sensitiser, therefore no separate determination is necessary	Acceptable For details see B 8.4 Fate and behaviour in water	Carpenter, M., (1989a) (Doc. No. 712-001)
B.2.1.18 Dissociation constant (pKa) (IIA 2.9.4)			Not relevant as Metaldehyde does not dissociate in water	

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.19 Stability in air, photochemical oxidative degradation (IIA 2.10)	Atkinson calculation	K_{OH} = 73.8 x 10^{-12} cm^3 x $molecule^{-1}$ x sec^{-1} half-life in troposphere: t½ : 5.3 hours	Acceptable For details see B 8.7.1 Fate and behaviour in air	Voget, M., (1994) (Doc. No. 782-001)
B.2.1.20 Flammability (IIA 2.11)	EEC/A10 GLP	Technical product purity: 99.5% (w/w) As the preliminary test was positive, the main test according to EEC/A10 was performed with the result, that Metaldehyde is highly flammable (the propagated combustion over the 100 mm was < 45 seconds)	Acceptable The study was performed with the TGAI of high purity. R11 for classification is required	Tremain, S.P., (2001) (Doc. No. 119-002)
B.2.1.21 Auto-flammability (IIA 2.11.2)	EEC/A16 GLP	Technical product purity: 99.5% (w/w) No self ignition up to 400 °C	Acceptable The study was performed with the TGAI of high purity.	Tremain, S.P., (2001) (Doc. No. 119-002)
B.2.1.22 Flash point (IIA 2.12)			Not applicable because material is a solid with a melting point > 40°C	

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.23 Explosive properties (IIA 2.13)	EEC/A14 GLP	Technical product purity: 99.5% (w/w) Thermal sensitivity test: no explosion after 5 minutes (nozzle diameter: 2.0 mm and 6.0 mm) Shock test: no explosion occurred within 6 tests using a mass of 10 kg from a height of 0.4 m Friction test: no explosion occurred within 6 tests using a 36 kgf (≈360 N) loading. A black mark on the porcelain plate and peg indicates decomposition	Acceptable The study was performed with the TGAI of high purity. Although dust explosion does not cover this annex point, the MSDS indicates that metaldehyde is classified as St(H)2: strong dust explosion, indicator 2	Tremain, S.P., (2001) (Doc. No. 119-002) Anonymous, (2001) (Doc No. 955-004)
B.2.1.24 Surface tension (IIA 2.14)	EEC/A5 Ring method GLP	Purified product purity: 99.5% (w/w) σ = 71.9 mN/m at 19.5 °C ± 0.5 °C (0.204 g/L aqueous solution)	Acceptable The compound is not considered as surface active	O'Connor, B.J., Mullee, D.M., (2001) (Doc. No. 119-001)
B.2.1.25 Oxidising properties (IIA 2.15)	Statement	Oxidizing properties are not expected, considering the overall chemical structure and the oxygen balance of Metaldehyde.	Acceptable	Weiss, A. (2009) (Doc. No. 143-001)

* not GLP: as no certificate of the relevant GLP authority is attached, but due to the date of study, not necessary.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for Classification and Labelling.

2.2 Identified uses

Metaldehyde is a molluscicide for the control of slugs and snails.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies (DSD)

Method	Results	Remarks	Reference
EEC/A10	highly flammable (the propagated combustion over the 100 mm was < 45 seconds)	-	Tremain, S.P., (2001) (Doc. No. 119-002)

3.1

3.1.1 Summary and discussion of physico-chemical properties

Current classification according to Annex VI, Table 3.1 in the CLP Regulation for Metaldehyde is Flam. Sol. 2, H228.

3.1.2 Comparison with criteria

Not relevant since no test/study is reported in Annex VI, Table 3.1 to be compared with method EEC/A10.

3.1.3 Conclusions on classification and labelling

Current classification according to Annex VI, Table 3.1 in the CLP Regulation for Metaldehyde is Flam. Sol. 2, H228.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

One single study on ADME of metaldehyde was conducted in Sprague-Dawley CD rats and included single oral dosing with 10 and 100 mg/kg and repeated oral dosing with 10 mg/kg. In this study, absorption, distribution, excretion as well as metabolism were investigated.

Absorption: Metaldehyde was absorbed rapidly and practically completely in male and female rats after single low and high dose (10 and 100 mg/kg) and repeated oral low doses (10 mg/kg). Maximum concentrations in blood were found within 1-2 h in males and 2-4 h in females, with calculated half-lives of 3.4 h for males and 8.8 h for females indicating a faster metabolism and excretion in males than in females. Oral absorption was essentially complete as the majority of radioactivity (approximately 80% after 48 h and 85% after 7 days) was expired as CO_2. 10% of the administered radioactivity was still found in the body after 7 days, and 2-5% of the dose was found in urine.

Distribution: Following single or repeated oral dosing, radioactivity was widely distributed into the whole body. The majority of radioactivity found in the body was detected in the carcass (approximately 80%) and the rest was distributed more or less evenly in tissues and organs investigated. Small amount of metaldehyde (0.5% males and 0.59% females) were found in the brain, indicating that metaldehyde can pass the blood-brain barrier. There was no indication of bioaccumulation as there was no increase of tissue residue levels after repeated administration compared with tissue levels after single application. The amount of radioactivity found in the whole body markedly decreased over time as demonstrated by the amounts found at peak blood level, first half-life, second half-life and 7 days after dosing.

Excretion: Most of the administered radioactivity was found in the expired air, regardless of single or repeated dosing: approximately 80% after 48 h and 85% after 7 days. The amounts found in urine (2-5%) and faeces (2-3%) were comparably low and independent from the dosing scheme. Most of the radioactivity was excreted during the first 24- 48 h after application, however, males expired the radioactivity more rapidly than females during this time period.

Metabolism: Analysis of blood samples collected at different time points showed that metaldehyde is metabolised to acetaldehyde. No other metabolites were found in the blood. When urine samples were analysed, ^{14}C residues appeared as multiple peaks with retention times associated with very polar compounds. No intact metaldehyde was detected in urine. The analysis of trapping solutions of the expired air showed that almost all of the radioactivity was expired as CO_2. Taken together it can be assumed that metaldehyde is extensively metabolised to acetaldehyde. The metabolic profile of acetaldehyde is well established and involves conversion of acetaldehyde to acetyl-CoA. The physiological molecule acetyl-CoA is oxidised through the Krebs cycle to CO_2 or utilised in the various anabolic reactions involved in the synthesis of cholesterol, fatty acids or other tissues constituents. The oxidation reactions account for the large amount of ^{14}C-labelled CO_2 found in the expired air while the anabolic reactions account for the slow decline of ^{14}C residues observed in the carcass.

4.1.2 Human information

Not available.

4.1.3 Summary and discussion on toxicokinetics

Absorption, distribution, excretion and metabolism (toxicokinetics)

Rate and extent of oral absorption	Rapid and essentially complete (>95%) based on excretion via air (80% within 48h; 85% within 7 days) and urinary excretion (2-5% within 7 days). Further 8-10% was still present in the body after 7 days.
Distribution	Widely distributed; most of radioactivity found in carcass and not in specific organs
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	After 7 days: expired air (85%); urine (2-5%); faeces (2-3%)
Metabolism in animals	85% metabolised to acetaldehyde and expired as CO_2; 2-5% metabolised and excreted via polar degradates in the urine (no parent compound in urine); 2-3% in faeces not identified.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Acute oral toxicity in the rat (OECD guideline 401)	LD_{50} = 283 mg/kg bw (M+F)	-	Jones, J., Collier, T.; 1987
Acute oral toxicity in the rat, up-and-down procedure (OECD guideline 425)	LD_{50} = 654 mg/kg bw (F)	This study was not requested by the RMS or any other MS.	Durando, J.; 2009
Acute oral toxicity in the mouse (OECD guideline 401)	LD_{50} = 411 mg/kg bw (M) LD_{50} = 443 mg/kg bw (F)	-	Coles, R.; 1990
Acute percutaneous toxicity in rats	LD_{50} > 5000 mg/kg bw	Limited validity	Davies, R., Collins, C.; 1974

Several information regarding acute toxicity can be found in public literature e.g. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+1735 or http://www.inchem.org/documents/pims/chemical/pim332.htm. However the dossier submitter does not have access to the original studies. Therefore no evaluation on reliability of the respective information could be performed and this information has not been included into the CLH-report of metaldehyde.

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Reference:	**P0071: OECD 401 Acute oral toxicity in the rat, Project Number 102/9A**
Author(s), year:	Jones J., Collier T., 1987
Report/Doc. number:	Doc.No. 521-002, Lonza Report No. 1354, Conducting laboratory: Safepharm Laboratories Limited, Derby, UK
Guideline(s):	OECD Guideline 401 (1981)
GLP:	Yes
Deviations:	No
Validity:	Valid

Material and Methods:

Groups of 5 rats/sex received single doses of 0 (vehicle control), 100, 200, 400 and 800 mg/kg bw metaldehyde (sponsor´s identification: P0071; batch no. 3157; purity 99.3 %) suspended in arachis oil by oral gavage. This dose selection was based upon a range-finding study where one male and one female rat per test group were dosed with 100, 250, 500, 1000, 2000 and 5000 mg/kg bw. At the beginning of the study the rats (strain: Sprague-Dawley CFY; source: Interfauna Ltd., UK) were approximately five to eight weeks old and weighed 126-148 g (males) and 114-148 g (females).

Animals were observed immediately after dosing and at 6, 12 and 18 h after dosing and subsequently once daily for 14 days. Death and evidence of clinical signs of toxicity were recorded at each observation. Bodyweights were determined on the day of treatment (day 0), days 7 and 14,

and at death. All animals were subjected to gross necropsy examination for any macroscopic abnormalities. No tissues were retained.

Findings:

Range finding study: No deaths were observed at 100 and 250 mg/kg, while 2 deaths were noted at doses of 500 mg/kg and above. Therefore mortality data indicated an oral LD_{50} between 250 and 500 mg/kg.

Main study: The observed death rates were 0/5 for males and females at 100 mg/kg, 2/5 for males and 1/5 for females at 200 mg/kg, 3/5 for males and 4/5 for females at 400 mg/kg, and 5/5 for both males and females at 800 mg/kg. Two males treated with 800 mg/kg and one males treated with 400 mg/kg were found dead immediately after dosing. All other deaths were noted six or twelve hours after dosing. The acute oral median lethal dose (LD_{50}) was calculated to be 283 mg/kg bodyweight. Clinical signs were observed at all dose levels immediately after dosing. Principal signs of toxicity noted in both decedents and surviving animals were hunched posture, pilo-erection, lethargy and decreased respiratory rate. Occasional or isolated signs were increased salivation, ptosis, (occasional) body tremors, red/brown staining around the eyes, snout and mouth with diuresis, diarrhoea, tonic convulsions, ataxia and coma. Surviving animals showed no signs 2-8 days after dosing. All surviving animals had expected gains in bodyweight over the study period. Common findings noted in decedents were red or haemorrhaged lungs, dark or patchy pallor of the liver and congestion of the small intestine. Sloughing of the gastric mucosa was also noted. No abnormalities were noted at necropsy of animals killed at the end of the study period.

Conclusion:

Metaldehyde (suspended in arachis oil) is of moderate toxicity to rats after oral administration. The acute oral LD_{50} was calculated to be 283 (210 – 382) mg/kg bw.

Reference:	**Acute Oral Toxicity Study with META Metaldehyde techn. CAS No. 108-62-3: Up-And-Down Procedure in Rats**
Author(s), year:	Durando J., 2009
Report/Doc. number:	Eurofins/Product Safety Laboratories, USA; Laboratory Identification No. 26776 Lonza Report No. 4377, Doc. No.: 521-003
Guideline(s):	US EPA, OPPTS 870.1100 OECD Guidelines for the Testing of Chemicals, Test No. 425
GLP:	Yes
Deviations:	No
Validity:	Valid

Material and methods:

Test material	META Metaldehyde techn.
Lot/Batch	38596
Purity	99.10%
Vehicle	Distilled water; Satellite group: corn oil
Species	Rat, females
Strain	Sprague-Dawley derived, Albino
Age	9-11 weeks
Weight at dosing	164-213 g

Source Ace Animals, Inc., Boyertown, PA, USA

Main Test: Based on the Sponsor´s estimation of an LD_{50} of 350 mg/kg, the test was conducted using a default starting dose level of 111 mg/kg (as a 30% w/w mixture in distilled water), which was administered to one healthy female rat by oral gavage. Following the Up and Down test procedure, additional females (three at each dose level) were tested at levels of 350 and 1110 mg/kg.

Confirmatory Test: Following completion of the main test using distilled water as a vehicle (the preferred vehicle according to the guideline), a confirmatory limit test at 350 mg/kg using corn oil was performed. A number of previous gavage toxicity studies with the test substance have been conducted with corn oil as the vehicle, and it was considered useful to confirm that the vehicle would not make a significant difference to toxicity. The test substance diluted in corn oil at 30 % w/w was administered to one female rat by oral gavage at 350 mg/kg. Due to the absence of mortality in this animal, four additional females were sequentially treated at the same dose level.

Females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males. All animals were observed for mortality, signs of gross toxicity, and behavioural changes for a 14-day period post dose administration or until death occurred. Body weights were recorded prior to administration and again on Study days 7 and 14 or after death. Necropsies were performed on all animals.

Findings:

Table 11: Main test (vehicle: distilled water)

Dosing Sequence	Animal No.	Sex dosed	Dose level (mg/kg)	Dose volume (ml)	Outcome*
1	3101	F	111	0.077	S
2	3102	F	350	0.21	S
3	3103	F	1110	0.59	D
4	3104	F	350	0.20	S
5	3105	F	1110	0.65	D
6	3106	F	350	0.20	S
7	3107	F	1110	0.63	D

* S – Survival, D – Death

Main test:

111 mg/kg Dose Level (1 animal): no clinical signs, no effect on body weight, no gross abnormalities

350 mg/kg Dose Level (3 animals): All animals survived and gained body weight during the study. Following administration, two animals were hypoactive and/or exhibited reduced fecal volume and the third animal was observed with piloerection. However, all animals recovered by Day 2 and appeared active and healthy for the remainder of the 14-day observation period. No gross abnormalities were noted for these animals.

1110 mg/kg Dose Level (3 animals): All animals died within one day of test substance administration. Prior to death these animals were hypoactive and/or exhibited hunched posture and tremors. Gross necropsy of the decedents revealed red discoloration of the lungs, red intestines, red oral discharge and/or ano-genital staining.

Table 12: Confirmatory Test (vehicle: corn oil)

Dosing Sequence	Animal No.	Sex dosed	Dose level (mg/kg)	Dose volume (ml)	Outcome*
1	3108	F	350	0.22	S
2	3109	F		0.24	S
3	3110	F		0.23	D
4	3111	F		0.24	S
5	3112	F		0.24	S

* S – Survival, D – Death

Confirmatory test:

One animal died within one day of test substance administration. Prior to death this animal was hypoactive and exhibited tremors. Following administration, the surviving animals were hypoactive, however, they recovered by Day 1, gained body weight and appeared active and healthy for the remainder of the 14-day observation period. Gross necropsy of the decedent revealed discoloration of the intestines and lungs. No gross abnormalities were noted for any of the euthanized animals when necropsied at the conclusion of the 14-day observation period.

Conclusion:

Based on the results of the study, the acute oral LD_{50} of the test substance is estimated to be 654 mg/kg in female rats with approximate 95% Confidence Limits of 1110 mg/kg (upper) and 350 mg/kg (lower). A confirmatory limit test indicated that animals are not substantially more sensitive to the test substance when administered in corn oil than when administered in water.

Reference:	**P0071: Acute oral toxicity test in the mouse, Project Number 102/50**
Author(s), year:	Coles R., 1990
Report/Doc. number:	Doc.No. 521-001, Lonza Report No. 1325, Conducting laboratory: Safepharm Laboratories Limited, Derby, UK
Guideline(s):	OECD Guideline 401 (1981)); US EPA Pesticide Assessment Guidelines Subdivision F, No 81-1; Annex V method B1 of EEC Commission Directive 84/449/EEC
GLP:	Yes
Deviations:	No
Validity:	Valid

Material and Methods:

Groups of 5 mice/sex received single doses of 0 (vehicle control), 400, 526, 693, 912 and 1200 mg/kg bw metaldehyde (sponsor´s identification: P0071; batch no. 5448; purity 99.3 %) suspended in arachis oil by oral gavage. As the mortality data derived from these dose groups did not permit calculation of the acute oral LD_{50} value, an additional group of 5 mice/sex were treated with 304 mg/kg. The dose selection was based upon a range-finding study when one male and one female mouse per test group were dosed with 500, 1000, 3000 and 5000 mg/kg bw. At the beginning of the study the mice (strain: BKW; source: Bantin & Kingman Ltd., Hull, UK) weighed 21-30 g (males) and 20-25 g (females), and were approximately six to eight weeks old. Animals were observed 1 and 4 hours after dosing and subsequently once daily for 14 days. Deaths and evidence of overt toxicity were recorded at each observation. Individual bodyweights were recorded on the day of treatment (day 0), days 7 and 14, or at death. All animals were subjected to gross necropsy. No tissues were retained.

Findings:

Range finding study: No deaths were observed at 500 mg/kg, while 2 deaths were noted at doses of 1000 mg/kg and above. The mortality data indicated an oral LD_{50} between 500 and 1000 mg/kg.

Main study: The observed death rates were 0/5 for both sexes at 304 mg/kg, 4/5 for males and 3/5 for females at 400 mg/kg, 3/5 for both sexes at 526 mg/kg, and 5/5 for males and 4/5 for females at 693 mg/kg, 5/5 for both sexes at 912 mg/kg, and 5/5 for males and 4/5 for females at 1200 mg/kg. Deaths were noted 1-4 hours after dosing and on day 1 (one female at 400 mg/kg) and day 7 (one male and one female at 526 mg/kg) after treatment. Common signs of toxicity included hunched posture, lethargy and piloerection. Additional or isolated signs of toxicity noted were ataxia, ptosis, pallor of the extremities, decreased respiratory rate, occasional body tremors and tonic convulsions. Surviving animals appeared normal 1, 2 or 7 days after treatment. All animals treated with 304 mg/kg appeared normal throughout the study. Incidents of reduced bodyweight gain and bodyweight loss were noted in all treatment groups during the study period. Macroscopic findings found at necropsy of animals that died during the study were red lungs, dark liver or patchy pallor of the liver, pale spleen, dark kidneys, haemorrhage of the glandular gastric epithelium and large intestine. No findings were noted at necropsy of surviving animals.

Conclusion:

Metaldehyde (suspended in arachis oil) is of moderate toxicity to mice after oral administration. The acute oral LD_{50} was calculated to be 411 (346-489) mg/kg bw for males and 443 (333-591) mg/kg bw for females.

4.2.1.2 Acute toxicity: inhalation

The available study on acute inhalation toxicity shows major limitations and was considered not to be of sufficient validity for assessing the acute inhalation toxicity of metaldehyde.

Reference:	**Acute inhalation toxicity to the rat of metaldehyde dust**
Author(s), year:	Berczy Z., Cobb L., Cherry C., 1973
Report/Doc. number:	Eurofins/Product Safety Laboratories, USA; Laboratory Identification No. 26776 Lonza Report No. 4377, Doc. No.: 521-003
Guideline(s):	No test guideline is mentioned in the study report.
GLP:	No. When the study was performed (1973), GLP was not compulsory
Deviations:	Several deviations with respect to current guidelines were noted: reduced number of animals/group; two dose levels; no information on substance identification; major reporting deficiencies like air flow, oxygen content, actual concentration, temperature, humidity and MMAD
Validity:	limited scientific validity

Material and Methods:

Groups of 4 male and 4 female rats (strain: Sprague Dawley; source: Charles River Ltd., UK) weighing 205-245 g (males) and 173-204 g (females) were exposed for a 4 hour period (whole body exposure) to dust aerosols of metaldehyde. Neither the purity of the test substance nor the batch no. is presented in the study report. The nominal concentrations were 0 (dry air), 1 and 15 mg a.i./L air. These concentrations were produced by the selection of suitable powder feed and air flow rates in

the dust generator. The test atmosphere dust was passed through a central air inlet into a 100 L exposure chamber containing a group of 8 rats separated into individual compartments by radial grills. Particle size distribution was monitored by collecting dust on a glass slide and examination of the slide by light microscopy. After the 4 hour exposure period the animals were kept under observation for 14 days. Body weights were measured on the day of exposure (day 1) and then on days 2, 5, 8, 12 and 14. At the end of the study macroscopy was performed on all animals.

Findings:

Regarding particle size it was reported that 83-90 % of the particles ranged in a size of 1-5 µm and therefore were considered sufficiently small to reach the respiratory surfaces of the lung. Another 4-7 % of the particles had a reported size of 5-15 µm and were regarded as inhalable. The remaining 6-10 % had a particle size larger than 15 µm.

One female rat died overnight following the exposure with the high dose of 15 mg/L air. Slight temporary dyspnea and occasional sneezing was observed during exposure of the low dose animals. Eye irritation, dyspnea, sneezing, discomfort and increased nasal and oral secretion were seen in animals exposed to the high dust concentration. After exposure, these signs of irritation to the respiratory system disappeared within one hour, but the animals remained lethargic for two days (low concentration and one week (high concentration), respectively, post-exposure. Slight reductions of bodyweight were recorded on the day following the exposure procedure in both test groups, but the rate of growth returned to normal for the rest of the observation period. In the rat that died following dust exposure, marked congestion of the lung and pleural fluid in the thorax was noted; however, these findings could possibly be due to autolytic changes. In surviving rats, no macroscopic findings were noted at termination of the study.

Conclusion:

The results of the study showed irritating effects to the respiratory system, however, it is not clear if this irritation was an intrinsic characteristic of the test compound or a secondary result from the dust distributed in the chamber in this study of limited validity. Only nominal but not actual concentrations are presented in the study report. No LC_{50} value was calculated. Regarding the observed death rate, the results indicate that the LC_{50} value (4 h whole body exposure) for male and female rats is in excess of 15 mg/L air (nominal concentration). However, considering the limitations of the study, no exact statement on the actually inhaled concentration of metaldehyde is possible.

An attempt was made to initiate a new inhalation toxicity study, but the particle size could not be reduced to meet the requirements of OECD 403 (Griffiths, 2009; summarized in the additional report to the DAR). Based on this study, it is not feasible to perform a valid inhalation toxicity study due to the physico-chemical properties of metaldehyde.

Reference:	**Outcome of technical pre-trials for an acute inhalation toxicity study with Metaldehyde**
Author(s), year:	Griffiths D.R., 2009
Report/Doc. number:	Harlan Laboratories Ltd., Derbyshire, UK; Ref: L260109-01/vm Lonza Report No. 4336, Doc. No.: 581-004
Guideline(s):	Not applicable
GLP:	Not applicable
Deviations:	Not applicable
Validity:	Valid

Material and Methods:

A report in the form of a letter (2 pages) was submitted. In this letter, the work which was carried out to generate appropriate test atmospheres for the purposes of performing an acute inhalation toxicity study as recommended in OECD Test Guideline 403 is described. With respect to the particle size it is referred to the Draft OECD Test Guideline 403 in the current version of 1996 which requests a Mean Mass Aerodynamic Diameter (MMAD) of < 4 µm. This reflects the state-of-the-art requirements for the particle size of a test item to be tested in an acute inhalation toxicity study.

Due to the crystalline nature of the test material it was considered unsuitable for the purposes of direct atmosphere generation. Therefore attempts were made to grind the test material.

Findings:

With different grinding techniques like a Braun mini grinder, a Retsch Centrifugal Ball Mill with a Wrights Dust Feeder or a SAG 410 Solid Aerosol Generator, the smallest particle size which could be achieved was larger than 4 µm (MMAD = 11.07 µm).

With a particle separator which was introduced before the aerosol entered the exposure chamber in order to remove large particles and thereby increase the inhalable portion of the generated aerosol, the MMAD was still 9.58 µm.

Conclusion:

It was considered that all techniques available were exhausted to reduce the MMAD of the test material. Thus, the MMAD of approximately 10 µm was the best that could be practically achieved in these series of experiments. It was concluded that the nature of the test material has been demonstrated to be unsuitable for the performance of an acute inhalation toxicity study according to the state-of-the-art requirements for particle sizes reflected in the Draft OECD 403 of 1996.

4.2.1.3 Acute toxicity: dermal

An acute percutaneous toxicity study was performed in rats. However, this study shows some deviations and is therefore considered to be of limited scientific validity: No test guideline is mentioned in the study report. It complies only partly with OECD guideline 402. Following deviations were noted: no specification of the test material; several reporting deficiences concerning housing and feeding conditions; no detailed clinical observation findings and individual necropsy findings. The study is not conform with GLP. When the study was performed (1974), GLP was not compulsory.

Despite of the mentioned deviations, a conclusion can be drawn from this study regarding dermal toxicity. Groups of 5 rats/sex received a topical application of metaldehyde (neither purity nor batch

no. is presented) suspended in water at dose levels of 0 (vehicle control) and 5000 mg/kg bw. The test substance was prepared as a 50% suspension in water and applied evenly to the intact clipped skin on the dorso-lumbar region, equivalent to 10% of the total body surface. The treated area was then covered with aluminium foil which was held in contact with waterproof plaster. After the 24 hour exposure period, the dressings were carefully removed and the treated skin washed with warm dilute soap solution and afterwards rinsed with clean warm water. The animals were observed during a period of 14 days. No mortality was noted at 5000 mg/kg. The only signs of clinical toxicity were slight lethargy and piloerection on the day of treatment (number of animals concerned not given). There was no local reaction like erythema or oedema. All treated animals and controls gained normal bodyweight during the study. Terminal autopsy revealed darkening of the liver and spleen together with pale or mottled kidneys (no number of incidences reported).

Conclusion: Several limitations of the study design were noted, however, the results demonstrated adequate evidence that the acute dermal toxicity of metaldehyde is low. The acute dermal toxicity LD_{50} of metaldehyde (suspended in water) is considered to be greater than the maximum dose tested (5000 mg/kg bw).

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

Many poisonings are reported after accidental or suicidal intake of metaldehyde with intoxications ranging from mild to lethal outcome. Clinical signs include gastrointestinal symptoms which may be followed by convulsions, somnolence, apnoe, cyanosis, coma and death.

Several cases of metaldehyde poisoning are reported and summarized in a dissertation from Borbely A. (1970, Doc.No. 592-001). They were derived from a total of 223 cases of metaldehyde intoxication which were reported to the Swiss Toxicological Information Centre between march 1966 and June 1969. The intoxications were all caused by metaldehyde tablets or snail pellets containing metaldehyde. The data were derived mainly from report forms filled in by the telephone information service and completed by the treating physician. 122 intoxications were due to metaldehyde tablets (pure metaldehyde), and 101 cases to snail pellets (5 - 7 %) metaldehyde. 189 children, 24 adults and 10 animals were involved. Most of the children affected were aged 2 – 4 years. Apart from 20 attempted suicides by adults, all the intoxications were accidental. The course of the poisoning is known in 128 cases: 87 cases were mild, 25 moderately severe, 14 severe and 2 fatal. 22 case histories illustrating particularly severe and typical intoxications are presented in the dissertation and reported here as follows:

Case 1

A 50 year old woman had taken approximately 10-15 metaldehyde tablets about two hours before hospitalisation. She complained about intense nausea and vomited white matter. Some degree of psychological contact was achieved with the patient but she was unable to give exact details about the number of tablets ingested or when. Gastric lavage with sodium bicarbonate, activated charcoal and Carlsbad salt was adopted. During day 1, mental confusion increased. Epileptiform seizures with tonic-clonic spasms and subsequent respiratory failure and cyanosis were observed. Deep respiration emerged in the evening. Calcium was administered because of tetany signs. In the morning of day 2, the patient developed respiratory acidosis and coma. During days 2 and 3, 47 seizures were counted. There was a gradual reduction in frequency of seizures terminally, but increased occurrence of respiratory failure. On day 4, there was a final respiratory failure of the patient in coma.

Case 2

While playing, a 2 year old boy discovered a portion of a metaldehyde bar which was not completely burnt and swallowed it. Shortly afterwards the boy began to vomit spontaneously. In the evening, he had mild diarrhea and spent a restless night, awakening frequently. When taken out of bed the next morning, the child could neither walk nor stand. The boy showed somewhat slow reactions; contact was possible. The neurological status was as follows: intact bilateral reflexes, no pathological reflexes, Chvostek´s sign and muscles slightly hypertonic. No other pathological findings were noted. The child was treated with Valium for immediate sedation and relevant measures were undertaken to promote diuresis. No further symptoms were observed. The child was discharged as cured the next day.

Case 3

A nearly 3 year old child ingested snail pellets containing metaldehyde five hours prior to hospitalization. On admission, the child was shivering from head to foot, with heavily flushed face. Tendon reflexes were markedly enhanced, but not diffuse. Chvostek´s sign were initially negative and later positive. Positive signs of ankle clonus were noted. As therapy gastric lavage with Glauber´s salts, charcoal, and Luminal by intravenous drip were chosen. On day 2, muscle rigidity and trembling were no longer observed but low-grade hyperreflexia was persistent. Blood alkalosis with otherwise normal Astrup values, presumably due to the vomiting, was noted. The child was discharged the following day.

Case 4

A 33 year old male patient swallowed 6-8 metaldehyde tablets four hours before hospitalisation. Vomiting of white mucus occurred shortly afterwards, followed by violent trembling and shaking over the whole body and intense motor restlessness. Hyperventilation with tetany spasms, loud groaning and clouding of consciousness, choreatic movements, Trousseau´s sign, bilateral dorsal flexion of toes, intermittent convulsive choking and white-coated tongue were observed. No enlargement of liver was noted and pulmonary and cardiac findings were normal. Generalized seizures with tonic-clonic spasms and transient loss of consciousness occurred three times. Treatment was performed with morphine-scopolamine and Valium; no signs of renal or hepatic damage were noted. After regression of acute symptoms, the patient remained free of seizures under Luminal.

Case 5

One hour before hospitalisation, the patient (male, 24 years old) had swallowed half a metaldehyde tablet. The status on admission was: intense sensation of burning in the stomach and intense gastric pain, nausea, mild cyanosis, spasms of all extremities, restlessness, anxiety, slight sensory dulling, hyperpnea, slurring of speech, pure cardiac sounds, tachycardia and occasional extrasystoles. No information on progress.

Case 6

A 2 year old girl had been playing with metaldehyde snail pellets. After an unspecified period it no longer responded on being spoken to, and began to twitch and convulse. Slight bouts of vomiting occurred. Findings on admission were: stiff, unconscious child, extension spasms, clonic spasms, pink skin, pupils moderately dilated with little reaction to light, deep, accelerated breathing, tachycardia, increased muscle tone, reacting to touch with spasms and enhanced tendon reflexes. The child was given Somnifen and Taractan and was intubated for gastric lavage which revealed snail pellets. On day 2 the girl was again bright and responsive. The neurological status on discharge was normal (five days after admission).

Case 7

While on military service, the patient (adult male) distributed metaldehyde tablets and then had a meal without first washing his hands. An hour and a half later, he complained of nausea and abdominal pain (data on the further course are lacking).

Case 8

At an unspecified time, the patient (adult male, 30 years old) had ingested metaldehyde tablets. His condition on being found was characterised by vomiting, salivation, epileptiform convulsions and risus sardonicus. He was unresponsive when addressed but did react to mild stimuli. The patient became completely responsive within three days.

Case 9

About two hours previously the patient (male, 18 years old) had swallowed four metaldehyde tablets. Facial twitching, particularly at the side of the mouth occurred. Chvostek and Trousseau signs were positive, increasing restlessness, spasm of the hands and then of the arms and legs were noticed. Debris of metaldehyde tablets were obtained after several gastric lavages and administration of large amounts of laxatives. No acidosis was detected. The spasm-like subsided under Valium and the patient was able to sleep. He was discharged as cured one week later.

Case 10

Two hours previously, the patient (male, 24 years old) had swallowed some metaldehyde powder (an amount approximately equivalent to one-tenth of a metaldehyde tablet) by mistake and complained about a burning sensation in the stomach. After gastric lavage with sodium bicarbonate and liquid paraffin, no further symptoms were observed.

Case 11

Sixteen hours prior to hospitalisation a 2 ½ year old child had swallowed half a metaldehyde tablet and had vomited three times. 24 hours after admission slight trembling of the upper limbs was noted which subsided after several hours.

Case 12

The mother of the patient (male, 17 years old) had prepared rat poison with metaldehyde in the following manner: she had crushed half a metaldehyde tablet, mixed it with some chocolate and made a chocolate truffle out of it. The son ate half of this by mistake. Gastric lavage was adopted. No symptoms of poisoning were detectable.

Case 13

On blowing out a lamp, the patient (adult male) had aspirated metaldehyde dust. Three and half hours later, he complained about nausea, stomach pain and headache. He was treated with Antrenyl and Torecan suppositories and was free of symptoms ten hours after the incident.

Case 14

A two year old girl had been sucking a piece of metaldehyde and had possibly swallowed a small amount. After 25 minutes gastric lavage with sodium bicarbonate and prophylactic treatment with penicillin were adopted. Apart from acetone in urine, no symptoms of poisoning occurred.

Case 15

A 2 ½ year old girl had eaten pieces of metaldehyde scattered in the garden as snail pellets. Some hours later, first spasms, which lasted about sixteen hours were observed despite administration of Luminal. No subsequent spasms occurred. The patient was discharged as cured after seven days.

Case 16

About two and a half hours after ingestion of some snail pellets, the parents noticed that the child´s hand was unsteady while drinking from a cup, often spilling the drink. The child (male, four years old) also walked unsteadily and exhibited muscle twitching. Findings on admission were red face, slight tachypnea and swollen abdominal wall. Neurological status was normal, apart from signs of spasticity of the lower limbs and muscle twitching. Within 24 hours after gastric lavage and administration of adsorbent charcoal, the symptoms had regressed. Two days after hospitalisation, however, rather extensive paravertebral hematomas on the back and over the left spina iliaca anterior superior occurred. Platelets, coagulation time and prothrombin time were normal. The patient was discharged as cured after four days.

Case 17

A 15 year old boy had swallowed an unknown amount of metaldehyde snail pellets 30 minutes previously. During the period of hospitalisation, no symptoms of poisoning occurred, apart from acidosis demonstrable only biochemically.

Case 18

One hour before gastric lavage, the a 2 year old boy has swallowed one metaldehyde tablet or less. No symptoms of poisoning apart transient exanthema after 24 hours were observed.

Case 19

An hour and a quarter prior to hospitalisation and gastric lavage, a 1 year old boy had swallowed no more than one half tablet of metaldehyde. Status and blood count were normal. Gastric lavage with water and then sodium bicarbonate as well as administration of charcoal were adopted. No symptoms of intoxication occurred. Metaldehyde in lavage returns was chemically demonstrated.

Case 20

A 4 year old child had swallowed an unknown amount of metaldehyde tablets. On admission, no symptoms of poisoning except for a reddened face were noted. No subsequent symptoms occurred.

Case 21

The patient (adult male) had been boiling water with metaldehyde for some time when he began to suffer from nausea, dizziness and vomiting (intoxication by aspiration). The dizziness subsided only 24 hours later, after he had vomited another eight times. The patient was treated with oral sodium bicarbonate.

Case 22

Several days previously the patient (no information on age and sex) had swallowed 10 tablets of metaldehyde. He then began to suffer from disturbed vision, seeing triangles and red, green and black images. The disturbances of vision were said to have occurred only on the day of ingestion; no subsequent symptoms were noted. The patient had undergone gastrectomy shortly before. Acid was nonetheless demonstrated in the residual stomach.

In a publication by Moody J. and Inglis F. (1992, Doc.No. 592-029), a single case of metaldehyde intoxication is presented. A 37 year old Asian male was admitted to hospital suffering from a

suspected drug overdose and in a comatose condition. He developed pyrexia (temperature up to 38.5°C) and multiple violent seizures which were treated with diazepam and phenytoin. On admission, blood urea, glucose and electrolytes were normal and gastric lavage returned clear fluid. He was treated with intravenous fluids (5 % dextrose and normal saline) but developed hypokalaemia and mild renal impairment. All indices of liver function were normal apart from the AST which was slightly raised on admission (43 U L-1), increasing to 660 U L-1 after 3 days and declining to 95 U L-1 on the fifth day. On the sixth day the patient had recovered sufficiently to be transferred to a psychiatric hospital. Relatives found two empty containers of Slugit liquid in the garage. Slugit liquid contained approximately 20 % metaldehyde and 9 % ethylene glycol. A minimum intake of 35-50 ml was estimated after losses from nausea and vomiting. Subsequent examination of serum and urine specimens by gas chromatography confirmed the presence of metaldehyde. The serum levels remained elevated for 35 hours. Ethylene glycol did not appear to have contributed significantly to toxicity.

Thompson J., Casey P. and Vale J. (1995; Doc.No. 592-030) reported a case of suicidal intake of two 250 ml bottles of Murphy Slugit Liquid (20 % Metaldehyde, 9 % ethylene glycol) and 1 ½ packets of Ratak (difenacoum). The 45 year old male patient vomited and subsequently became comatose and developed convulsions. The patient was ventilated mechanically but died after one week. He was treated with vitamin K and his prothrombin time remained normal. The conclusion of the authors was that the clinical and post mortem findings were in keeping with metaldehyde poisoning, though ethylene glycol (not confirmed by analysis) may also have contributed to the fatal outcome.

A case report of massive metaldehyde poisioning is presented by Longstreth W. and Pierson D. (1982; Doc.No. 592-033). A 32 year old woman swallowed approximately 470 ml of a commercial slug bait that contained 4 % metaldehyde. In total, 28.9 g metaldehyde or approximately 330 mg/kg bw was ingested. All other ingredients were reported to be inert without known toxicity. The patient soon had nausea and vomiting. Two hours after ingestions she had the first of many generalised convulsions. Initially she was treated at a local hospital with gastric lavage, activated charcoal and diazepam, but later that day was transferred to a Medical Center because of continuing convulsions, decreased mental status and muscle spasms. By the time transfer was completed the patient was comatose. On neurologic examination she was noted to be unresponsive to voice or painful stimuli. Pupils were reactive, eye movements were full on horizontal oculocephalic reflexes and corneal reflexes were present. Tone and tendon reflexes were diffusely increased though there were flexor plantar responses. Chvostek´s sign was present. The arterial blood gas determinations showed respiratory alkalosis with pH 7.57. Urine pH was 5.5 with ketones present. Serum calcium and magnesium levels were normal. Toxicologic studies on blood and gastric contents were negative except for an unidentified substance. The hospital course was complicated by severe muscle spasms and repeated generalised convulsions despite therapeutic concentrations of phenytoin and phenobarbital in the blood. Diazepam administered intravenously only briefly controlled the spasms and convulsions. Although the serum creatine kinase level was elevated to four times normal, significant myoglobinuria did not develop. She also had pneumonia, increased oral and tracheobronchial secretions and elevated serum transaminase levels, which peaked during the second week and returned to normal by the time of discharge. Convulsions continued for three days, during which time an interictal electroencephalogram showed diffuse slowing with scattered epileptiform discharges. The coma lasted for seven days but tracheal intubation was prolonged to nine days due to general weakness and excessive secretions. When communication became possible the patient was noted to have pronounced memory deficits, an exaggerated glabellar reflex and prominent snout and palmomental reflexes. Three months before this admission, the findings of a detailed neurologic examination (done during a hospital stay for a suicide attempt) had been entirely normal. The patient´s strength improved and administration of anticonvulsant drugs was discontinued. Findings on lumbar puncture and contrast-enhanced cranial computerised tomography were normal. Although her mental

status improved slowly, at best she had a poor recent and remote memory, flat affect and pronounced latency of response. She was subsequently transferred to the psychiatric service. A repeat electroencephalogram was normal. Primarily she had an adaptive problem-solving impairment and severe impairment of memory in both verbal and visual-spatial areas. The patient was discharged 51 days after the attempted suicide. One year after the poisoning, the patient reported by telephone that her memory had returned almost to normal.

In a review publication by Booze T. and Oehme F., (1985; Doc.No. 592-025), 2 cases of metaldehyde poisoning are cited, of which one case is identical with the case above (32 year old woman) presented by Longstreth W. and Pierson D. (1982; Doc.No. 592-033). The other cited case was derived from EPA database and concerned a 30 year old female which ingested 16 – 19 g of a liquid slug bait. The clinical signs included convulsions for 3 days, fever, coma, memory loss, respiratory depression, frontal lobe damage, regression to infantile reflexes and general apathy. It was not reported if the patient recovered.

No detrimental effects were reported on health in manufacturing personnel.

Conclusion:

Data regarding human intoxication are primarily available from exposures to single doses after accidental or suicidal oral intake. Chronic poisonings at low doses are not known and are not likely to occur because of the rapid elimination of metaldehyde. The course of the intoxication is characterised by a first phase involving gastrointestinal signs such as nausea, salivation, vomiting and later abdominal pain and diarrhoea. This phase may be followed by convulsions, somnolence, coma, apnoea, cyanosis, memory loss and decreased blood pressure.

4.2.3 Summary and discussion of acute toxicity

Two acute oral toxicity tests in rats showed that metaldehyde is of moderate acute oral toxicity. Also in mice moderate acute oral toxicity was demonstrated. At high dose levels, unspecific symptoms like reduced activity and lethargy, hunched posture and decreased respiratory rate but also convulsions and ataxia were observed in both species tested. Target organs in these studies were the lungs, liver and gastrointestinal tract.

Despite some limitations of the study with acute dermal exposure to rats, the results obtained demonstrated low acute toxicity of metaldehyde by this route of administration. The available study on acute inhalation toxicity (Berczy et al., 1973) shows major limitations and was considered not to be of sufficient validity for assessing the acute inhalation toxicity of metaldehyde. An attempt was made to initiate a new inhalation toxicity study, but the particle size could not be reduced to meet the requirements of OECD 403 (Griffiths, 2009). Based on the new experience (Griffiths, 2009) it is not feasible to perform a valid inhalation toxicity study due to the physico-chemical properties of metaldehyde.

4.2.4 Comparison with criteria

The lowest LD_{50} value of 283 mg/kg for acute oral toxicity was found in rats. According to Regulation (EC) No. 1272/2008 metaldehyde belongs to acute toxicity category 3 ($50 < ATE \leq 300$ mg/kg bw) and requires classification and labelling with **H301 "Toxic if swallowed"**.

For acute dermal toxicity the LD_{50} value was greater than 5000 mg/kg in rabbits. According to Regulation (EC) No. 1272/2008, metaldehyde does not require any classification and labelling for acute dermal toxicity.

4.2.5 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: Acute Tox. 3, H301

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Information from humans

Numerous case reports and notifications to Poison Centers on poisonings after accidental or suicidal intake of metaldehyde were documented by the Dossier Submitter (DS). The reported incidents involved 189 children and 24 adults. Of these, 122 intoxications were due to ingestion of metaldehyde tablets (pure metaldehyde), and 101 cases to snail pellets (containing 5 - 7% metaldehyde). The severity of the poisoning was known in 128 cases: 87 cases were mild, 25 moderately severe, 14 severe and 2 were fatal. From these cases, 22 case histories were presented in the CLH dossier. The course of the intoxication is characterised by a first phase involving gastrointestinal effects such as nausea, salivation, vomiting and later abdominal pain and diarrhoea. This phase may be followed by convulsions, somnolence, coma, apnoea, cyanosis, memory loss and decreased blood pressure.

Acute toxicity

Oral

The substance has an existing minimum classification for acute oral toxicity as Acute Tox. 4*.

The Dossier Submitter presented 3 acute oral toxicity studies. Two were OECD TG 401 compliant using rats and mice (Jones and Collier, 1987 and Coles, 1990, respectively) and the third was a TG 425 compliant study (Durando, 2009).

The two acute oral toxicity tests in rats showed that metaldehyde is of moderate acute oral toxicity. In mice, moderate acute oral toxicity (LD_{50} values of 411 mg/kg bw and 443 mg/kg bw in males and females, respectively) was demonstrated. The lowest LD_{50} value of 283 mg/kg bw for acute oral toxicity was found in rats (calculated for both sexes, Jones and Collier, 1987). At dose levels from 100 mg/kg bw and above, non-specific symptoms such as reduced activity and lethargy, hunched posture and decreased respiratory rate, but also convulsions and ataxia were observed in both species tested. Surviving rats and mice recovered by day 8 after treatment. Target organs in these studies were the lungs, liver and gastrointestinal tract.

Dermal

In a dermal acute toxicity study of limited validity (only partly compliant with OECD TG 402), groups of 5 rats/sex received a topical application of metaldehyde (without data on purity or batch no.) suspended in water at dose levels of 0 (vehicle control) and 5000 mg/kg bw (Davies and Collins, 1974). No mortality was observed during the 14-day observation period. The only signs of clinical toxicity were slight lethargy and piloerection on the day of treatment (the number of animals affected was not given). There were no

local reactions, such as erythema or oedema. Terminal autopsy revealed darkening of the liver and spleen together with pale or mottled kidneys (incidences were not reported).

Comments received during public consultation

Two MSCAs agreed with the proposed classification for acute oral toxicity.

Assessment and comparison with the classification criteria

Human information

Data was available on numerous cases of human intoxications after single accidental or suicidal oral intake. These case reports do not provide sufficient detail to enable the dose swallowed to be estimated, except in one case report stating that 29 g (appr. 330 mg/kg bw) was ingested (Longstreth and Pierson, 1982). Thus, it cannot be assessed whether human data would justify a lower category than category 3 resulting from the lowest LD_{50} estimated in animals (see below).

Oral

The lowest LD_{50} value of 283 mg/kg bw for acute oral toxicity was found in an OECD TG 401 compliant study in male/female rats dosed at 100-800 mg/kg bw in arachis oil (Jones and Collier, 1987). According to Regulation (EC) No. 1272/2008, metaldehyde therefore meets the criteria for classification in acute toxicity category 3 ($50 < ATE \leq 300$ mg/kg bw);H301 "Toxic if swallowed".

The most recent, OECD TG 425 compliant study (Durando, 2009) was considered to provide supportive information. It was conducted on female rats only with the highest dose of 1110 mg/kg bw (in water) and with two test groups given 350 mg/kg bw using water and corn oil as vehicle. The LD_{50} was estimated to be 654 mg/kg bw.

The classification proposal is supported by observations from two acute neurotoxicity studies of mortalities in rats after single oral administration of 250 mg/kg bw (Haferkorn, 2009; Jones, Finn, Mullee, 2003).

Additionally, in a developmental toxicity study (Neeper-Bradley and Chun, 1990), mortality (6/25) was observed in pregnant dams in the initial 1-2 days of treatment (150 mg/kg bw by oral gavage).

Inhalation

The available acute inhalation study (Berczy *et al.*, 1973) was of limited quality and did not allow to estimate a LC_{50} value. Based on this information, no conclusion on the need for classification can be drawn for this endpoint.

Dermal

Taking the limitations of the acute dermal study in rats into account and the absence of mortalities during the 14 days observation period, it is concluded that these data do not support a need for classification for this endpoint.

Overall, RAC agrees with the proposal of the DS, and considers that **metaldehyde should be classified as Acute Tox. 3; H301**.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Table 13: Summary of effects observed in rats, mice dogs and rabbits in comparison to cut off vales

Species- Route (Reference)	Maximum applications	Cut off value Cat 1 STOT SE (1272/2008) [mg/kg bw]	Cut off value Cat 2 STOT SE (1272/2008) [mg/kg bw]	Effects below cut off value	Significance of toxicological effect (1272/2008) below cut off value
Rat- acute oral (gavage) (Jones J. et al., 1987)	1	300	2000	- ≥ 200 mg/kg bw: death, hunched posture, pilo-erection, lethargy, decreased respiratory rate	Lethal effect, already covered by acute toxicity classification
Rat- acute oral (gavage) (Durando J., 2009)	1	300	2000	- ≥ 350 mg/kg bw: hypoactive and/or reduced fecal volume, piloerection - ≥ 1100 mg/kg bw: death, hypoactive, hunched posture, tremors	Absence of significant toxicity at 350 mg/kg bw Lethal effect, already covered by acute toxicity classification
Mouse- acute oral (gavage) (Coles R., 1990)	1	300	2000	- ≥ 400 mg/kg bw: death, hunched posture, lethargy, piloerection, ataxia, ptosis, pallor of the extremities, decreased respiratory rate, tremors, tonic convulsions	Lethal effect, already covered by acute toxicity classification
Mouse- 90 days oral (Gill M. et al., 1990)	1-2	300	2000	- ≥ 743 (F) mg/kg bw: death - 1919 (M)- 2996 (F) mg/kg bw: death	Lethal effect, already covered by acute toxicity classification
Dog- 28 days oral (Leuschner J., 2002)	1	300	2000	- ≥ 60 mg/kg bw/d: reduced motility, clonic convulsions, increased respiratory rate, emesis - ≥ 75 mg/kg bw/d: tonoclonic convulsions, mydriasis, inflated stomach, slight tremor - 90 mg/kg bw/d: ataxia, salivation, abdominal/lateral position, pale gingivial; moribund condition of 1/3 females:	The intensity/severity of the symptoms declined with time and had almost disappeared towards the end of the 4-week treatment Moribund condition, already covered by acute toxicity classification

Species- Route (Reference)	**Maximum applications**	**Cut off value Cat 1 STOT SE (1272/2008) [mg/kg bw]**	**Cut off value Cat 2 STOT SE (1272/2008) [mg/kg bw]**	**Effects below cut off value**	**Significance of toxicological effect (1272/2008) below cut off value**
				shaking of the head, lateral position, difficulty in breathing- no pathological findings (macroscopic) after necropsy	
Dog- 52 weeks oral (Leuscher J., 2003)	1-2	300	2000	- 90 mg/kg bw: ataxia, reduced motility, emesis, tremor, twitching, salivation- no changes in histopathology	Incidence and severity declined from study week 19 onwards. No changes in histopathology.
Rat- developmental (Neeper-Bradley T. et al., 1990)	1-2	300	2000	Dams: - 150 mg/kg bw: 6/25 death, 6/25 ataxia, 3/25 tremor, 3/25 twitching, 1/25 hyperactive, 1/25 prostration, 1/25 paresis	Lethal effect, already covered by acute toxicity classification
Rabbit- developmental (Neeper-Bradley T. , 1990a)	1-2	300	2000	Dams: - ≥ 100 mg/kg bw: 1/5 tremor - ≥ 200 mg/kg bw: 2/5 death, 3/5 tremor, 2/5 ataxia, 1/5 paresis, 1/5 broken vertebrae - ≥ 350 mg/kg bw: 2/5 death, 2/5 tremor, 2/5 ataxia, 1/5 convulsions, 1/5 prostration, 1/5 broken vertebrae - 500 mg/kg bw: 4/5 death, 3/5 tremor, 4/5 ataxia, 3/5 twitch, 2/5 broken vertebrae	Absence of significant toxicity at 100 mg/kg bw. Lethal effect ≥ 200 mg/kg bw, already covered by acute toxicity classification
Rat- oral (gavage) acute neurotoxicity (Haferkorn J., 2009)	1	300	2000	- ≥ 150 mg/kg bw: slight tremor (F), piloerection, diarrhea, impaired ability for wired manoeuvre, impaired gait (F), ↓ resistance during limb rotation (F) - 250 mg/kg bw: 5/10 death (F), reduced motility (F), ataxia (F), tremor (M+F), reduced muscle tone (F), tonic convulsions (F), ↑ body temperature, ↓	Reversible changes in clinical signs Transient findings in neurological screening No macroscopic or microscopic findings in the nervous tissue or in the vasculature of the nervous tissue

Species- Route (Reference)	**Maximum applications**	**Cut off value Cat 1 STOT SE (1272/2008) [mg/kg bw]**	**Cut off value Cat 2 STOT SE (1272/2008) [mg/kg bw]**	**Effects below cut off value**	**Significance of toxicological effect (1272/2008) below cut off value**
				hindleg splay (F), ↓ righting reflex (F), ↓ toe/tail pinch response (F)	Lethal effect ≥ 250 mg/kg bw, already covered by acute toxicity classification
Rat- oral (gavage) acute neurotoxicity (Herberth M.T., 2011)	1	300	2000	- ≥150 mg/kg bw: tremors (M+F) and lacrimation (F); lower total and ambulatory locomotor activity (F) - ≥ 250 mg/kg bw: mortality (M+F), tremors (M+F), clonic and tonic convulsions (F), impaired mobility (F), altered gait (F), low and very low arousal (F), shorter hindlimb footsplay (F), higher mean time to first step (F), increased mean catalepsy time (F), and lower mean body temperatures (M+F); lower total and ambulatory locomotor activity (M+F)	No clinical signs Transient findings in neurological screening No macroscopic or microscopic findings in the nervous tissue Lethal effect ≥ 250 mg/kg bw, already covered by acute toxicity classification
Rat- oral 90 day repeated dose neurotoxicity (Jones L. et al. 2003)	10	300	2000	- 240 mg/kg bw: 1 female: loss of limb function with no sign of recovery (premature sacrifice on day 22) considered to result from spinal cord injury	No macroscopic or microscopic findings Premature sacrifice at 240 mg/kg bw, already covered by acute toxicity classification

4.3.2 Comparison with criteria

There was no evidence of any specific, non-lethal target organ toxicity arising from a single exposure to metaldehyde. (Reversible) clinical signs of toxicity were observed after single exposures to metaldehyde but were considered to be non-specific signs of general acute toxicity. According to the Guidance on the Application of the CLP Criteria (ECHA 2009): “Acute toxicity refers to lethality and STOT-SE to non lethal effects. However, care should be taken not to assign both classes for the same toxic effect, essentially giving a “double classification”, even where the criteria for both classes are fulfilled”. The dose-response curve for metaldehyde seems to be rather steep, meaning that significant acute toxicity occurs only at doses which already lead to mortality. Therefore no classification as STOT SE is proposed.

4.3.3 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: no classification proposed

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The Dossier Submitter concluded that there was no evidence of any specific, non-lethal target organ toxicity arising from a single exposure to metaldehyde. Reversible clinical signs of toxicity were observed after single exposures to metaldehyde, but these were considered to be non-specific signs related to general acute toxicity.

The CLH report evaluates neurotoxic effects from acute and repeated neurotoxicity studies in Chapter 4.12.1.1 (Other effects). The DS concluded that neurofunctional effects (e.g. tremor, convulsions, ataxia, paresis) following exposure to metaldehyde are considered to occur only at doses which are clearly acutely toxic. These effects did not persist (with the exception of hind limb paresis following spinal cord injury) and were not consistent with sustained dysfunctions normally induced by classical neurotoxins. Because of the distinct differences between metaldehyde and classical neurotoxins, the term "neurotoxic effect" was not considered adequate to characterize the toxicity profile of metaldehyde. Therefore in the EFSA peer review, it has been concluded that metaldehyde does not require classification as a neurotoxicant. The conclusion drawn by EFSA is as follows: "*Acute toxic effects following metaldehyde administration include partly pronounced neurological symptoms, without specific neurotoxic mechanism leading to degeneration or other toxic damage to the central or peripheral nerve tissue. Therefore, these reversible effects at high doses are not relevant for classification and labelling as neurotoxicant*."

One acute inhalation toxicity study of limited validity (Berczy, 1973) showed some effects suggesting irritation of the respiratory system: eye irritation, dyspnea, sneezing, discomfort and increased nasal and oral secretion were seen in animals exposed to high dust concentrations. After exposure, these signs of irritation to the respiratory system disappeared within one hour.

No classification was proposed for STOT SE.

Comments received during public consultation

Two MSCAs agreed with the proposal for no classification for STOT SE.

Assessment and comparison with the classification criteria

No effects other than reversible non-specific clinical signs were reported in the acute toxicity studies at doses without mortalities.

Neurological abnormalities at the lethal dose of 250 mg/kg bw and at the non-lethal dose of 150 mg/kg bw from two acute neurotoxicity studies may be indicative of neurotoxicity. However, as 150 mg/kg bw is close to the lethal dose (factor < 2) and comparable

abnormalities were seen at lethal/non-lethal doses, the neurotoxic effects are considered to be covered by the acute toxicity classification.

RAC considers that dyspnea, sneezing, discomfort and increased nasal and oral secretion could be indicative of an transient irritative response of the respiratory tract. However, 15 mg/L was tested in the acute inhalation study and at this high dose it remains uncertain whether the high particle load caused the effects or whether it could be a substance-related (sensory) irritation. No information on lower test concentrations were available. RAC agrees with the Dossier Submitter that **no classification for STOT SE is warranted**.

Supplemental information - In depth analyses by RAC

A range of clinical symptoms were summarised in the CLH report as observations from human intoxications (such as confusion, restlessness, haziness, drowsiness, coma, spasms, tremor, muscle twitching, chorea, abnormal reflexes, ataxia, elevated muscle tone, hypersensitivity, Chvostek's sign, Trousseau's sign, disturbed vision, amnesia, respiratory arrest, *risus sardonicus*). Although indications of convulsions/tremor/ataxia were seen in humans and animals, it is neither possible to conclude on the similarities between species nor on the mode of actions behind them.

Clinical/neurological signs were seen in rats in an acute neurotoxicity (OECD TG 424) study (Haferkorn, 2009) at doses of 150 mg/kg bw and 250 mg/kg bw. While 5/10 female rats died after a dose of 250 mg/kg bw within 24h after administration, no mortalities were seen at 150 mg/kg bw. Neurological screening revealed several changes such as pilo-erection, tremor, diarrhea, increased body temperature, convulsions, impaired ability for wire manoeuvre, impaired gait, decreased resistance during limb rotation, increased hindleg splay, reduced righting reflex and/or a reduced toe/tail pinch response. The histomorphological examination of the nervous system did not reveal any pathological evidence.

Mortalities were also noted at 250 mg/kg bw in 1/10 male and 2/10 female rats in a second OECD TG 424 study (Herberth, 2011). The predominant findings during the FOB evaluations in the 250 mg/kg bw group at the time of peak effect (6 h after dosing) on study day 0 included tremors, clonic and tonic convulsions, impaired mobility, altered gait, low and very low arousal, shorter hindlimb footsplay, higher mean time to first step, increased mean catalepsy time, and/or lower mean body temperatures. FOB findings in the 150 mg/kg bw group included tremors and lacrimation. On study day 0, lower total and ambulatory locomotor activity was noted in the 150 mg/kg bw group females and 250 mg/kg bw group males and females during the first 10 or 20 minutes of testing and slower habituation was noted for the 250 mg/kg bw group males and females. There were no treatment-related neurological findings noted on study days 7 and 14, demonstrating that the acute effects of metaldehyde were reversible. There was no evidence of morphological or neuropathological changes at any dosage level. Based on the results of this study, the no-observed-adverse-effect-level (NOAEL) for neurotoxicity of a single dose of metaldehyde to rats was 75 mg/kg bw for males and females.

4.4 Irritation

4.4.1 Skin irritation

Table 14: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation and corrosivity study in rabbits	Not irritating	-	Jones, J.; 1983

4.4.1.1 Non-human information

A primary skin irritation and corrosivity study was performed in female New Zealand White rabbits. No guideline is mentioned in the study report, but the design complies to a large extent with the requirements of the OECD guideline 404. The study is conform with GLP and is considered to be scientifically valid and acceptable.

A portion of metaldehyde (0.5 g moistened with water) was applied to the clipped dorsal skin. The patch was occluded (no further details given) and secured with a strip of impermeable adhesive tape for a 4-hour exposure period. Following the exposure period, the patches were removed and the skin wiped with a paper towel moistened with water to remove any remaining test material. The skin sites were examined 60 minutes after treatment and then daily for 3 days. Skin reactions were evaluated according to Draize. No skin reactions were noted on any treated site during the observation period.

4.4.1.2 Human information

The irritation and sensitisation potential of 36 substances including metaldehyde was tested (*Lisi P., Carafini S., Assalve D.; 1987; Doc.No. 592-002*). The number of persons tested for metaldehyde was 442, of whom 89 were agricultural workers, 30 ex-agricultural workers, and 323 others. Patch tests were performed on the upper back and were read after 48 and 72 h. Irritant and allergic reactions were evaluated according to Cronin`s criteria. The concentration tested was 1%. Neither irritant nor allergic reactions were observed in the test persons.

4.4.1.3 Summary and discussion of skin irritation

Metaldehyde (moistened with water) is not irritating to the skin.

4.4.1.4 Comparison with criteria

Estimated skin irritation scores are below the criteria for triggering classification and labelling.

4.4.1.5 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: no classification proposed

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The Dossier Submitter summarised data from Lisi *et al.* (1987) indicating that 442 persons (a third of them were current or former agricultural workers) tested with 1% metaldehyde in patch tests showed neither irritant nor allergic reactions.

A study on skin irritation/corrosion in rabbits (similar to OECD TG 404) with occlusive administration of 0.5 g metaldehyde did not reveal any skin reactions after 60 minutes or 1, 2 and 3 days after treatment (Jones, 1983). The Dossier Submitter proposed no classification.

Comments received during public consultation

One MSCA agreed with the proposal for no classification.

Assessment and comparison with the classification criteria

RAC agrees with the proposal of the Dossier Submitter that based on the available data **no classification as a skin irritant is warranted**.

4.4.2 Eye irritation

Table 15: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Acute eye irritation testing rabbits	Very slightly irritang	-	Coles, R.; 1990

4.4.2.1 Non-human information

An acute eye irritation test was performed in three adult female New Zealand White rabbits. The study was conducted according to GLP and to OECD guideline 405. The study is considered scientific valid and acceptable.

The animals received single applications of 0.1 mL (equivalent to approximately 82 mg) of the undiluted test substance (batch no. 5448, purity not given) into the conjunctival sac of the right eye. The eyelids were held together for one second following application. The left eyes remained untreated and served for control purposes. The eyes were examined for ocular reactions according to Draize (1 hour after the instillation and then 1, 2, and 3 days thereafter).

Metaldehyde (undiluted) was found to be slightly irritating to the eyes of rabbits. Iridial inflammation (grade 1) was noted in all treated animals 1 hour after treatment but no longer at 24, 48 and 72 h. Minimal conjunctival irritation (grade 1) was noted in all treated eyes 1 and 24 hours

after treatment. Conjunctival chemosis and discharge were found only 1 h after treatment. All treated eyes appeared normal after 48 h. No corneal effects were noted during the study.

4.4.2.2 Human information

Not available.

4.4.2.3 Summary and discussion of eye irritation

Metaldehyde (undiluted) was slightly irritating to the eyes of rabbits.

4.4.2.4 Comparison with criteria

Estimated eye irritation scores (24 – 72 hours; 0 (conjunctival chemosis), 0.33 (conjunctival redness) and 0 (iritis) and 0 (corneal opacity) are below the criteria for triggering classification and labelling.

4.4.2.5 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: no classification proposed

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

In an acute eye irritation test, which was compliant with OECD TG 405, slight irritation to the eyes of rabbits were observed. Iridial inflammation (grade 1) was noted in all treated animals 1 h after treatment but no longer at 24, 48 and 72 h. Minimal conjunctival irritation (grade 1) was noted in all treated eyes 1 and 24 h after treatment. Conjunctival chemosis and discharge were found only 1 h after treatment. All treated eyes appeared normal after 48 h. No corneal effects were noted during the study.

The Dossier Submitter proposed no classification as the estimated scores (24 – 72 h) were 0 (conjunctival chemosis), 0.33 (conjunctival redness) and 0 (iritis) and 0 (corneal opacity), and these scores did not meet the criteria for classification in the CLP Regulation.

Comments received during public consultation

Two MSCAs agreed with the proposal for no classification.

Assessment and comparison with the classification criteria

RAC agrees with the Dossier Submitter's proposal that based on the low severity grades of irritation and oedema of the conjunctiva and the reversibility of the mild effects after 1 h or 24 h, **no classification for eye irritation/eye damage is warranted**.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

One acute inhalation toxicity study of limited validity (Berczy, Z.; 1973) showed some irritating effects to the respiratory system: Eye irritation, dyspnea, sneezing, discomfort and increased nasal and oral secretion were seen in animals exposed to the high dust concentration. After exposure, these signs of irritation to the respiratory system disappeared within one hour.

4.4.3.2 Human information

Not available.

4.4.3.3 Summary and discussion of respiratory tract irritation

Mild and transient signs of respiratory tract irritation were observed in one non-valid acute inhalation toxicity study.

4.4.3.4 Comparison with criteria

Mild and transient signs of respiratory tract irritation seen in a non-valid acute inhalation toxicity study are not sufficient for classifying as respiratory tract irritant.

4.4.3.5 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: no classification proposed

4.5 Corrosivity

See chapter *4.4.1 Skin irritation*.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 16: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Buehler test (guinea pig)	Not sensitising	Limited validity, some major deviations	Nitka, S.; 1984
Local Lymph Node Assay (mouse)	Not sensitising	-	Bull, A. D.; 2007
Local Lymph Node Assay (mouse)	Not sensitising	-	Dreher D.M.; 2008

4.6.1.1 Non-human information

Skin sensitization in the guinea pig was investigated in a study according the methods of Buehler (1965) but not according to current guidelines. Although no sensitizing effects were observed, the study was considered of limited validity and not adequate to draw conclusions on the sensitizing properties of metaldehyde. Therefore, a new Local Lymph Node Assay was performed. After sending the CLH dossier of metaldehyde to ECHA, AGES (Austrian Agency for Health and Food Safety, who is the dossier submitter for metaldehyde) received another Local Lymph Node Assay submitted by the Metaldehyde Task Force during an equivalence check.

Reference:	**LZ1060 Metaldehyde. Assessment of skin sensitization potential using the Local Lymph Node Assay in the mouse**
Author(s), year:	Bull A.D., 2007
Report/Doc. number:	Huntingdon Life Sciences Limited, Cambridgeshire UK; LZA 0292/064237/LN Lonza Report No. 4064, Doc. No.: 567-003
Guideline(s):	OECD Guideline 429 (2002)
GLP:	Yes
Deviations:	No
Validity:	Valid

Material and methods:

Test material	LZ1060 Metaldehyde
Lot/Batch	36809
Purity	99.5%
Vehicle	Acetone : olive oil (4:1 v/v)
Species	Mouse, females
Strain	CBA/Ca
Age	8-12 weeks
Weight at dosing	18.8 – 21.9 g
Source	Harlan UK Ltd., Bicester, Oxon, England

Metaldehyde was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay (LLNA). The assay determines the level of T-lymphocyte proliferation in the lymph nodes draining the site of chemical application, by measuring the amount of radiolabelled thymidine incorporated into the dividing cells. The criterion for a positive response is that one or more of the concentrations tested should elicit a 3-fold or greater increase in isotope incorporation relative to the vehicle control group.

During the study all animals were observed daily for signs of ear-irritation, toxicity or illness.

A preliminary study where groups of one female each were treated with 5, 10 and 25% metaldehyde preparations was performed to select the doses for the main study. The test conditions thereby were similar to the main study.

In the main study 25 µl metaldehyde was applied as 5%, 10% or 25% w/v preparations in acetone : olive oil (4:1 v/v) to the dorsal surfaces of the ears of groups of 4 female mice each. A further vehicle control group of 4 mice received acetone : olive oil (4:1) alone in the same manner. The procedure was repeated daily for 3 consecutive days.

In contemporaneous studies hexyl cinnamic aldehyde, a known skin sensitiser, served as positive control, using 10%, 25% or 50% preparations in acetone : olive oil (4:1).

Five days after the first application (day 6), all mice were injected via the tail vein with 250 µl phosphate buffered saline (PBS) containing 80 µCi/ml ^{3}H-methylthymidine (specific activity 2 Ci/mmol), giving a nominal concentration of 20 µCi to each mouse. 5 hours later, the animals were terminated and the draining auricular lymph nodes were excised and pooled for each experimental group. 1ml PBS was added to the pooled lymph nodes. Thereafter, single cell suspensions of lymph node cells were prepared by mechanical disaggregation. The cells were washed 3 times with 10 ml PBS and were resuspended thereafter in 3 ml trichloroacetic acid (5% TCA). After overnight incubation with TCA at 4°C, the samples were pelleted by centrifugation and the supernatant was discarded. The cells were then resuspended in 1 ml 5% TCA and transferred to scintillation vials containing 10 ml Ultimate gold scintillation fluid. Finally ^{3}H-methylthymidine incorporation was measured by β-scintillation counting.

Findings:

There were no deaths, no signs of toxicity or irritation noted during the preliminary or main study. In the main study greasy fur (cranial region) was found for all test and control animals as well as particles on ears in all treated animals, both effects resolved up to day 6 or 4, respectively.

The application of metaldehyde at concentrations of 5%, 10% or 25% w/v in acetone : olive oil (4:1) resulted in an isotope incorporation which was less than 3-fold at all three concentrations (0.4, 0.9 and 1.0, respectively).

The application of hexyl cinnamic aldehyde (positive control) at concentrations of 10%, 25% or 50% in acetone : olive oil (4:1) resulted in a clear concentration dependent increase in the stimulation index.

No skin sensitisation was seen in the vehicle control group.

Table 17: Radiolabel incorporation into lymph-nodes of mice treated with metaldehyde

Concentration of metaldehyde (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test control ratio
5	8	1272.3	159.04	0.4
10	8	3206.1	400.76	0.9
25	8	3441.3	430.16	1
Control vehicle	8	3527.7	440.96	N/A

N/A: not applicable

Table 18: Radiolabel incorporation into lymph-nodes of mice treated with hexyl cinnamic aldehyde (positive control)

Concentration of hexyl cinnamic aldehyde (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test control ratio
10	8	16123.9	2015.49	7.9
25	8	27440.8	3430.10	13.5
50	8	40300.6	5037.58	19.8
Control vehicle	8	2034.7	254.34	N/A

N/A: not applicable

Conclusion:

Metaldehyde is not regarded as a potential skin sensitizer when using the LLNA test system.

Reference:	**Metaldehyde: local lymph node assay in the mouse (pooled method) and Amendment 1**
Author(s), year:	Dreher D.M.., 2007
Report/Doc. number:	Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, UK Unpublished report No.: 2904-001
Guideline(s):	OECD Guideline 429 (2002)
GLP:	Yes
Deviations:	No
Validity:	Valid

Materials and Methods

Test material: Metaldehyde
Description: white powder
Source: Metaldehyde Task Force
Lot/Batch No.: 070605
Purity: 99.6±2 %
CAS No.: 108-62-3
Stability of test compound: Stable for period of study
Vehicle: Dimethyl sulphoxide (DMSO)

Test Animal
Species: Mouse
Strain: CBA/CaCrl
Age: 9 – 10 weeks old
Weight at dosing: 17 – 19 g
Source: Charles River (UK) Ltd.
Acclimation period: 14 days
Diet: SQC(E) Rat and Mouse Maintenance Diet No. 1
Water: Mains water, ad libitum
Housing: Group house during acclimatisation and individually housed from Day 1 in cages conforming to the 'Code of Practice for the Housing and Care of Animals Used in Scientific Procedures' (Home Office, London, 1989)

Environmental conditions:
Temperature: 19 – 25°C
Humidity: 40 – 70%
Air changes: at least 15 changes per hour
Photoperiod: 12 hours light; 12 hours dark

Animal assignment and treatment

In a preliminary screening test, one mouse was treated by daily application of 25 µL of metaldehyde at 5% w/v in DMSO applied to the dorsal surface of each ear for three consecutive days. DMSO was chosen as the vehicle because it produced the highest suitable concentration of metaldehyde. The mouse was observed daily for five days.

In the main test four groups of four female mice were treated with one of four treatments: vehicle (DMSO) control, 1% w/v, 2.5% w/v and 5% w/v of metaldehyde in DMSO. Topical applications were applied to the outer aspect of the auditory pinnae daily on Days 1, 2 and 3. On Day 6, the mice were placed in a thermocage to dilate the peripheral blood vasculature to aid intravenous dosing of

0.25 mL of tritiated ^{3}H-methyl thymidine injected (in phosphate buffered saline) to each mouse (final concentration of 80 μCi/mL injected). Five hours after the intravenous injection, all mice were killed by exposure to a rising concentration of carbon dioxide. The auricular lymph nodes were then removed and placed in 5 mL of phosphate buffered saline. The nodes from each group were pooled and suspensions of the cellular components of the lymph nodes were processed in phosphate buffered saline followed by 5% w/v aqueous trichloroacetic acid. The samples were analysed using a scintillation counter. The Stimulation Index (ratio of the mean scintillation count per test group relative to the control) was used to determine any stimulation effect. The distintegrations per minute (DPM) values were transformed into DLM (disintegrations per minute per lymph node) to provide the Stimulation Index (SI) value for each test group.

Results and Discussion:

There were no mortalities, signs of systemic effects or change in body weights during the study. The Stimulation Indices were 0.6, 1.0 and 1.5 for concentrations of 1%, 2.5% and 5% w/v metaldehyde respectively. These values are below the threshold level of 3.0 and so are not indicative of a skin sensitisation response.

Conclusion:

The local lymph node assay demonstrated that metaldehyde does not have the potential to cause skin sensitisation under the conditions of the study.

4.6.1.2 Human information

The irritation and sensitisation potential of 36 substances including metaldehyde was tested (*Lisi P., Carafini S., Assalve D.; 1987; Doc.No. 592-002*). The number of persons tested for metaldehyde was 442, of whom 89 were agricultural workers, 30 ex-agricultural workers, and 323 others. Patch tests were performed on the upper back and were read after 48 and 72 h. Irritant and allergic reactions were evaluated according to Cronin`s criteria. The concentration tested was 1%. Neither irritant nor allergic reactions were observed in the test persons.

4.6.1.3 Summary and discussion of skin sensitisation

Metaldehyde was not sensitising to the skin in two Local Lymph Node Assays, in a Buehler test of limited validity and in a patch test performed in human volunteers.

4.6.1.4 Comparison with criteria

Metaldehyde was not sensitising to the skin in two Local Lymph Node Assays.

4.6.1.5 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: no classification proposed

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The Dossier Submitter referred to the publication of Lisi *et al.* (1987), who reported that 442 persons (a third of them were current or former agricultural workers) tested with 1% metaldehyde in patch tests showed neither irritant nor allergic reactions.

The CLH report presented data from a Buehler test of limited validity conducted in guinea pigs (Nitka, 1984) and two Local Lymph Node Assays (LLNA) in mice (Bull, 2007; Dreher, 2008).

In the main study of Bull (2007), doses of 25 µL metaldehyde in 5%, 10% or 25% w/v preparations in acetone: olive oil (4:1 v/v), were administered onto the dorsal surface of the ear. This resulted in isotope incorporation of 3H-methylthymidine marker at ratios of 0.4, 0.9 and 1, while the positive control (10, 25, and 50% hexyl cinnamic aldehyde) revealed stimulation indices (SI) of 7.9 and higher.

SI of 0.6, 1.0 and 1.5 at concentrations of 1, 2.5 and 5% metaldehyde in DMSO were seen in the second LLNA (Dreher, 2008).

The Dossier Submitter concluded that metaldehyde was not sensitising in two LLNA and one Buehler test of limited validity and no classification was proposed.

Comments received during public consultation

Two MSCAs agreed with the proposal for no classification.

Assessment and comparison with the classification criteria

RAC agreed with the Dossier Submitter that the SI of ≤ 1.5 from the two LLNA are below the SI of 3 that may trigger classification.

The Buehler test cannot be considered for classification purposes as insufficient detail information was provided in the CLH report.

The negative Patch test findings of the Lisi study were consistent with the negative LLNA results.

No classification for skin sensitization is warranted.

4.6.2 Respiratory sensitisation

No data available.

4.7 Repeated dose toxicity

Table 19: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Sprague-Dawley rat 4 weeks oral (dietary)	0, 2500, 5000, 10000 and 20000 ppm/diet (equivalent to 0, 197, 382, 761 and 1547 mg/kg bw/d for males; 0, 233, 454 and 875 mg/kg bw/d for females) NOAEL could not be determined LOAEL = 2500 ppm based on: - increased liver weights - histopathological findings in the liver (hepatocellular hypertrophy)	Dose finding study, some deviations, supplementary information only	Van Miller, J.; 1989
Sprague-Dawley rat 90 days oral (dietary)	0, 250, 750 and 2500 ppm/diet (equivalent to 0, 21, 65 and 215 mg/kg bw/d for males and females) NOAEL = 250 ppm Effects at LOAEL: - histopathological findings in the liver (hepatocellular hypertrophy)	-	Thomas, O., Bartlett, A., Brooks, P.; 1998
CD-1 mouse 90 day oral (dietary)	0, 100, 300, 1000, 3000 and 10000 ppm/diet (equivalent to 0, 19, 54, 178, 560 and 1919 mg/kg bw/d for males; 0, 24, 70, 235, 743 and 2996 mg/kg bw/d for females) NOAEL could not be determined LOAEL = 100 ppm based on: - increased liver weights - histopathological findings in the liver (hepatocellular hypertrophy, necrosis, acute inflammation, anisokaryosis)	Dose finding study, limited investigations, supplementary information only	Gill, M., Wagner, C.; 1990
Beagle dog 4 weeks oral (dietary)	Escalating doses: 30, 60, 75 and 90 mg/kg bw/d Fixed doses: 75 and 90 mg/kg bw/d NOAEL could not be determined Observed effects: - mortality at 90 mg/kg bw/day - clinical signs: reduced motility, tremor, convulsions, ataxia, emesis, increased respiratory rate, lateral/ abdominal position, moderate salivation, pale gingival, mydriasis	Dose finding study	Leuschner, J.; 2002
Beagle dog 26 weeks oral (dietary)	0, 20, 60 and 90 mg/kg bw/d NOAEL = 20 mg/kg bw/d Effects at LOAEL: - histopathological findings in the testes: diffuse atrophy of the germinative epithelium - histopathological findings in the prostate: diffuse atrophy	-	Neumann, W.; 1980, 1991 + Re-evaluation of histopathological findings in the testes Leuschner, J.; 2009

Beagle dog 52 weeks oral (dietary)	0, 10, 30 and 90 mg/kg bw/d via diet NOAEL = 10 mg/kg bw/d Effects at LOAEL: - histopathological findings in the testes (atrophy of the germinal epithelium) - mortality	-	Leuschner, J.; 2003 + Re-evaluation of histopathological findings in the testes Leuschner, J., Drommer, W.; 2009
Beagle dog 52 weeks oral (dietary)	0, 1, 3.5 and 15 mg/kg bw/day NOAEL > 15 mg/kg bw/day	-	Gauvin, G.V., 2010
NZW rabbit 21 day dermal	0, 100, 300 and 1000 mg/kg bw/d NOAEL = 1000 mg/kg bw/d	-	Hermansky, S., Wagner, D.; 1991

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

28-day toxicity (rat)

Reference:	**Twenty-Eight day Dietary Oral Toxicity Study with Metaldehyde in rats**
Author(s), year:	Van Miller J., 1989
Report/Doc. number:	Lonza Report No. 1380, Doc.No. 532-001, Conducting laboratory: Bushy Run Research Center, Pennsylvania, USA
Guideline(s):	No guideline is mentioned in the study report but the design is similar to OECD guideline 407.
GLP:	Yes
Deviations:	limited histopathology, no NOAEL could be determined
Validity:	This dose finding study is scientific valid but due to the deviations mentioned considered as supplementary information only.

Material and Methods:

Groups of 10 male and 10 female rats of approximately 8 weeks of age (strain: Sprague-Dawley CD; source: Charles River Breeding Laboratories, MI) received diets containing 0, 2500, 5000, 10000 or 20000 ppm metaldehyde (batch no. 5448; purity 99.0 %), equivalent to 0, 197, 382, 761 and 1547 mg/kg bw/d in males and 0, 233, 454 and 875 mg/kg bw/d in females, resp., for 4 weeks. Due to 100 % mortality in females at the top dose, no daily intakes could be calculated for this group. Diets were prepared weekly; concentrations of metaldehyde in the diet, and stability and homogenicity of the test substance were confirmed by analysis.

Animals were observed twice daily for clinical signs or reaction to treatment. Detailed clinical observations were performed each week. Body weight and food consumption data were collected weekly. Prior to sacrifice, blood samples were taken from fasted animals for haematological investigations (haematocrit, haemoglobin, RBC, erythrocyte indices, reticulocyte count, total leucocyte count, WBC differential, platelets count) and clinical chemistry investigations (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), gamma glutamyl transpeptidase, total protein, albumin, globulin, A/G ratio, blood urea nitrogen, creatinine, glucose, total, direct and indirect bilirubin, sodium, potassium, chloride, calcium, inorganic phosphorus). No urinalysis was performed.

At necropsy, suriving animals were subjected to a detailed gross pathological examination, and weights of selected organs (adrenals, brain with stem, heart, kidneys, liver, ovaries, spleen, testes) were recorded for each animal. Histopathological examinations were limited to adrenals, brain, kidneys, liver, spinal cord and peripheral nerve (sciatic) from all control animals (both sexes), from male rats of the 20000 ppm group and from females of the 10000 ppm group.

Findings:

General observations: At the highest dose level of 20000 ppm, mortality occurred in 100 % of females and 40 % of males. Death occurred in the females during the first week of treatment (1 animal on day 2, 1 on day 3, 1 on day 4, 1 on day 5, 4 on day 6, 2 on day 7) while no males died prior to day 6. Also, 60 % of the females receiving 10000 ppm died (between day 4 and 21) or were sacrificed in a moribund condition. Animals were sacrificed when found with hind limb paralysis/paresis. In all cases the paralysis/paresis was associated with spinal fracture/luxation which resulted in pinching of the spinal cord (see pathology). According to the study report it was assumed that convulsions, although observed in only one high dose female (on day 2), occurred prior to and resulted directly in the fractures/luxations. No other clinical signs of toxicity were considered related to metaldehyde toxicity.

Concerning food consumption, there was some dose-related decrease in both sexes from all treatment groups during the first week of treatment and food intake tended to be slightly lower throughout the remainder of the study. However, these differences were considered to be the result of moderate aversion to the test diet, particularly in the first week. There were also dose-related decreases in body weight gain for males from the 5000, 10000 and 20000 ppm groups, and for females from the 5000 and 10000 ppm groups during the first week of the study. Mean weight and the total weight gain for all groups, however, were similar to controls at the end of the study.

Table 20: 28 day feeding study in rats Food consumption and body weight / body weight gain (mean values)

	0 ppm	2500 ppm	5000 ppm	10000 ppm	20000 ppm
Males					
body weight (g)					
week 0	255.9	255.5	255.1	254.7	254.7
week 1	298.1	293.8	286.1*	280.8**	270.4**
week 2	332.2	329.5	319.7	314.5*	307.1**
week 3	356.3	357.2	347.8	342.0	342.9
week 4	376.8	380.2	370.9	365.1	369.1
body weight gain (g)					
week 0 - 1	42.1	38.2	31.0*	26.1**	13.8**
week 0 - 4	121.3	124.6	115.7	110.4	111.0
food consumption (g/animal/day)					
week 0 - 1	25.4	24.1*	22.6**	21.6**	20.1**
week 3 - 4	26.3	26.4	25.0	24.3*	25.4
Females					
body weight (g)					
week 0	164.4	163.5	162.9	164.0	162.8
week 1	186.5	181.1	177.9	172.7	-
week 2	202.7	198.4	196.4	193.7	-
week 3	214.3	210.3	210.1	211.1	-
week 4	226.8	219.5	217.7	218.6	-
body weight gain (g)					
week 0 - 1	22.1	17.6	15.0*	6.8**	-
week 0 - 4	62.4	56.0	54.9	53.2	-
food consumption (g/animal/day)					

	0 ppm	2500 ppm	5000 ppm	10000 ppm	20000 ppm
week 0 - 1	19.0	16.9**	15.3**	13.3**	-
week 3 - 4	18.9	18.2	18.5	18.2	-

** ($p \leq 0.01$), * ($p \leq 0.05$); significantly different from controls

Haematological investigations at study termination revealed a decrease in erythrocyte count for males from the 10000 and 20000 ppm groups. In addition, a trend (not statistically significant) towards decreased values of hemoglobin and hematocrit was observed in all treatment groups of males and in the 2500 and 10000 ppm groups of females. Investigations on clinical chemistry parameters demonstrated a significant increase in urea nitrogen for females from the 2500 and 10000 ppm groups. It was stated in the report that the biological significance of all these findings mentioned is unclear. No other differences between treatment groups and controls were considered treatment-related.

Table 21: 28 day feeding study in rats Haematological and clinical chemistry findings (mean values)

	0 ppm	2500 ppm	5000 ppm	10000 ppm	20000 ppm
Males					
Erythrocyte (106/µL)	7.8	7.7	7.7	7.3*	7.2*
Hematocrit (%)	43.6	42.8	42.8	41.8	41.0
Hemoglobin (g/dL)	16.1	15.7	15.9	15.8	14.9
Urea nitrogen (mg/L)	144	153	148	153	135
Females					
Erythrocyte (106/µL)	7.1	6.9	7.0	6.7	-
Hematocrit (%)	40.1	39.4	40.2	38.9	-
Hemoglobin (g/dL)	15.0	14.8	15.0	14.3	-
Urea nitrogen (mg/L)	141	162*	155	180**	-

** ($p \leq 0.01$), * ($p \leq 0.05$); significantly different from controls

Pathology: Gross lesions related to treatment were restricted to the animals that died or were sacrificed in a moribund condition. These lesions were consistent with those to be expected in animals that suffered convulsions and with the observations of hind limb paralysis/paresis. The lesions included color change (congestion and/or haemorrhage) of various organs, traumatic injuries to the back and spinal cord (hemorrhages and fractures/luxations) and lesions of the urinary tract (hydronephrosis, distended bladders and blood ingested urine). The urinary tract lesions were presumably secondary to urine stasis resulting from paralysis.

Organ weights: There was a dose-dependent increase in absolute as well as relative liver weights in all dose groups as compared to controls. There were also increased relative kidney weights in males at 5000 and 20000 ppm. Due to lack of dose response in the 10000 ppm group and lack of histological changes in kidneys, these findings were considered of questionable toxicological significance.

Histopathology: The microscopic lesions found, particularly those in rats which died or were sacrificed moribund, confirmed the lesions observed grossly. They included hemorrhage and/or congestion of various organs, vertebral luxations or fractures, paravertebral and spinal cord hemorrhage and cord compression, vacuolization and malacia. Hydronephrosis, nephritis and urinary bladder hemorrhage occurred more frequently in rats with spinal trauma than in controls. In addition to the above lesions which were mainly the secondary results of convulsive episodes, most of the surviving animals showed dose-related hepatocellular hypertrophy which was rated mild to moderate. Similar findings were observed in animals that died or were sacrificed prior to termination of the study, although the lesions were more evident and severe in rats that survived longer. Furthermore, sporadic foci of hepatocellular degeneration were noted in animals that survived to termination of the study.

Table 22: 28 day feeding study in rats Organ weights and histopathological findings

	0 ppm	2500 ppm	5000 ppm	10000 ppm	20000 ppm
Males					
Liver weight:					
absolute (g)	9.696	12.335**	13.017**	12.751**	16.205**
relative (% of bw)	2.755	3.458**	3.738**	3.700**	4.665*
Hepatocellular hypertrophy	0/10	8/10	9/10	10/10	6/6
Kidney weight:	2.716	2.895	3.001	2.826	3.076
absolute (g)	0.774	0.813	0.863**	0.820	0.886**
relative (% of bw)					
Females					
Liver weight:					
absolute (g)	6.652	7.053	7.328	8.077	-
relative (% of bw)	3.144	3.389	3.557**	3.922**	-
Hepatocellular hypertrophy	0/10	10/10	10/10	4/4	-

** ($p \leq 0.01$), * ($p \leq 0.05$); significantly different from controls

Conclusion:

Continuous treatment of rats with metaldehyde at dietary concentrations of 2500 ppm or more over 28 days caused systemic toxicity as shown by increased liver weight and hepatocellular hypertrophy. At higher dose levels (10000 ppm for females and 20000 ppm for both sexes), mortality and spinal injuries, presumably as a result of convulsions, occurred. Changes in haematology (decrease in erythrocytes) were found in males at 10000 ppm and above. In addition, transient reduction of body weight gain and food consumption was noted at higher dose levels. A NOAEL could not be determined in this study. The LOAEL was 2500 ppm (equivalent to 197 mg/kg bw/d for males and 233 mg/kg bw/d for females).

90-day toxicity (rat)

Reference:	**P0071: Ninety day sub-chronic oral (dietary) toxicity study in the rat**
Author(s), year:	Thomas O., Bartlett A., Brooks P., 1998
Report/Doc. number:	Lonza Report No. 2974, Doc.No. 533-003, Conducting laboratory: Safepharm Laboratories Limited, Derby, UK
Guideline(s):	Japanese MAFF Guidelines for Toxicological Studies 59 No 4200, 1985
GLP:	Yes
Deviations:	No
Validity:	Yes

Material and Methods:

10 rats/sex/group (strain: Sprague-Dawley Crl:CD BR; source: Charles River Ltd., Kent, UK) received dietary concentrations of 0, 250, 750 or 2500 ppm metaldehyde (P0071; batch no. 22654; purity 99.1 %) over a period of 90 days. The mean achieved dose levels were 0, 21, 65 or 215 mg/kg bw/day for both sexes. At the start of the treatment the animals were approximately 5 – 8 weeks old and weighed 156 – 212 g (males) and 132 – 194 g (females). Diets were prepared at monthly intervals and stability and homogeneity of the test material in the diet were determined and confirmed by analysis.

All animals were examined for clinical signs once daily. Bodyweights were recorded at weekly intervals and at terminal kill. Food consumption was recorded for each cage group weekly throughout the study. Water intake was observed daily for each cage group by visual inspection of

the water bottles for any overt change. The eyes of all control and high dose animals were examined at the beginning and at the end of the study. Prior to sacrifice, blood samples were taken from non-fasted animals from the lateral tail vein. Hematology included haemoglobin, erythrocyte count, hematocrit, MCH, MCV, MCHC, total and differential leucocyte count, platelet count, reticulocyte count, prothrombin time and partial thromboplastin time. Clinical chemistry parameters assessed were urea, glucose, total protein, albumin, albumin/globulin ratio, sodium, potassium, chloride, calcium, inorganic phosphorus, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), creatinine and total bilirubin. The following parameters were measured in freshly collected urine: volume, specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, reducing substances, blood and microscopic examination of the sediment. All animals were subjected to a full external and internal macroscopic examination. Organ weights were determined from adrenals, brain, heart, kidneys, liver, ovaries, spleen and testes. Histopathological examinations included samples of the following tissues and organs of all animals: adrenals, aorta (thoracic), bone & bone marrow (femur including stifle joint, sternum), brain (three levels), caecum, colon, duodenum, exorbital lachrymal gland, eyes, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs with bronchi, lymph nodes (cervical and mesenteric), mammary gland, skeletal muscle, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands (submaxillary), sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes with epididymides, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder and uterus.

Preliminary study: The dose levels were chosen after the conduction of a 14-day preliminary study with 3 rats/sex/group receiving 0, 800, 2500 or 7500 ppm (estimated to be equivalent to 0, 40, 125 or 375 mg/kg bw/d). One female of the highest dose group developed clinical signs on study day 10 including dehydration, pallor of the extremities, hindlimb paralysis, piloerection, ptosis and body tremors and was sacrificed in extremis immediately. Bodyweight gain and also body weight was reduced during week 1 in females receiving 7500 ppm. One female from either the remaining treatment groups showed a bodyweight loss at day 4. A reduction in bodyweight gain was also apparent amongst males receiving 7500 ppm but only slightly in lower dose groups. Animals receiving 7500 ppm and females receiving 2500 ppm showed a reduced dietary intake during the first week of the study. Absolute and relative liver weights were elevated in both sexes at 7500 ppm. Microscopic examination revealed centrilobular hepatocyte enlargement in both sexes at 2500 and 7500 ppm and in two males at 800 ppm.

Findings:

General observations: One female from the highest dose group was found dead on study day 11 without any clinical precursors that might indicate a deterioration in health. Also histopathological examination failed to determine a precise cause of this death. There were no clinical signs of toxicity detected during the study.

Body weight: Females treated with 2500 ppm showed a statistically significant reduced bodyweight gain during the first week of treatment when compared with controls. Bodyweight development returned to normal thereafter although these animals completed the treatment period with a lower terminal bodyweight than controls.

Food consumption: There was no adverse effect on dietary intake during the study. Females treated with 2500 ppm did, however, show a reduction in food efficiency (ratio of bodyweight gain to dietary intake) during week 1 which recovered thereafter. No overt intergroup differences in water consumption were found during the study.

Table 23: 90 day feeding study in rats: Body weight / body weight gain

	Dose group level (ppm)							
	Males				Females			
	0	250	750	2500	0	250	750	2500
Body weight gain (g)								
- week 1	55	56	57	53	27	24	24	17**
- week 2	51	45	48	47	21	18	20	19
Body weight (g)								
- day 0	189	188	190	182	166	163	158	156
- day 90	501	502	493	493	310	299	294	269

** ($p < 0.01$); significantly different from controls

Ophthalmoscopic examination: There were no treatment-related effects observed.

Hematology, clinical chemistry and urinalysis: No treatment-related effects or any significant intergroup differences were observed.

Organ weights: Individual males and females of the 2500 ppm group showed a slight increase in relative liver weight compared to controls, however, statistical significance was not achieved. Females receiving 2500 ppm showed a statistically significant reduction in absolute heart and spleen weight. In the absence of a reduction of relative organ weights and histopathological findings, these differences were considered to be incidental or as a result of lower terminal bodyweight of these animals. Females of this group showed also a significant increase in relative brain weight which is often reported when bodyweight was reduced during treatment. A reduction of relative adrenal weights in females receiving 750 ppm was clearly not dose related and considered to be of no toxicological significance.

Table 24: 90 day feeding study in rats: Absolute and relative (% of bodyweight) organ weights

	Dose group level (ppm)							
	Males				Females			
	0	250	750	2500	0	250	750	2500
Liver								
- absolute	16.07	15.43	15.46	17.05	10.39	10.03	9.27	9.61
- relative (% bw)	3.21	3.08	3.14	3.45	3.36	3.37	3.19	3.66
Heart								
- absolute	1.63	1.55	1.62	1.70	1.07	0.99	0.98	0.89**
- relative (% bw)	0.33	0.31	0.33	0.35	0.35	0.34	0.34	0.34
Spleen								
- absolute	0.88	0.82	0.80	0.81	0.59	0.52	0.52	0.46**
- relative (% bw)	0.18	0.16	0.16	0.17	0.19	0.18	0.18	0.18
Brain								
- absolute	2.21	2.15	2.09	2.30	1.94	1.95	1.87	1.97
- relative (% bw)	0.45	0.43	0.43	0.48	0.64	0.67	0.65	0.76**
Adrenals								
- absolute	0.062	0.070	0.065	0.068	0.085	0.077	0.072**	0.075
- relative (% bw)	0.012	0.014	0.013	0.014	0.028	0.026	0.025	0.029

** ($p < 0.01$); significantly different from control group

Necropsy: Enlarged liver was reported for 5 males receiving 2500 ppm although this was only partly reflected in the liver weights. The female found dead (2500 ppm) showed pale kidneys and spleen together with normally expected post-mortem changes. No other treatment-related findings were noted.

Histopathology: Centrilobular hepatocyte enlargement was noted in both sexes at doses of 750 ppm and above. No further treatment-related effects were found.

Table 25: 90 day feeding study in rats: Histopathological findings in the liver

	Dose group level (ppm)							
	Males				Females			
	0	250	750	2500	0	250	750	2500
Centrilobular hepatocyte enlargement								
- minimal	0/10	0/10	5/10	1/10	0/10	0/10	5/10	4/10
- slight	0/10	0/10	0/10	8/10	0/10	0/10	0/10	5/10

Conclusion:

The predominant treatment-related effects were found in the liver. Centrilobular hepatocyte enlargement was observed at 750 ppm and above while liver enlargement and increased relative liver weight were noted in individual animals only in the high dose group (2500 ppm). Females receiving 2500 ppm showed a reduction of body weight gain and food efficiency during the first week of treatment which resulted in a reduced (not statistically significant) terminal body weight. In conclusion, the NOAEL is considered to be 250 ppm (equivalent to 21 mg/kg bw/d for both sexes).

90-day toxicity (mouse)

Reference:	**Ninety-Day Dietary Dose Range Finding Study with Metaldehyde in Mice**
Author(s), year:	Gill M. and Wagner C., 1990
Report/Doc. number:	Lonza Report No. 1546, Doc.No. 533-002, Conducting laboratory: Bushy Run Research Center, Pennsylvania, USA
Guideline(s):	Dose finding study, no guideline is mentioned in the study report
GLP:	Yes
Deviations:	No haematology and clinical/urine chemistry were performed.
Validity:	This dose finding study is scientific valid but due to the limited investigations considered as supplementary information only

Material and Methods:

In this dose finding study for an oncogenicity study 15 male and 15 female mice per dose group received diets containing 0, 100, 300, 1000, 3000 or 10000 ppm metaldehyde (batch no. 5448, purity: 99.0 %) over 90 days. The actual intake of metaldehyde was 0, 19, 54, 178, 560 and 1919 mg/kg bw/d for males and 0, 24, 70, 235, 743 and 2996 mg/kg bw/d for females. The animals (strain: CD-1, source: Charles River Breeding Laboratories, MI, USA) were approximately 7 weeks of age at first dosing. Stability and homogeneity of the test material in the diet were determined and confirmed by analysis.

During the treatment period, observations for mortality were made twice daily. Detailed clinical observations were performed once each a week, and observations for overt clinical signs were made on all other days. Body weight and food consumption data were collected weekly. No haematology, clinical chemistry or urinalysis were performed. All animals were subjected to complete necropsy. Organ weights (absolute, relative to body weight, relative to brain weight) were determined for liver, kidneys, brain with stem and testes. Histopathological examinations were performed on the following tissues and organs from 10 animals/sex/group randomly selected from the surviving animals of the control and 10000 ppm group. In addition, these tissues were examined for all animals that died during the study: gross lesions, brain (cerebral cortex, cerebellar cortex, medulla/pons), pituitary, thyroid-parathyroid complex, thymic region, lungs with mainstream bronchi, heart, liver (three lobes), spleen, esophagus, stomach, duodenum, jejunum, ileum, cecum,

colon, representative lymph nodes (mesenteric and submandibular), kidneys, adrenals, pancreas, testes, epididymis and ovaries. From the other dose groups, only selected organs were examined for 10 animals/sex/group: liver, kidneys, testes, stomach, lungs, duodenum and gross lesions.

Findings:

General observations: There were no treatment-related clinical signs observed during the study. However, mortality occurred in 5 males and 1 female of the 10000 ppm group: 3 males on study day 1, one female on study day 2, one male on study day 4 and one male on study day 8. A female from the 3000 ppm treatment group was found dead on study day 24. The cause of death for these animals was not apparent based on gross or microscopic examination. In addition, one male from the controls died of disseminated lymphosarcoma on study day 80. Finally, a female receiving 3000 ppm died as a result of a cage accident on study day 36.

Food consumption: There was no treatment-related effect on food consumption.

Body weight: Body weight gain was lower in males receiving 10000 ppm during the first week of the study. Body weight for females in the 3000 and 10000 ppm groups tended to be higher than for controls beginning in the 4th week of the study and occasional statistically significant differences from control were observed thereafter for body weight and/or body weight gain. Body weight findings probably should be considered together with the increases in liver weight observed during the study. For example, body weight reduction in males receiving 10000 ppm could be masked by the significant increase in liver weight. In addition, the increase in body weight for females may reflect increases observed in liver weight.

Table 26: 90 day feeding study in mice: Body weight / body weight gain

	Dose group level (ppm)					
	0	**100**	**300**	**1000**	**3000**	**10000**
Males						
body weight gain (g)						
-week 0-1	1.2	1.1	1.1	0.8	1.5	0.4**
-week 0-13	6.6	6.7	6.5	7.6	7.4	6.7
Females						
body weight gain (g)						
-week 0-1	1.1	0.8	0.9	1.2	0.9	0.8
-week 0-13	5.5	5.0	6.1	6.1	7.0**	6.7**

** ($p \leq 0.01$), significantly different from controls

Organ weights: Dose-related increases (mostly statistically significant) in absolute and relative liver weights were observed in both sexes at dose levels of 300 ppm and above. The percent increase for absolute liver weights compared to controls ranged from 6-181 % for males and 12-104 % for females. 15-17 % decreases in absolute and relative kidney weights were observed in males in the 10000 ppm treatment group.

Table 27: 90 day feeding study in mice: Absolute and relative (% of bodyweight, % of brain weight) organ weights

	Dose group level (ppm)					
	0	**100**	**300**	**1000**	**3000**	**10000**
Males:						
Liver weight						
- absolute	1.902	1.930	2.018	2.288**	2.926**	5.347**
- relative (% body weight)	5.525	5.645	5.895**	6.520**	8.340**	15.558**
- relative (% brain weight)	398	409	431*	468**	602**	1100**
Kidney weight						
- absolute	0.650	0.648	0.611	0.642	0.627	0.549

	Dose group level (ppm)					
	0	**100**	**300**	**1000**	**3000**	**10000**
- relative (% body weight)	1.890	1.909	1.787	1.827	1.795	1.607**
- relative (% brain weight)	136	138	130	131	129	113**
Females:						
Liver weight						
- absolute	1.495	1.552	1.677**	1.840**	2.168**	3.049**
- relative (% body weight)	5.461	5.589	5.856*	6.536**	7.556**	10.607**
- relative (% brain weight)	311	310	333	371**	432**	615**
Kidney weight						
- absolute	0.408	0.404	0.430	0.389	0.404	0.407
- relative (% body weight)	1.496	1.454	1.502	1.395	1.434	1.419
- relative (% brain weight)	85	80	85	79	81	82

* ($p \leq 0.05$); ** ($p \leq 0.01$); significantly different from controls

Necropsy: Treatment-related gross lesions were limited to the livers of males in the 10000 ppm treatment group (7 swollen and/or size increased) and 300 ppm treatment group (2 swollen).

Histopathology: Hepatic lesions were observed in all treatment groups and included hepatocellular necrosis, hypertrophy and/or hyperplasia, inflammation, anisokaryosis, hepatocellular vacuolization, cholestasis and biliary hyperplasia. The number of the lesions observed for individual animals, the number of animals affected within a treatment group, and the severity of individual lesions increased in a dose-related manner for animals in all treatment groups. The severity of lesions were minimal for the 100 and 300 ppm groups, minimal and/or moderate for the 1000 and 3000 ppm groups, and in the range of minimal to severe for animals in the 10000 ppm group.

Table 28: 90 day feeding study in mice: Histopathological findings in the liver

	Dose group level (ppm)					
	0	**100**	**300**	**1000**	**3000**	**10000**
Males:						
Liver, total number examined	11	10	10	10	10	15
hepatocellular hypertrophy	-	1	2	10	10	11
hepatocellular hyperplasia	-	-	-	-	-	3
hepatocellular necrosis	-	4	3	8	10	10
inflammation, chronic	2	1	-	2	2	4
inflammation, acute	-	4	4	7	10	11
vacuolization	-	-	1	0	1	7
anisokaryosis	-	1	5	10	10	11
cholestasis	-	-	-	-	-	4
biliary hyperplasia	-	-	1	1	3	3
Females:						
Liver, total number examined	10	10	10	10	11	11
hepatocellular hypertrophy	-	1	4	10	11	10
hepatocellular hyperplasia	-	-	-	-	-	1
hepatocellular necrosis	2	2	3	6	7	8
inflammation, chronic	2	4	2	5	3	8
inflammation, acute	3	3	3	5	7	8
vacuolization	-	-	1	-	-	7
anisokaryosis	-	3	8	9	11	11
cholestasis	-	-	-	-	-	-
biliary hyperplasia	-	-	-	-	-	2

Conclusion:

Treatment with 10000 ppm metaldehyde in the diet resulted in the death of 5 males and 1 female within the first eight days of treatment. Effects on the liver were observed in all treatment groups (100 ppm and above): increased liver weight, swelling of the liver, hepatocellular hypertrophy, hyperplasia, necrosis, inflammation, anisokaryosis, vacuolization, cholestasis and biliary hyperplasia. Increases in body weight in females at 3000 and 10000 ppm were possibly related to

the increases in liver weights. The decreases in absolute and relative kidney weights in males of the 10000 ppm treatment group were not associated with microscopic lesions in the kidney. Based on the results of this study, dosage levels of 20, 100 and 300 ppm were selected for a subsequent 18-month oncogenicity study. No NOAEL could be derived from this study. The LOAEL was 100 ppm (equivalent to 19 mg/kg bw/d for males and 24 mg/kg bw/d for females).

4-weeks toxicity (dog)

Reference:	**4-week dose-range-finding study for a 52-week chronic toxicity study of metaldehyde by oral administration via the diet to Beagle dogs**
Author(s), year:	Leuschner J., 2002
Report/Doc. number:	LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany; LPT Report No. 14543/01 Lonza Report No. 3506, Doc. No.: 532-003
Guideline(s):	Dose-range-finding study based on OECD Guideline 452
GLP:	Yes
Deviations:	Not applicable
Validity:	Valid

Material and methods:

Test material	Metaldehyde
Lot/Batch	30202
Purity	98.3%
Vehicle	Diet
Species	Dog
Strain	Beagle
Age	7 months
Weight at dosing	6.5 – 10.5 kg (males), 6.4 – 9.6 kg (females)
Source	Stefano Morini, S. Polo D´Enza, Italy

A dose-range-finding study was conducted in order to select the dose levels for a 52-week chronic toxicity study of metaldehyde in dogs. The study was divided into two experiments. The first experiment was called “Escalating dose levels” and lasted 22 days in total. In this experiment, metaldehyde was administered orally via the diet to 4 animals (2 males, 3 females as 1 female was sacrificed prematurely) initially at a dose level of 90 mg/kg bw/day and subsequently by escalating dose levels of 30, 60, 75 and 90 mg/kg bw/day. Each of the doses was administered for 3 days followed by a 2-day wash-out period. Due to sings of toxicity at the initial dose of 90 mg/kg bw/day, the dosing was stopped after the first administration and after a regular wash-out period the dosing was continued as described above. The second experiment called “Fixed dose levels” was a four week treatment. One group of animals (2 males, 2 females) received 75 mg/kg bw/day and a second group (2 males, 2 females) received 90 mg/kg bw/day orally via the diet. Parameters evaluated in all animals included body weight, food and drinking water consumption, clinical signs and mortality. Blood samples for haematology and clinical biochemistry were taken from all phase 2 animals (fixed dose levels) fasted overnight before the first administration and at the end of test week 4. Additionally, recording of heart rate (ECG) for all animals of the fixed dose level experiment was carried out on test day 1 (before feeding, and 2h and 4h after start of feeding) and at the end of test week 4 (before feeding and 2h after start of feeding). At the end of the study all

surviving animals were allowed to recover. Autopsy and macroscopic inspection of the animal which died prematurely was carried out as soon as possible after exitus.

The substance-food mixture was freshly prepared daily. The metaldehyde concentration was adjusted for each dosing interval or for each week to the weekly mean food consumption per group employing the food consumption data of the previous week. Daily intakes were not calculated.

Due to the low number of animals, a statistical evaluation of the results was not possible.

Table 29: Study design of the dose-range-finding study

Group	Duration of administration (days)	Dose in mg/kg bw/day via diet	Number and sex of animals
Phase 1: Escalating dose levels			
1	1*	90	2 males 2 females
	3-day wash-out period		
	3	30	
	2-day wash-out period		
	3	60	
	2-day wash-out period		
	3	75	
	2-day wash-out period		
	3	90	
Phase 2: Fixed dose level			
2	28	75	2 males, 2 females
3	28	90	2 males, 2 females

* Due to signs of toxicity at the intended lowest dose level of 90 mg/kg bw/day, the dosing was stopped after the first administration. One female animal had to be sacrificed prematurely in a moribund condition approximately 6 hours after application. The study was continued starting with a new lowest dose level of 30 mg/kg bw/day and another female animal to keep a group size of 2 males and 2 females.

Findings:

Clinical signs and mortality: Due to signs of toxicity at the intended lowest dose level of 90 mg/kg bw/day the dosing was stopped after the first administration. The following clinical symptoms were observed: Ataxia and clonic convulsions were noted in both male and female animals. Additionaly, both female animals exhibited emesis. In female animal no. 3, lateral position, difficulty in breathing and shaking of the head were noted 4 hours after application. The animal had to be sacrificed prematurely in moribund condition approximately 6 hours after application. Macroscopic inspection at necropsy revealed no substance-related pathological findings.

During the escalating dose period no behavioural changes were noted following the 3-day administration of 30 mg/kg bw/day. Reduced motility was observed in both male and female animals and clonic convulsions in one male and two females on the first day of administering 60 mg/kg bw/day. In addition, increased respiratory rate and emesis were noted in one female animal. The 3-day treatment with 75 mg/kg bw/day led to reduced motility in one male and one female animal on two or all three administration days and to tonoclonic convulsions in one male and one female animal on two or all three administration days. Emesis was also observed in one female on all three administration days. A further increase in the dose to 90 mg/kg bw/day led to ataxia in all animals and to occasional or regular slight tremor in one male and the two females during the treatment days. In addition, tonoclonic convulsion and emesis were observed in all female animals and salivation in one female animal during the application period. Abdominal position was noted in both females on the first administration day. None of the animals of the escalating dose phase starting at 30 mg/kg bw/day died.

In the fixed dose level experiment, treatment with 75 mg/kg bw/day led to slight tremor, inflated stomach, emesis, mydriasis and increased respiratory rate of both male and female dogs. All the effects were observed starting 1-2 hours after administration and lasted up to 6 hours. The intensity of the symptoms subsided with time. No signs of toxicity were observed from day 7 onwards.

Treatment with 90 mg/kg bw/day led to slight tremor, clonic and tonoclonic convulsions, inflated stomach, emesis, increased respiratory rate, slight ataxia, lateral position, abdominal position, moderate salivation, pale gingival and mydriasis of both male and females starting on day 1 onwards. All the effects were observed starting from 20-60 min after administration and lasted up to 6 hours. Overall, the severity of all symptoms declined with time and the signs of toxicity had almost disappeared towards the end of the 4-week treatment. No mortality occurred in the fixed dose level experiment.

Body weight: No substance-related effect was measured in the escalating dose experiment. In the fixed dose level experiment, male dogs treated with 90 mg/kg bw/day revealed a slight reduction of body weight gain approximately -5% relative to the 75 mg/kg bw/day dose group.

Food and water consumption: Both were not influenced by administration of the test substance in both experiments.

Electrocardiography: The visual assessment of the electrical complexes of the ECG and the determination of the heart rate did not show any substance-related changes at 75 or 90 mg/kg bw/day during the 28-day treatment.

Haematology: No substance-related influence was observed for haemoglobin content, number of erythrocytes and leucocytes, differential blood count, haematocrit value, platelet and reticulocyte counts, thromboplastin time, activated partial thromboplastin time, erythrocyte sedimentation rate, MCV, MCH and MCHC.

Clinical biochemistry: In test week 4, increased cholesterol, creatinine and glucose levels compared to pre-dose levels were found in all male and female dogs. However, no dose-relationship was observed and no concurrent control group was included in this test. Thus no conclusion on treatment-relationship is possible.

There was no influence on bile acids, bilirubin (total), protein (total), protein electrophoresis, urea (in blood), calcium, chloride, potassium and sodium. The activity of ALAT/GPT, ALP, ASAT/GOT and LDH was not increased compared to pre-dose levels.

Conclusion:

On the basis of the results of this study, dose levels of 10, 30 and 90 mg/kg bw/day were proposed for the 52-week main study by the study director.

26-weeks toxicity (dog)

Reference:	**26-weeks-toxicity of metaldehyde 99% - called "Metaldehyd" - in Beagle dogs after oral administration** **and** **Supplement No. 1 for 26-weeks-toxicity of metaldehyde 99% - called "Metaldehyd" - in Beagle dogs after oral administration**
Author(s), year:	Neumann W., 1980 and Neumann W., 1991
Report/Doc. number:	LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany; LPT Lonza Report No. 1379 Part 1, Doc. No.: 533-001 and Lonza Report No. 1379 Part 2, Doc. No.: 533-001
Guideline(s):	No, study was performed before adoption of OECD Guideline 452 (adopted May 1981)
GLP:	No, study was performed before implementation of GLP
Deviations:	Not applicable
Validity:	Valid

Reference:	**Histological re-examination of the testes and re-evaluation of the findings of the 26-week toxicity of metaldehyde 99% in Beagle dogs after oral administration (LPT Study report dated March 31, 1980)**
Author(s), year:	Leuschner J., 2009
Report/Doc. number:	LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany; LPT Report No. 24158, Doc. No.: 581-005
Guideline(s):	Not applicable, as the report is a histological re-examination and re-evaluation of samples generated in the study of Neumann W., 1980
GLP:	Not applicable
Deviations:	Not applicable
Validity:	Valid

Material and methods:

Test material	Metaldehyde
Lot/Batch	No batch number; test material obtained from current production in January 1979
Purity	>99%
Vehicle	Diet
Species	Dog
Strain	Beagle
Age	8 months
Weight at dosing	Not reported
Source	Chr. Fred Leuschner & Co., Laboratory of Toxicological and Pharmacological Examinations, Loehndorf/Post Wankendorf, Germany

Metaldehyde was administered orally via diet to male and female Beagle dogs (6 dogs/sex/dose) to achieve dose levels of 0, 20, 60 and 90 mg/kg bw/day for a period of 26 weeks. All animals were adapted to laboratory condition for a period of four weeks prior to study initiation. The dose levels were set on the basis of a 14-day preliminary test (1979) using 1 male and 1 female per dose at the

dose levels of 90 and 180 mg/kg bw/day. No intolerance was observed at 90 mg/kg bw/day but the dose level of 180 mg/kg bw/day caused vomiting and was within the lethal range. The test substance was admixed to the food homogeneously every day for each dose group. 50 g/kg bw/day of the food was offered to each dog for one hour between 8 and 9 a.m. In case of animals with poor appetite, the food was served for a longer period up to 8 hours. The residue was removed and weighed.

The following examinations were carried out:

- Behaviour including examination of general reflexes and external appearance, faeces: daily
- Food consumption, drinking water: daily
- Body weight: once a week (for the calculation of the daily dose of the test compound, the weight was determined daily without registration)
- Haematology: before the first administration and after 4, 8, 16, 20 and 26 test weeks in all animals: haemoglobin, erythrocytes, leucocytes, differential blood count, haematocrit value, thromboplastin time, erythrocyte sedimentation rate, blood clotting time, platelets, reticulocytes
- Clinical biochemistry: before the first administration and after 4, 8, 16, 20 and 26 test weeks in all animals: ALAT, ASAT, ALP, blood urea, glucose, sodium, potassium, calcium, chloride, total protein, uric acid, total bilirubin, albumin, globulin, liver function, creatinine, free cholesterol, total cholesterol, non-esterified fatty acids, esterified fatty acids, LDH, direct bilirubin, gamma-GT
- Urinalysis: before the first administration and after 8, 16 and 26 test weeks in all animals: colour, specific weight, protein, glucose, bilirubin, haemoglobin, ketone bodies, pH, urinary sediment
- Electrocardiography: on the first test day and after 13 and 26 weeks in all animals; examination before and 2 hours after administration on the lying animal in the limb leads I-III; limb lead II was evaluated and heart rate was determined
- Examination of circulary functions: after 26 test weeks in the animals of the highest dose group and the control; the dogs were kept under narcosis and after surgical interventions, systolic and diastolic pressure in general and pulmonary circulation were measured before and after administration of norepinephrine.
- Ophthalmological, auditory and dental examinations: before the first administration and after 4, 8, 16, 20 and 26 test weeks in all animals: ophthalmological examination included cornea, anterior chamber, pupil, lens, vitreous body, fundus of the eye; the auditory check was done with a simple noise test
- Macroscopic and microscopic evaluation: after 26 weeks in all animals; macroscopic inspection; organ weights: heart, liver, lungs, spleen, kidneys, adrenals, thymus, pituitary, gonads, thyroid, brain; histopathological examination: heart, lungs, liver, spleen, kidneys, adrenals, thymus, pituitary, gonads, thyroid, brain, prostate/uterus, stomach, duodenum, jejunum, ileum, colon, rectum, salivary glands, eye, urinary bladder, bone marrow, trachea, aorta, oesophagus, pancreas, lymph node, peripheral nerve, skeletal muscle, skin, tongue, spinal cord, gall bladder, bone, mamma

- Statistical evaluation: Analysis of variance and Student´s t-test were carried out. Limits of significance were $p \leq 0.01$
- Timeframe: the experiments were performed between June 15th 1979 and December 15th 1979

In a supplement to the main report, a re-evaluation of the macroscopic and microscopic pathology data of liver, testes and prostate and reformatted individual animal data were presented.

Findings:

- Behaviour including examination of general reflexes and external appearance, faeces: At all three dose levels, behaviour and external appearance were unchanged during 26 weeks of treatment. The neurological examinations did not lead to pathological findings. Pupillary light reflex, corneal reflex, patellar reflex, flexor, extensor and postular reflexes were not changed.
- Food consumption, drinking water: Food and drinking water consumption were within the normal range during the 26 week test period. The real intake of metaldehyde is shown in the following table:

Table 30: Real intake of metaldehyde in the 26-week dog study (mg)

Group	Males	Females
20 mg/kg bw/day	20.2 ± 0.4	19.7 ± 2.8
60 mg/kg bw/day	61.5 ± 4.1	62.2 ± 5.6
90 mg/kg bw/day	91.8 ± 7.8	86.7 ± 3.8

- Body weight: no effect on body weight or body weight gain
- Haematology, clinical biochemistry and urinalysis: no effects of test substance were found
- Electrocardiography and examination of circulatory functions: No effect attributable to the test substance was observed in the ECG. The minimal changes in the functional picture were within the normal range and explained by the fact that the ECG in the dogs could not be recorded in a state of actual rest. At all tested dose levels systolic and diastolic pressure was unchanged in both general and pulmonary circulation.
- Ophthalmological, auditory and dental examinations: no effects were observed during the test period
- Macroscopic and microscopic evaluation:

  Results of macroscopic evaluation, organ weighing and histopathology were presented in the original report. However, due to the request from US EPA, a Supplement of the study (Neumann W., 1991) was provided including detailed individual tables, redesigned summary tables and information on the severity and extent of all lesions described. A review on liver, testes and prostate findings was undertaken at request from the sponsor, which basically confirmed the findings of the original report. No effects were noted at macroscopic evaluation and organ weighing.

Histopathology:

Liver: The overall evaluation of the histopathological findings in the liver did not show any treatment-related effect. The few very slight lesions recorded are considered to be spontaneous findings occurring in dogs.

Testes: Diffuse atrophy of the testes was found only in the mid (2/6 males) and high dose group (4/6 males). The atrophy seen in one control animal was a consequence of cryptorchism. One animal from the low dose group had very slight focal atrophy of the tubules. This was considered a spontaneous finding because it occurred also in one untreated control animal. The dose level of 20 mg/kg bw/d was therefore considered the NOAEL for the testes effects.

Prostate: Diffuse atrophy of the prostate was found in the mid dose group (4/6 males) and the high dose group (2/6 males). One male from the high dose group also showed focal atrophy. No such effects were seen in the control or low dose group.

Mesenteric lymph nodes: Examination of the lymph nodes revealed a considerable infestation with parasites, especially in the higher dose groups, which could have possibly contributed to the higher incidences of findings at these dose levels. For precautionary reasons a clear NOAEL of 20 mg/kg bw/day was set.

Table 31: Histopathological findings in the 26-week dog study (according to Supplement 1 of the original study, Neumann W., 1980, 1991)

	Control		**20 mg/kg bw/day**		**60 mg/kg bw/day**		**90 mg/kg bw/day**	
	♂	♀	♂	♀	♂	♀	♂	♀
LIVER								
examined	6	6	6	6	6	6	6	6
no abnormalities detected	5	4	5	2	4	5	4	2
very slight single cells necrosis with predominantly mononuclear cell proliferation	0	2	1	3	2	1	1	2
very slight periportal infiltration of lymphocytes, histiocytes and eosinophilic granulocytes	0	0	0	1	0	0	2	2
very slight hydropic swelling in focal areas (H.E. staining)	1	0	0	0	0	1	2	2
PROSTATE								
examined	6	-	6	-	6	-	6	-
no abnormalities detected	6	-	6	-	2	-	3	-
atrophy, diffuse	0	-	0	-	4	-	2	-
atrophy, focal	0	-	0	-	0	-	1	-
TESTICLES								
examined	7#	-	6	-	7#	-	6	-
no abnormalities detected	5	-	5	-	2	-	2	-
diffuse atrophy	1	-	0	-	2	-	4	-
focal atrophic tubules	1	-	1	-	3	-	0	-
LYMPH NODE, MES.								
examined	6	6	6	6	6	6	6	6
no abnormalities detected	4	4	4	5	3	2	1	1
follicular hyperplasia	0	0	0	0	0	0	3	3
inflammation - chronic	1	1	1	1	3	3	1	1
erythrocytes (medullary funicle)	1	1	1	0	0	1	0	0
lymphocytes depletion - moderate	0	0	0	0	0	0	2	0
parasitic granuloma	1	1	2	1	3	4	4	2

[#] one animal of this group was examined in both testicles

In the original 26-week-toxicity study of metaldehyde in Beagle dogs (Neumann W., 1980, Doc. No. 533-001), the findings in the testes were not graded according to severity. Thus, a microscopical re-examination of H. & E. stained paraffin and PAS-stained sections of the testes of all 24 male dogs of the 26-week toxicity study was performed in 2009 with the objective to score findings using a current severity grading system. This up-grade was considered necessary to gain direct comparability of findings in the different dog reports (Leuschner J., 2009, Doc. No. 581-005).

Findings of the histological re-examination of the testes and re-evaluation:

Lesions related to the administration of the test substance: Cryptorchism and a moderate atrophy of the testes (1 of 6 animals) and a mild to moderate focal atrophy of the germinative epithelium (3 of 6 animals) were noted for the animals of the 60 mg/kg bw/d dose group. The changes in the 90 mg/kg bw/d dose group consisted of a mild to moderate diffuse atrophy/degeneration of the germinative epithelium. Four of 6 animals were affected, the mean severity grade of the high dose animals for diffuse atrophy of the left and right testes was 1.67 and 1.83 compared to 0.00 and 0.00 in the control. The changes are morphologically clearly distinct from the spontaneous findings in the 20 mg/kg bw/d dose group and in the control group and therefore attributable to the treatment with the test item.

Lesions unrelated to the administration of the test item: A focal minimal to mild atrophy of the germinative epithelium of the testes was noted in control animals and animals from the 20 mg/kg bw/d dose group. Incidence and severity indicate an incidental distribution of this finding for the control and for the low dose treated animals.

Table 32: Histological re-examination of testes (Leuschner J., 2009)

Dose group	**Control**		**20 mg/kg bw/day**		**60 mg/kg bw/day**		**90 mg/kg bw/day**	
No. animals	**6**		**6**		**6**		**6**	
	#	**SEV**	**#**		**#**	**SEV**	**#**	**SEV**
Testis (I)								
Atrophy of germin. epith., focal	4	0.67	2	0.50	3	1.33	1	0.17
Atrophy of germin. epith., diff.	0	-	0	-	0	-	4	1.67
Atrophy of testis	1	0.33	0	-	1	0.50	0	-
Cryptorchism	1	-	0	-	1	-	0	-
Testis (II)								
Atrophy of germin. epith., focal	2	0.33	2	0.50	4	1.50	1	0.17
Atrophy of germin. epith., diff.	0	-	0	-	0	-	4	1.83
Testis (I) PAS-stain								
Atrophy of germin. epith., focal	4	0.67	2	0.50	3	1.33	1	0.17
Atrophy of germin. epith., diff.	0	-	0	-	0	-	4	1.67
Atrophy of testis	1	0.33	0	-	1	0.50	0	-
Cryptorchism	1	-	0	-	1	-	0	-
Testis (II) PAS-stain								
Atrophy of germin. epith., focal	2	0.33	2	0.50	4	1.50	1	0.17
Atrophy of germin. epith., diff.	0	-	0	-	0	-	4	1.67

Severity grading (SEV): 1= minimal, 2= mild, 3= moderate, 4= marked, mean values are presented

Conclusion:

A NOAEL of 20 mg/kg bw/day is set for this 26-week-toxicity study in Beagle dogs based on the histopathological findings in testes (mild to moderate atrophy/degeneration of the germinative epithelium) and prostate (diffuse atrophy). The histopathological re-examination of the testes confirmed the pattern of testicular findings: the severity score of focal findings was increased at 60 mg/kg bw/day while at the high dose level of 90 mg/kg bw/day a clear increase of diffuse atrophic changes was found. The NOAEL for this study was therefore confirmed to be 20 mg/kg bw/day.

52-weeks toxicity (dog)

Reference:	**52-week chronic toxicity study of metaldehyde by repeated oral administration via the diet to Beagle dogs**
Author(s), year:	Leuschner J., 2003
Report/Doc. number:	Lonza Report No. 3657, Doc.No. 537-003, Conducting laboratory: LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany
Guideline(s):	OECD Guideline 452 (1981); EC Guideline L133 Part B, Chronic Toxicity Test (1988); Japanese MAFF, 12 NohSan No. 8147 (2000)
GLP:	Yes
Deviations:	No
Validity:	Yes

Material and Methods:

Metaldehyde (batch no. 30202, purity: 98.3 %) was administered with the diet to 4 male and 4 female Beagle dogs per treatment group at dose levels of 0, 10, 30 and 90 mg/kg bw/d over a period of 52 weeks. The Beagle dogs (source: Stefano Morini, Reggio Emilia, Italy) were approximately 6 months of age at the beginning of the study and weighed 5.4-9.2 kg (males) and 5.8-9.2 kg (females). The amount of the test substance given was adjusted to each animal´s actual body weight and mean food consumption weekly. The test substance-diet mixture was freshly prepared daily. The diet was checked for stability, concentration and homogeneity at the beginning of the study and thereafter every 3 months. The dose levels for this study were selected based on the results of a 4-week dose finding study in dogs.

All animals were checked at least once daily for clinical signs. These observations included skin/fur, eyes, mucous membranes, respiratory and circulatory systems, somatomotor activity and behaviour patterns. Mortality was checked twice daily, and as soon as possible after exitus, post-mortem examination was performed. Body weights were recorded at study initiation and thereafter in weekly intervals. Food consumption was recorded on a daily basis throughout the experimental period. The report included weekly mean values. Daily monitoring by visual appraisal of the drinking water consumption was maintained throughout the study. In case of food not consumed during the first part of the daily feeding, the quantity of remaining food was recorded and the amount of test substance intake of the animal was corrected accordingly. The report includes weekly mean values of uptake of the test substance. Blood samples were taken from animals fasted overnight at the beginning of the study and at the end of test weeks 13, 26 and 52. Haematology included erythrocytes, hematocrit, haemoglobin, MCV, MCH, MCHC, leucocytes, differential blood count, reticulocytes, platelets, thromboplastin time, activated partial thromboplastin time and erythrocyte sedimentation rate. The following clinical chemistry parameters were determined: albumin, albumin/globulin ratio, total bilirubin, total cholesterol, creatinine, glucose, total protein, urea in blood, calcium, chloride, potassium, sodium, alanine amino transferase (ALAT), aspartate

amino transferase (ASAT), alkaline phosphatase (ALP), gamma-glutamyl-transferase (GGT), triglycerides and inorganic phosphorus. Urine was collected from all animals in a metabolism cage in the morning of the appropriate test day 3 hours prior to the administration after the dogs received 50 mL tap water/kg bw orally by gavage. Urinalysis was performed at the beginning of the study and at the end of test weeks 13, 26 and 52 and included volume, pH, specific gravity, protein, glucose, bilirubin, urobilinogen, ketones, haemoglobin, nitrite, color and microscopic examination. Ophthalmological and auditory examinations were performed prior to the first administration and during test weeks 13, 26 and 52. All animals were examined at necropsy. The weights of the following organs were determined: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes and thyroids including parathyroids. The following organs and tissues were examined histopathologically: adrenals, aorta abdominalis, bone (sternum and os femoris with joint), bone marrow (sternum and femur), brain (transverse section through optic chiasma, infundibulum, midbrain, brain stem and cerebellum), caecum, epididymis, eye with optic nerve, gall bladder, gross lesions, heart (3 levels: left and right ventricle, septum), small intestine (duodenum, jejunum, ileum), large intestine (colon, rectum), kidney and ureter, lacrimal gland, liver, lungs (with mainstream bronchi and bronichioles), lymph node (cervical, mesenteric), mammary gland, muscle (skeletal, thigh and tibia), nerve (sciatic and tibial), esophagus, ovaries, pancreas, pituitary, prostate, salivary glands (mandibular, parotid, sublingual), seminal duct, skin (left flank), spinal cord (cervical, thoracic, lubar) incl. spinal ganglion and spinal root, spleen, stomach, testes, thymus, thyroids incl. parathyroids, tissues masses or tumours, trachea incl. larynx, urinary bladder, uterus (incl. cervix and oviducts) and vagina.

Findings:

General observations: Clinical signs were only observed in the highest dose group from study week 1 onwards. The following symptoms were noted: ataxia, reduced motility, emesis, tremor, twitching and salivation. Individual to all animals were affected. Incidence and severity appeared to decline from study week 19 onwards with none to two animals only affected.

Mortalilty: 1 male and 1 female from the 30 mg/kg dose group and 1 female of the 90 mg/kg dose group were found dead between study days 260 and 322. The deaths are regarded to be test-substance related. No premortal symptoms were recorded.

Body weight: The body weight of the treated animals was not influenced compared to controls. Body weight gain appeared to be reduced in the high dose group, however, no statistical significance was noted.

Food consumption: During some weeks of the study there was a slight statistically significant increase of food intake noted for male and female animals of the high dose group. This effect might be due to the slight decrease in body weight gain noted in this dose group. No overt intergroup differences in water consumption were found during the study.

Table 33: 52 week feeding study in dogs Body weight / body weight gain

	Dose group level (mg/kg bw/d)							
	Males				**Females**			
	0	**10**	**30**	**90**	**0**	**10**	**30**	**90**
Body weight (kg) - week 0 - week 52	 7.70 13.33	 7.55 11.43	 7.55 11.77	 8.00 11.00	 7.33 10.83	 6.85 10.68	 6.83 10.07	 6.70 9.13
Body weight gain - absolute (kg) - relative (%)	 5.63 73	 3.88 51	 4.22 56	 3.00 38	 3.5 48	 3.83 56	 3.24 47	 2.43 36

Ophthalmoscopic examination: There were no treatment-related effects observed.

Auditory examination: In test week 13 one male and one female dog treated with 90 mg/kg did not react to the noise test. None of the animals of this dose group was affected in test weeks 26 or 52. There was no indication of any impairment in animals of lower dose groups.

Haematology: Test substance-related changes were seen as an increase in haemoglobin, erythrocytes and haematocrit of animals of the highest dose groups. This effect was observed in both sexes in study week 13 (statistically significant only for females) and in females only in study week 26 (not longer statistically significant). At the end of the study, no changes were noted for any of the haematological parameters. The decrease of activated thromboplastin time in males of the highest dose group in week 13 is regarded as spontaneous and within the normal variability.

Table 34: 52 week feeding study in dogs Relevant haematological findings

	Dose group level (mg/kg bw/d)							
	Males				**Females**			
	0	**10**	**30**	**90**	**0**	**10**	**30**	**90**
Erythrocytes (1012/L)								
- Week 0	5.38	5.45	5.23	5.33	5.35	5.18	5.40	5.60
- Week 13	5.45	5.85	5.45	6.23	5.73	5.70	6.08	7.15**
- Week 26	5.70	6.13	5.65	5.55	5.48	5.58	5.70	6.35
- Week 52	6.43	6.78	6.60	6.25	6.45	6.18	7.13	6.27
Hemoglobin (mmol/L)								
- Week 0	7.33	7.48	7.13	7.10	7.23	6.95	7.28	7.45
- Week 13	7.50	8.08	7.58	8.88	7.95	7.80	8.28	9.90**
- Week 26	7.95	8.70	8.00	8.28	7.93	7.78	7.90	8.95
- Week 52	9.00	9.30	9.33	9.18	9.18	8.58	9.80	8.47
Hematocrit (%)								
- Week 0	38.3	38.5	36.8	36.8	37.0	35.3	37.3	38.3
- Week 13	36.3	39.3	37.3	43.0	39.0	38.0	40.8	49.3**
- Week 26	38.5	42.0	38.8	39.3	38.0	37.5	37.5	43.0
- Week 52	43.0	45.3	45.0	43.8	43.8	41.0	47.0	40.7

** ($p < 0.01$); significantly different from control group

Clinical chemistry: In the highest dose group, test substance-related changes were noted for bilirubin (females, week 13), triglycerides (females, weeks 13 and 26) and AP levels (both sexes, weeks 13, 26 and 52).

Table 35: 52 week feeding study in dogs Relevant clinical chemistry findings

	Dose group level (mg/kg bw/d)							
	Males				**Females**			
	0	**10**	**30**	**90**	**0**	**10**	**30**	**90**
Bilirubin (µmol/L)								
- Week 0	3.73	2.83	3.68	3.23	2.78	3.30	2.95	3.28
- Week 13	2.48	2.30	2.95	2.85	2.53	2.33	2.95	3.98**
- Week 26	3.30	3.38	3.00	2.60	3.00	3.05	3.33	3.35
- Week 52	3.88	3.65	3.53	3.73	3.43	3.38	3.27	3.93
Triglycerides (mmol/L)								
- Week 0	0.243	0.215	0.218	0.200	0.195	0.190	0.180	0.200
- Week 13	0.265	0.220	0.285	0.258	0.238	0.260	0.195	0.490**
- Week 26	0.305	0.280	0.258	0.283	0.235	0.340	0.245	0.553**
- Week 52	0.230	0.215	0.223	0.278	0.208	0.235	0.217	0.280
AP (U/L)								
- Week 0	250	258	287	302	259	235	240	220
- Week 13	143	149	174	296**	147	135	138	236
- Week 26	85	102	108	221**	79	97	79	156
- Week 52	71	94	94	216**	66	79	70	151**

** (p< 0.01); significantly different from control group

Urinalysis: No substance-related changes were noted at urinalysis. The slightly decreased specific gravity of the urine noted for the intermediate males in week 52 is regarded as fortuitous as no dose-relationship was noted. The slightly decreased pH-value observed for high dose males in week 52 is regarded to be within the normal variability.

Necropsy: The macroscopic necropsy examination during dissection revealed no findings in the organs and tissues in any treatment group. The gross findings such as indurated, emphysematous and/or discoloured lungs were observed in 2 mid dose animals and 1 high dose animal and were regarded as spontaneous.

Organ weights: A treatment-related increase of absolute and relative liver weights was found in males and females of the mid and high dose groups whith statistical significance achieved only at the high dose group.

Table 36: 52 week feeding study in dogs Absolute and relative (% of bodyweight) organ weights

	Dose group level (mg/kg bw/d)							
	Males				**Females**			
	0	**10**	**30**	**90**	**0**	**10**	**30**	**90**
Liver								
- absolute	430	360	457	541	351	350	362	418
- relative (% bw)	34.2	33.9	41.2	51.6**	33.6	34.5	38.1	48.8**

** (p< 0.01); significantly different from control group

Histopathology: The histopathological findings in testes were re-examined and discussed in the "*Expert statement on the histological findings (giant cells, atrophy and degeneration of the germinative epithelium) in the 52-week toxicity study in Beagle dogs with metaldehyde"* (Leuschner J., Drommer W., 2006), see below.

Mild atrophy of the prostate was noted in 3 of 4 male animals of the high dose group and 1 of 4 animals from the mid dose group.

No treatment-related findings were noted in any other organ examined. In particular, no changes were noted in the spinal cord with ganglion/root (cervical, thoracal and lumbar region). A minimal fatty infiltration of hepatocytes without degeneration was observed in the liver of control and test animals. None of the histopathological findings in the male of the mid dose group which died prematurely could be seen as a cause of death. Both females which died prematurely (1 from the mid dose and 1 from the high dose group) revealed microscopic findings in the lungs: moderate interstitial pneumonia and moderate bronchopneumonia were found which might have contributed to the deaths of the animals, although they seemed to be not directly related to the test substance. The study authors regarded the premature deaths of these animals as substance-related.

Table 37: 52 week feeding study in dogs Relevant histopathological findings (testes and prostate)

	0 mg/kg	**10 mg/kg**	**30 mg/kg**	**90 mg/kg**
Testis I (not reported if left or right testis) no. examined	**4**	**4**	**41)**	**4**
Atrophy of the germinal epithelium (animal no., grade)	-	-	2 (no.18 moderate no.20 mild)	2 (no.25 mild no.27 marked)

	0 mg/kg	**10 mg/kg**	**30 mg/kg**	**90 mg/kg**
Degeneration of the germinal epithelium (animal no., grade)	-	1 (no.10 mild)	-	3 (no.26 marked no.27 mild no.28 mild)
Giant cells (animal no., grade)	-	2 (no.10 mild, no.12 minimal)	1 (no.18 mild)	2 (no.25 minimal no.28 mild)
Juvenile testis (animal no.)	-	1 (no.12)	-	-
Testis II (not reported if left or right testis) no. examined	**4**	**4**	**41)**	**4**
Atrophy of the germinal epithelium (animal no., grade)	-	-	2 (no.18 minimal no.20 minimal)	3 (no.25 mild no.27 marked no.28 moderate)
Degeneration of the germinal epithelium (animal no., grade)	-	1 (no.10 mild)	-	3 (no.26 marked no.27 mild no.28 mild)
Giant cells (animal no., grade)	-	1 (no.10 mild)	-	2 (no.25 mild no.28 minmal)
Juvenile testis (animal no.)	-	1 (no.12)	-	-
Prostate no. examined	**4**	**4**	**41)**	**4**
Atrophy (animal no., grade)	-	-	1 (no.19 mild)	3 (no.25 mild no.26 mild no.28 mild)
Lymphocytic infiltration (animal no., grade)	1 (no.3 minimal)	1 (no.9 moderate)	-	-
Suppurative prostatitis (animal no., grade)	-	1 (no.9 minimal)	-	-
Autolysis (animal no., grade)	-	-	1 (no18 moderate)	-
Cysts (animal no., grade)	-	-	1 (no.19 mild)	1 (no.27 minimal)

Severity grading: minimal, mild, moderate, marked
1) including animal no. 18 which died prematurely. In animal no. 18, atrophy of the germinal epithelium in both testes (minimal to moderate), giant cells in testis I (mild), and autolysis in the prostate (moderate) were recorded.

Conclusion:

Clinical signs of toxicity (ataxia, reduced motility, emesis, tremor, twitching, salivation) were observed at the high dose group (90 mg/kg) from study week 1 onwards with incidence and severity declining over the time. 1 male and 1 female animal from the mid dose group (30 mg/kg) and 1 female from the high dose group were found dead between study day 260 and 322. As no obvious cause of death could be determined, the deaths were considered to be related to treatment. A reduction of body weight gain was noted in high dose animals but without statistical significance.

Also in the high dose group, 1 male and 1 female animal did not react to the noise test in week 13 but were not affected in week 26 and 52. Red blood cell parameters (increased erythrocytes, haemoglobin and hematocrit) were affected predominantely in females at the highest dose group. The effects were strongest at week 13 but declined until week 26 and were no longer seen at week 52. A similar situation was observed for bilirubin and triglyceride values in females of the high dose group, which were increased at week 13 but returned to normal until the end of the study. In contrast, elevated levels of alkaline phosphatase were observed in males and females at weeks 13, 26 and 52. Relative and absolute liver weights were increased in both sexes of the high dose group. In histopathology, in one animal of the mid dose group and 3 animals of the high dose group mild atrophy of the prostate was observed. The histopathological findings in testes and setting of the NOAEL is reported below.

Reference:	**Metaldehyd: A 52 week dietary toxicity study in beagle dogs**
Author(s), year:	Gauvin, G. V., 2010
Report/Doc. number:	Report No. 1714-001 Conducting laboratory: MPI Research, Inc., Mattawan, Michigan
Guideline(s):	OECD Guideline 452 (2009) US EPA, OPPTS 870.4100 (1998)
GLP:	Yes
Deviations:	No
Validity:	Yes

Material and Methodes:

Test material	Metaldehyde
Lot/Batch	080702
Purity	100.0%
Vehicle	LabDiet 5007 Certified Canine Diet Meal
Species	Beagle dogs
Strain	-
Age	7 to 7.5 weeks at receipt + 14 days acclimation period
Weight at dosing	Males: 8.10 - 10.45 kg; Females: 7.45 - 8.50 kg
Source	Covance Research Products, Inc., Kalamazoo, Michigan

The animals were housed individually. The test material Metaldehyde was administered orally for 52 weeks as a diet additive. Diet and tap water were available ad libitum. Dose levels of 1, 3.5 and 15 mg/kg/day were tested by using dose groups of each 4 male and 4 female animals (Table 38).

Table 38: 52 week dietary toxicity study in dogs – Study design

		Number of Animals	
Group Number		**Male**	**Female**
1	0	4	4
2	1	4	4
3	3.5	4	4
4	15	4	4

Prior to study: A complete physical examination was conducted on all animals by a staff veterinarian prior to initiation of test article administration.

All animals received an electrocardiographic examination prior to placement on study. Insofar as possible, care was taken to avoid causing undue excitement of the animals before the recording of electrocardiograms (ECGs) in order to minimize extreme fluctuations or artifacts in these measurements. Standard ECGs (10 Lead) were recorded at 50 mm/sec. A consultant veterinary cardiologist interpreted the ECGs and made a recommendation for each animal for placement on study.

General Observations: All animals were observed for morbidity, mortality, injury, the availability of food and water as well as gross neurofunctional and neurobehavioral signs twice daily throughout the duration of the study with the exception of Day 201 on which observations were recorded only once. On occasion, veterinary consultations were conducted during the course of the study. Body weights for all animals were measured and recorded within two days of receipt, twice prior to randomization (Days -8 and -1), and weekly during the study. The body weights recorded within two days of receipt and on Day -8 are not reported. Food consumption was measured and recorded daily during the study and reported weekly. Food efficiency was calculated for each week that food consumption was measured.

Detailed Clinical Observations: Detailed Clinical Observations of each animal were performed once predose and weekly during the study. On occasion, clinical observations were recorded at unscheduled intervals. The observations included, but were not limited to, evaluation of the skin, fur, eyes, mucous membranes, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size). Changes in level of activity, gait, posture, altered strength, and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation) were recorded.

Ophthalmoscopic examinations: Ophthalmoscopic examinations were conducted on all animals predose and prior to the terminal necropsy.

Clinical Pathology: Clinical pathology evaluations were conducted on all animals predose, 3, 6, and 9 months, and prior to the terminal necropsy. The animals had access to drinking water but were fasted overnight prior to sample collection. Blood samples were collected from the jugular vein. Urine samples were collected using steel pans placed under the cages for approximately 16 hours. Hematology (Leukocyte count (total and absolute differential), Erythrocyte count, Hemoglobin, Hematocrit, Mean corpuscular hemoglobin, Mean corpuscular volume, Mean corpuscular hemoglobin concentration, Absolute reticulocytes, Platelet count and Blood cell morphology), Coagulation (Prothrombin time and Activated partial thromboplastin time), Clinical Chemistry (Alkaline phosphatase, Total bilirubin (with direct bilirubin if total bilirubin exceeds 1 mg/dl), Aspartate aminotransferase, Alanine aminotransferase, Gamma glutamyl transferase, Sorbitol dehydrogenase, Urea nitrogen, Creatinine, Total protein, Albumin, Globulin and A/G (albumin/globulin) ratio, Glucose, Total cholesterol, Triglycerides, Electrolytes (sodium, potassium, chloride), Calcium Phosphorus Creatine kinase) and Urinalysis parameters (Volume Specific gravity, pH, Color and appearance Protein, Glucose, Bilirubin, Ketones, Blood, Urobilinogen, Microscopy of centrifuged sediment) were evaluated.

Necropsy: All animals were euthanized and examined carefully for external abnormalities including palpable masses. The skin was reflected from a ventral midline incision and any abnormalities were identified and correlated with antemortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities and the organs removed, examined, and, where required, placed in fixative. All designated tissues were fixed in neutral buffered formalin, except for the eye (including the optic nerve) and testes, which were fixed using a modified Davidson’s fixative. Formalin was infused into the lung. A full complement of tissues and organs was collected from all animals.

Organ weight: Body weights and protocol-designated organ weights were recorded for all animals at the scheduled necropsy and appropriate organ weight ratios were calculated (relative to body and brain weights). Paired organs were weighed together except only the right mandibular salivary gland was weighed. A combined weight for the thyroid and parathyroid glands was collected.

Histopathology: Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed of tissues. A four-step grading system was utilized to define gradable lesions for comparison between dose groups.

Results:

Examinations prior to study: Complete physical examinations found the dogs to be in good, stable, and healthy condition prior to dose initiation on this study.

Pre-study electrocardiograms conducted in all dogs were evaluated by the Study Director and a board-certified veterinary cardiologist. All dogs were found to have normal sinus rhythm within normal limits and with no apparent arrhythmias. All dogs placed on study were considered healthy, cardiovascularly stable, and were subsequently placed on study.

Mortality: There were no deaths on study. All animals survived the 1 year duration of the study. All animals were humanely euthanized at the end of study and tissues were submitted by scheduled necropsy.

Body Weight: All dogs on study demonstrated normal growth and development over the 52 week course of the study. The body weights were well within the normal growth charts for this breed of dog provided by the supplier. There was neither consistent dose- nor time-dependent changes in the week-to-week body weight gains or losses recorded in this study.

There were no statistically significant differences in group mean body weights in male dogs measured across the 52 week duration of this study. The 3.5 mg/kg/day treatment group of female dogs demonstrated a short period of significantly greater mean body weight in Weeks 18 and 19 when compared to their standard diet control group cohorts. The body weight differences, while statistically significant, were not considered to be physiologically relevant. Overall, there were no biologically meaningful changes in group mean body weights induced by metaldehyde consumption in this study.

Food Consumption: There were no biologically significant changes in group mean food consumption induced by dietary administration of metaldehyde to male and female beagle dogs up to 15 mg/kg/day of metaldehyde.

Detailed Clinical Observations: There were no clinical signs attributed to test article administration. All clinical signs reported were present in all treatment groups including the standard diet control group. There were reports of mucoid, soft, and watery feces over the 52 week duration of this feed admixture study. The incidence rate is summarized in Table 39 and expressed as the total number of incidences over the 52 week period of the study as a function of the total number of animals in the group expressing the finding (max = 4).

Table 39: 52 week dietary toxicity study in dogs – Incidence rate of fecal alterations

Group-	Mucoid Feces		Soft Faces		Watery Feces	
	Male	Female	Male	Female	Male	Female

Dose Level (mg/kg/day)						
Control	31/3	3/3	118/3	4/3	4/1	2/2
1	36/4	32/4	60/3	34/4	4/2	1/1
3.5	27/4	14/3	27/4	13/4	2/2	1/1
15	35/4	40/4	27/4	54/4	1/1	2/2

The incidence rates reported in the "control" groups of other long term studies conducted in beagle dogs at the conducting laboratory show that these findings can be very variable. Although the incidence rates of mucoid feces in female treated groups were higher than those of the concurrent control group they were similar to the male control incidence and showed no dose response relationship. It should be noted that these findings regarding fecal output were short-lived, resolved without treatment, were not limited to test article treatment groups, were not dose-related, and were not considered to be outside the scope of normal variations in this purpose-bred laboratory beagle from the source of supply (Covance Laboratories, USA) used on this study.

Ophthalmoscopic examinations: There were no abnormalities detected prior to or after 52 weeks of chronic daily exposure to doses of up to 15 mg/kg/day of metaldehyde in male or female beagle dogs on this study.

Clinical Pathology: There were no test article-related effects on hematology parameters and clinical chemistry analytes. Sporadic statistical differences were seen that were considered not meaningful due to the magnitude or direction of change, the lack of consistency, and/or the interval involved (pretest).
There were no test article-related effects on coagulation parameters. Sporadic statistical differences were seen that were considered not meaningful due to the magnitude or direction of change and/or the interval involved (pretest).
There were no test article-related effects on urinalysis parameters. Urine volumes were moderately variable within and between groups and intervals, but there were no dose-dependent patterns. Sporadic statistical differences were seen that were considered not meaningful due to the magnitude or direction of change.

Necropsy: There were no metaldehyde-related macroscopic findings at any dose level in either sex. All macroscopic observations were considered incidental based on lack of microscopic correlates and/or lack of dose dependency.

Organ weight: There were no metaldehyde-related organ weight changes. All organ weight differences were considered reflective of biological variation based on lack of statistical significance, lack of dose dependency and/or lack of microscopic correlates.

Histopathology: There were no metaldehyde-related microscopic changes. There was a slight increase in severity of thymus gland generalized lymphoid depletion in 15 mg/kg/day males and females; the differences were minimal and did not correlate with any statistically significant thymic weight reductions. Generalized lymphoid depletion is observed as a part of normal aging-associated thymic involution, and the onset and degree varies significantly in beagle dogs[9]. The degree of thymic lymphoid depletion observed on this study was within the expected range for beagles of this age and was not considered to be related to metaldehyde administration. All microscopic findings were considered incidental and of the type occasionally observed in beagle dogs of this age.

[9]Ploemen J-P HTM, Ravesloot WTM, Van Esch E. The incidence of thymic B lymphoid follicles in healthy beagle dogs. Toxicol Pathol 2003; 32:214-219.

Conclusion:

There were no adverse behavioral, physiological, or anatomical effects of 52 week dietary administration of metaldehyde to adult beagle dogs at dose levels of 1, 3.5, or 15 mg/kg/day. A no-observed-effect-level (NOEL) of at least 15 mg/kg/day of metaldehyde has been established in this study.

Reference:	**Expert statement on the histological findings (giant cells, atrophy and degeneration of the germinative epithelium) in the 52-week toxicity study in Beagle dogs with metaldehyde**
Author(s), year:	Leuschner J., Drommer W., 2006
Report/Doc. number:	LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany; LPT Report No. 15050/01, Doc. No.: 581-001
Guideline(s):	Not applicable, as the report is a histological re-examination and re-evaluation of samples generated in the 52-week study in dogs (Leuschner J., 2003)
GLP:	Not applicable
Deviations:	Not applicable
Validity:	Valid

Findings:

A complete picture of the results of the original 52-week toxicity study in dogs is presented in the DAR. In this Report, an additional table is included describing the clinical signs observed at the beginning of this 52-week toxicity study in detail. Furthermore, the histopathological findings in testes are discussed for the setting of the NOAEL in this 52-week toxicity study.

General observations/clinical signs

Clinical signs were only observed in the highest dose group (90 mg/kg bw/d) from study week 1 onwards. The following symptoms were noted: ataxia, reduced motility, emesis, tremor, twitching and salivation. Individual to all animals were affected. Incidence and severity appeared to decline from study week 19 onwards with none to two animals only affected.

Table 40: Clinical signs observed during Week 1 in the 52-week dog study in the highest dose group of 90 mg/kg bw/d

Animal number, sex	Observed on test day	Total number of days	Observation
25 m	1, 3-7	6	Ataxia
	2-5, 7	5	Tremor
	1-7	7	Emesis
	1-4	4	Salivation
	1-5, 7	6	Twitching
26 m	1, 3-5, 7	5	Ataxia
	1-7	7	Tremor
	1-5	5	Emesis
	1-2	2	Salivation
	2-7	6	Twitching
27 m	1-7	7	Ataxia
	1-5, 7	6	Tremor
	1-5	5	Emesis
	2	1	Salivation
	2-7	6	Twitching

	2-3	2	Lateral position
28 m	1, 3-7	6	Ataxia
	2-7	6	Tremor
	1-2, 6-7	4	Emesis
	1-2	2	Salivation
	1-5, 7	6	Twitching
	1	1	Lateral position
29 f	1-7	7	Ataxia
	1-7	7	Tremor
	1-3, 5-7	6	Emesis
	1	1	Salivation
	1-4, 6-7	6	Twitching
30 f	1-4, 6-7	6	Ataxia
	1-7	7	Tremor
	1-7	7	Emesis
	1	1	Salivation
	1-7	7	Twitching
31 f	1-4, 6-7	6	Ataxia
	1-7	7	Tremor
	7	1	Emesis
	1-7	7	Twitching
32 f	6-7	2	Ataxia
	1-7	7	Tremor
	4-6	3	Emesis
	1-7	7	Twitching

Histopathological findings in testes

Table 41: Atrophy and/or degeneration of the germinative epithelium in the testes (Leuschner J., 2003- see table 37)

	0 mg/kg	**10 mg/kg**	**30 mg/kg**	**90 mg/kg**
Testis I (not reported if left or right testis) no. examined	**4**	**4**	**41)**	**4**
Atrophy of the germinal epithelium (animal no., grade)	-	-	2 (no.18 moderate no.20 mild)	2 (no.25 mild no.27 marked)
Degeneration of the germinal epithelium (animal no., grade)	-	1 (no.10 mild)	-	3 (no.26 marked no.27 mild no.28 mild)
Giant cells (animal no., grade)	-	2 (no.10 mild, no.12 minimal)	1 (no.18 mild)	2 (no.25 minimal no.28 mild)
Juvenile testis (animal no.)	-	1 (no.12)	-	-
Testis II (not reported if left or right testis) no. examined	**4**	**4**	**41)**	**4**
Atrophy of the germinal epithelium (animal no., grade)	-	-	2 (no.18 minimal no.20 minimal)	3 (no.25 mild no.27 marked no.28 moderate)
Degeneration of the germinal epithelium (animal no., grade)	-	1 (no.10 mild)	-	3 (no.26 marked no.27 mild no.28 mild)

	0 mg/kg	**10 mg/kg**	**30 mg/kg**	**90 mg/kg**
Giant cells (animal no., grade)	-	1 (no.10 mild)	-	2 (no.25 mild no.28 minmal)
Juvenile testis (animal no.)	-	1 (no.12)	-	-

Severity grading: minimal, mild, moderate, marked
1) including animal no. 18 which died prematurely.

Upon request of the notifier the histopathological slides of the testes were re-examined by the study pathologist in order to verify the original diagnosis. The results of the re-examination were presented in an expert statement. In this paper it was explained that the histological changes of testes seen at the highest dose (90 mg/kg), where all animals were affected, consisted of mainly moderate to marked diffuse atrophy and/or degeneration of the germinative epithelium. At the two lower dose levels (10 and 30 mg/kg), findings were more focal in nature and of minimal to mild severity.

Concerning the "moderate severity" finding in one animal (No. 18), which died premature, of the mid dose (30 mg/kg), it was explained that the finding was limited to only one testis (the other testis showed only minimal focal atrophy) and was therefore regarded to be a spontaneous change.

Furthermore, it is stated in the position paper that the histopathological findings seen in testes at 10 and 30 mg/kg bw/day are of spontaneous nature and morphologically distinct from the treatment related findings seen at 90 mg/kg bw/day. Historical background data for atrophy and degeneration of the germinative epithelium performed at LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany during the years 2003-2006 were made available, which should demonstrate that incidences observed at the low and mid dose level were comparable to historical control data.

In conclusion, it was proposed in the position paper to set the NOAEL at 10 mg/kg bw/d based on mortality occurring at 30 and 90 mg/kg bw/d.

Table 42: Historical background data of atrophy and/or degeneration of the germinative epithelium in the testes of control animals (Leuschner J., Drommer W., 2006)

Finding	**Study 1**	**Study 2**	**Study 3**	**Study 4**	**Study 5**	**Study 6**
Testis I (not reported if left or right testis) no. examined	**4**	**5**	**5**	**5**	**5**	**4**
Atrophy of the germinal epithelium	0	0	0	1	1	0
Degeneration of the germinal epithelium	0	0	0	0	0	0
Giant cells	1	0	0	1	1	1
Testis II (not reported if left or right testis) no. examined	**4**	**5**	**5**	**5**	**5**	**4**
Atrophy of the germinal epithelium	0	0	0	3	3	0
Degeneration of the germinal epithelium	0	0	0	0	0	1
Giant cells	2	2	0	1	1	1

Conclusion:

Considering the historical control data on testes findings, the NOAEL of the 52-week study is set at 10 mg/kg bw/d based on testes findings (atrophy of the germinal epithelium) and mortality observed at 30 mg/kg.

4.7.1.2 Repeated dose toxicity: inhalation

No data available. It is not feasible to perform a valid inhalation toxicity study due to the physico-chemical properties of metaldehyde (see also section 4.2.1.2: Acute toxicity: inhalation).

4.7.1.3 Repeated dose toxicity: dermal

Reference:	**21-day repeated cutaneous dose toxicity study with metaldehyde in New Zealand White rabbits**
Author(s), year:	Hermansky S., Wagner C., 1991
Report/Doc. number:	Bushy Run Research Center, Pennsylvania, USA Lonza Report No. 1800
Guideline(s):	EPA Guideline Subdivision F, Section 82-2, 1984; OECD Guideline 410
GLP:	Yes
Deviations:	None
Validity:	Valid

Material and Methods:

5 New Zealand White rabbits per sex and dose group were treated cutaneously with 0, 100, 300 and 1000 mg metaldehyde/kg bw/d. The animals (source: Hazelton Research Products Inc., Denver, USA) were approximately 25 weeks of age at the first dosing. The test substance (batch no. 5448, purity 99.0 %) was administered as a dry powder applied directly to the back and covered with a gauze dressing moistened with Milli-Q water. Additional Milli-Q water was then added to the gauze to ensure consistent and sustained contact with the skin. Afterwards a bandage and tape were used to fix the gauze. After 6 hours of treatment, the wrapping was removed and the back of each animal was wiped with a damp cloth. The animals were dosed daily from Monday through Friday for three consecutive weeks. All animals were sacrificed on Monday of the fourth week.

During the 21-day study period, observations for mortality were made twice daily while detailed clinical observations including skin irritation were performed daily. Body weight data were collected for all animals on study day 1, day 8, day 15 and day 22. Food consumption data was reported for three intervals (days 1-8, days 8-15, days 15-22). Blood was collected after overnight fasting. Hematology included erythrocyte count, haemoglobin, hematocrit, erythrocyte indices, platelet count, total leukocyte count and differential leukocyte count. In clinical chemistry the following parameters were assessed: glucose, urea nitrogenk creatinine, total protein, albumin, globulin, total bilirubin, direct bilirubin, indirect bilirubin, calcium, phosphorus, sodium, potassium, chloride, ASAT, ALAT, gamma-glutamyl-transferase and alkaline phosphatase. A complete necropsy was performed on all animals. The organ weights of liver, kidneys, adrenals and testes were determined. The following tissues and organs were examined histopathologically from all animals of the control and the high dose group: gross lesions, liver (2 lobes with gallbladder), kidneys and skin (application site and non-application site).

Findings:

General observations: No animal died during the study. There were no signs of systemic toxicity observed.
Local irritation: The occluded area was divided into two components for observations and reporting. The “treatment area” refers to the area of metaldehyde application and “back” refers to the remainder of the occluded area of the back that was not designated for contact with metaldehyde. Excoriation was observed in both male and female animals of control and treatment groups. This was primarily observed in areas that were not treated with metaldehyde and was attributed to the dosing and occlusion procedures. There was no erythema or edema observed throughout the study in either male or female animals.

Food consumption and body weight: There were no treatment-related effects noted throughout the study.

Hematology and clinical chemistry: There were no treatment-related differences between control and treated animals observed.

Organ weights: There were no effects attributable to treatment observed.

Necropsy: No gross lesions were identified which were considered related to treatment. Focal areas of erythema and/or edema of treated and untreated (but prepared similarly as treated) skin were observed in most or all animals of all groups including controls. The differences between in-life observations and necropsy findings regarding the presence or absence of erythema and/or edema were attributed to the prosectors identifying small focal areas of erythema and/or edema which were not considered to be remarkable by the technician performing the in-life observation.

Histopathology: In both males and females, minor microscopic lesions were noted which were attributed to preparation of the sites and/or artefacts of the dosing procedure.

Conclusion:

No treatment-related effects were found at any dose in this study. Slight local irritation was observed in control and treatment groups and was attributed to dosing and occlusion procedures. The NOAEL for subchronic dermal toxicity in rabbits therefore is larger than 1000 mg/kg bw/d.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

Not available.

4.7.1.6 Other relevant information

Not available.

4.7.1.7 Summary and discussion of repeated dose toxicity

Rat, oral

28 days:

Sprague-Dawley CD rats received diets containing 0, 2500, 5000, 10000 and 20000 ppm metaldehyde (equivalent to 0, 197, 382, 761 and 1547 mg/kg bw/d for males; 0, 233, 454 and 875

mg/kg bw/d for females). Continuous treatment of rats with metaldehyde at dietary concentrations of 2500 ppm or more over 28 days caused systemic toxicity as shown by increased liver weight and hepatocellular hypertrophy. At the highest dose level of 20000 ppm, mortality occurred in 100% of females and 40% of males. Death occurred in the females during the first week of treatment while no males died prior to day 6. Also, 60% of the females receiving 10000 ppm died (between day 4 and 21) or were sacrificed in a moribund condition. In most cases, animals were sacrificed when found with hind limb paralysis/paresis presumably resulting from convulsions since these findings were associated with spinal fracture/luxation and pinching of the spinal cord. Changes in haematology (decrease in erythrocytes) were found in males at 10000 ppm and above. In addition, transient reduction of body weight gain and food consumption was noted at higher dose levels. A NOAEL could not be determined in this study. The LOAEL was 2500 ppm (equivalent to 197 mg/kg bw/d for males and 233 mg/kg bw/d for females).

90 days:

Sprague-Dawley rats received dietary concentrations of 0, 250, 750 and 2500 ppm metaldehyde (equivalent to 0, 21, 65 and 215 mg/kg bw/d for males and females) for 90 days. The predominant treatment-related effects were found in the liver. Centrilobular hepatocyte enlargement was observed at 750 ppm and above while liver enlargement and increased relative liver weight were noted in individual animals only in the high dose group (2500 ppm, not statistically significant). Females receiving 2500 ppm showed a reduction of body weight gain and food efficiency during the first week of treatment which resulted in a reduced (not statistically significant) terminal body weight. In conclusion, the NOAEL is considered to be 250 ppm (equivalent to 21 mg/kg bw/d for both sexes).

Mouse, oral

90 days:

CD-1 mice received 0, 100, 300, 1000, 3000 and 10000 ppm per diet (equivalent to 0, 19, 54, 178, 560 and 1919 mg/kg bw/d for males; 0, 24, 70, 235, 743 and 2996 mg/kg bw/d for females). Treatment with 10000 ppm metaldehyde in the diet resulted in the death of 5 males and 1 female within the first eight days of treatment. Effects on the liver were observed in all treatment groups (100 ppm and above): increased liver weight, swelling of the liver, hepatocellular hypertrophy, hyperplasia, necrosis, inflammation, anisokaryosis, vacuolization, cholestasis and biliary hyperplasia. Increases in body weight in females at 3000 and 10000 ppm were possibly related to the increases in liver weights. The decreases in absolute and relative kidney weights in males of the 10000 ppm treatment group were not associated with microscopic lesions in the kidney. Based on the results of this study, dosage levels of 20, 100 and 300 ppm were selected for a subsequent 18-month oncogenicity study. No NOAEL could be derived from this study. The LOAEL was 100 ppm (equivalent to 19 mg/kg bw/d for males and 24 mg/kg bw/d for females). For details see DAR.

Dog, oral

Originally, only the 52-week study in Beagle dogs was submitted. For the resubmission of metaldehyde, also an oral 28-day study and an oral 26-week study in Beagle dogs were submitted.

In the 28-day dose-range-finding study, severe clinical symptoms occurred at 90 mg/kg in the escalating dose experiment so that one female dog was sacrificed in moribund condition. In the fixed dose experiment, 75 and 90 mg/kg led to clinical symptoms but no mortality occurred. No histopathology was performed.

In the 26-week toxicity study, no clinical symptoms were observed at all three dose levels of 20, 60 and 90 mg/kg. No effects were noted for body weights, haematology, clinical biochemistry, urinalysis, ophthalmology, auditory examination, macroscopic evaluation and organ weights. At

histopathological examination, diffuse atrophy of the testes was found in the mid (2/6 males) and high dose group (4/6 males). A histopathological re-examination was performed in 2009, when also severity scores were investigated. The re-examination confirmed the pattern of testicular findings: the severity score of focal findings was increased at 60 mg/kg bw/day while at the high dose level of 90 mg/kg a clear increase of diffuse atrophic changes was found. The NOAEL for the 26-week study was therefore confirmed to be 20 mg/kg.

In the 52-week study in Beagle dogs (10, 30 and 90 mg/kg), mortality occurred in the mid and high dose group which was considered treatment-related. Clinical signs of toxicity (ataxia, reduced motility, emesis, tremor, twitching, salivation) were observed at the high dose group (90 mg/kg) from study week 1 onwards with incidence and severity declining over the time. Body weight gain was only slightly but not statistically significant reduced in the high dose group. There was indication of hearing impairment at the high dose group. Also in the high dose group, increases of erythrocytes, haemoglobin, hematocrit, bilirubin and triglycerides were observed during the conduct of the study but had largely resolved until the end of the study. In contrast, increased levels of alkaline phosphatase were observed in this group until the end of the study. The increase in liver weight in both sexes of the high dose group was not correlated with any histopathological finding. Male reproductive organs (testes, prostate) were target organs of metaldehyde as incidence and severity of microscopic lesions increased with dose. Prostate atrophy was observed in 1/4 males (25%) of the mid dose group and 3/4 (75%) males from the high dose group. In testes, atrophy and/or degeneration of the germinal epithelium was observed which mostly correlated with the occurrence of giant cells. The incidences for atrophy and/or degeneration of the germinal epithelium were 1/4 (25%) in the low dose, 2/4 (50%) in the mid dose and 4/4 (100%) in the high dose group. The single finding of juvenile testes in one animal of the low dose group appeared together with giant cells. Upon request of the notifier, the histopathological slides of the testes were re-examined by the study pathologist in 2006 and the results presented in an expert statement. In this statement the effects occurring at 10 and 30 mg/kg were judged to be of spontaneous nature and morphologically distinct from the findings at 90 mg/kg. Historical background data were also submitted. In conclusion, it was proposed in the expert statement to set the NOAEL at 10 mg/kg bw/day based on mortality occurring at 30 and 90 mg/kg.
Considering the historical control data on testes findings, the RMS followed the majority of comments received from the Member States, EFSA and the notifier to set the NOAEL of the 52-week study at 10 mg/kg bw/d based on testes findings (atrophy of the germinal epithelium) and mortality observed at 30 mg/kg.

inhalative

It is not feasible to perform a valid inhalation toxicity study due to the physico-chemical properties of metaldehyde (see also section 4.2.1.2: Acute toxicity: inhalation).

Rabbit, dermal

In a 21-day dermal toxicity study in rabbits, no substance-related effects were observed. Signs of local irritation occurred in control and treatment groups and were attributed to the dosing and occlusion procedures. The NOAEL for repeated dermal toxicity in rabbits was 1000 mg/kg bw/d (highest dose tested).

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Table 43: Summary of effects observed in rats, mice, dogs and rabbits in comparison to cut off values

Species- Route (Reference)	**Study duration**	**Cut off value Cat 1 STOT RE (1272/2008) [mg/kg bw/d]**	**Cut off value Cat 2 STOT RE (1272/2008) [mg/kg bw/d]**	**Effects below cut off value**	**Significance of toxicological effect (1272/2008) below cut off value**
Rat- oral (van Miller J.,1989)	28 days	30	300	- ≥ 197 (M)- 233 (F) mg/kg bw/d: ↑ liver weights, hepatocelluar hypertrophy	Changes in liver weight with no evidence of organ dysfunction
Rat- oral (Thomas O. et al., 1998)	90 days	10	100	- 65 mg/kg bw/d only: centrilobular hepatocyte enlargement	No evidence of organ dysfunction
Mouse- oral (Gill M. et al., 1990)	90 days	10	100	≥ 19 (M)-24 (F) mg/kg bw/d: hepatocellular hypertrophy, necrosis, inflammation, anisokaryosis - ≥ 54 (M)-70 (F) mg/kg bw/d: ↑ liver weight, vacuolisation, bilary hyperplasia	Liver lesions of minimal severity- no evidence of organ dysfunction
Dog- oral (Leuschner J., 2002)	28 days	? *	? *	- ≥ 60 mg/kg bw/d: reduced motility, clonic convulsions, increased respiratory rate, emesis - ≥ 75 mg/kg bw/d: tonoclonic convulsions, mydriasis, inflated stomach, slight tremor - 90 mg/kg bw/d: ataxia, salivation, abdominal/lateral position, pale gingivial; moribund condition of 1/3 females: shaking of the head, lateral position, difficulty in breathing- no pathological findings (macroscopic) after necropsy	The intensity/severity of the symptoms declined with time and had almost disappeared towards the end of the 4-week treatment Moribund condition, already covered by acute toxicity classification
Dog- oral (Neumann W., 1980,	26 weeks	? *	? *	- ≥ 60 mg/kg bw/d: **diffuse atrophy pf the prostate**, moderate	**Severe organ damage**

Species-Route (Reference)	**Study duration**	**Cut off value Cat 1 STOT RE (1272/2008) [mg/kg bw/d]**	**Cut off value Cat 2 STOT RE (1272/2008) [mg/kg bw/d]**	**Effects below cut off value**	**Significance of toxicological effect (1272/2008) below cut off value**
1991, Leuschner J., 2009)				atrophy of the testes (1/6), mild to moderate focal atrophy of the germinative epithelium (3/6), parasitic granuloma in mes. lymph nodes - 90 mg/kg bw/d: **mild to moderate diffuse atrophy of the germinative epithelium**, follicular hyperplasia mes. lymph nodes	
Dog- oral (Leuscher J., 2003)	51 weeks	? *	? *	- ≥ 30 mg/kg bw/d: **mortality** - 90 mg/kg bw: ataxia, reduced motility, emesis, tremor, twitching, salivation- no changes in histopathology, transient hearing loss, ↑ AP, **moderate to marked diffuse atrophy and/or degeneration of the germinative epithelium above the historical control data**	Incidence and severity of clinical signs declined from study week 19 onwards. No changes in histopathology. **Mortality** **Severe organ damage**
Dog oral (Gauvin G.V., 2010)	52 weeks	? *	? *	- ≥ 15 mg/kg bw/d: no effects	No clinical, macroscopic or microscopic signs
Dog oral (Gauvin G.V., 2010)	90 days	? *	? *	- ≥ 2500 ppm: liver weight changes (M) and microscopic hepatocellular centrilobular hypertrophy (M+F)	Liver effects
Rabbit- oral (gavage) (Neeper-Bradley T., 1990a)	Developmental (12 days of dosing)	30?	300?	Dams: - ≥ 100 mg/kg bw: 1/5 tremor, 1/5 hypoactive and death	No gross lesions observed. No dose response regarding mortality after administration of repeated doses

* For cut off values in dog studies, the only available document is ECBI/64/06 "Dose limits for classification with R48 based on dogs studies", 2006. In this document it is proposed that the cut off values for dog studies should be below the limit dose for the rat.

At histopathological examination in the 26-week dog toxicity study diffuse atrophy of the testes was found in the mid (2/6 males) and high dose group (4/6 males). A histopathological re-examination

was performed in 2009, when also severity scores were investigated. The re-examination confirmed the pattern of testicular findings: the severity score of focal findings was increased at 60 mg/kg bw/day while at the high dose level of 90 mg/kg a clear increase of diffuse atrophic changes was found.

In the 52-week study in Beagle dogs (10, 30 and 90 mg/kg), mortality occurred in the mid and high dose group which was considered treatment-related. All animals showed histological changes of testes at the highest dose (90 mg/kg), which consisted of mainly moderate to marked diffuse atrophy and/or degeneration of the germinative epithelium. At the two lower dose levels (10 and 30 mg/kg), findings were more focal and of minimal to mild severity and were thus considered to be of spontaneous nature and morphologically distinct from the treatment related findings seen at 90 mg/kg bw/day. Historical background data for atrophy and degeneration of the germinative epithelium demonstrated that incidences observed at the low and mid dose level were comparable to historical control data.

According to Regulation (EC) No. 1272/2008, mortality occurring at 30 mg/kg bw/d in a 52-week dog study and histopathological testes findings observed at 60 and 90 mg/kg bw/d in a 26-week and 52-week dog study, respectively trigger classification with Category 2 for Specific target organ toxicity – repeated exposure, **H373 "May cause damage to organs through prolonged or repeated exposure (if swallowed)"**.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Metaldehyde requires classification with **H373 "May cause damage to organs through prolonged or repeated exposure (if swallowed)"** based on the findings of mortality at 30 mg/kg bw/d and testicular findings at 60 and 90 mg/kg bw/d in dogs.

Criteria as specified in the Regulation (EC) No. 1272/2008: For the oral route the guidance values to assist in Category 2 classification are 10 < dose ≤ 100 mg/kg bodyweight/day. These guidance values refer to effects seen in a standard 90-day toxicity study conducted in rats.

It is not feasible to perform a valid inhalation toxicity study due to the physico-chemical properties of metaldehyde (see also section 4.2.1.2: Acute toxicity: inhalation). No substance-related effects were observed in a 21-day dermal toxicity study in rabbits.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Regulation (EC) No. 1272/2008: STOT RE 2, H373

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)
Summary of the Dossier Submitter's proposal The DS summarised the metaldehyde effects observed in repeated dose oral toxicity studies on rats, mice, dogs and rabbits in comparison with guidance values (see the Table below and Table 43, CLH report).

Mortalities and testicular toxicity seen in chronic studies in dogs were found by the DS to be adverse effects to justify classification in Category 2 for Specific target organ toxicity – repeated exposure, H373 "May cause damage to organs through prolonged or repeated exposure (if swallowed)".

The study results were summarised as follows:

In the histopathological examination in the 26-week dog toxicity study, diffuse atrophy of the testes was found in the mid (2/6 males) and high dose group (4/6 males). A histopathological re-examination was performed in 2009, when also severity scores were investigated. The re-examination confirmed the pattern of testicular findings: the severity score of focal findings was increased at 60 mg/kg bw/day while at the high dose of 90 mg/kg bw/day, a clear increase in diffuse atrophic changes was found.

In the 52-week study in Beagle dogs (10, 30 and 90 mg/kg bw/day), mortality occurred in the mid and high dose group which was considered treatment-related. All animals showed histological changes of testes at the highest dose (90 mg/kg bw/day), which consisted of mainly moderate to marked diffuse atrophy and/or degeneration of the germinative epithelium. At the two lower dose levels (10 and 30 mg/kg bw/day), findings were more focal and of minimal to mild severity and were thus considered to be spontaneous in nature and morphologically distinct from the treatment related findings seen at 90 mg/kg bw/day. Historical background data for atrophy and degeneration of the germinative epithelium demonstrated that incidences observed at the low and mid dose levels were comparable to historical control data.

No data on repeated inhalation toxicity was available.

No treatment-related effects were found at any dose in a 21-day dermal study in rabbits (Hermansky and Wagner, 1991). Slight local irritation was observed in control and treatment groups which was attributed to dosing and occlusion procedures.

Comments received during public consultation

Two comments from Industry expressed disagreement with the classification proposal, the interpretation of the effects in the dog studies and the adjustment of cut-off levels for the effects seen in dogs.

In one comment it was indicated that the pathology report of the 52-week dog study speculated that the cause of deaths were secondary to pulmonary exposure as a result of emesis. Effects on the testes were interpreted to be unrelated to treatment.

Two MSCAs agreed with the proposal to classify the substance as STOT RE 2; H373.

Assessment and comparison with the classification criteria

The available studies on repeated oral toxicity were presented in Table 43 of the CLH report and these data are presented in modified form in the Table below.

Table: Summary of effects observed in rats, mice, dogs and rabbits in comparison to guidance values (corresponding to Table 43 in the CLH Dossier, modified)

Species-Route (Reference)	Study duration	Guidance value Cat 1 STOT RE (1272/2008) [mg/kg bw/day]	Guidance value Cat 2 STOT RE (1272/2008) [mg/kg bw/day]	Effects at or below guidance value	Significance of toxicological effect (1272/2008) below guidance value
Rat- oral (van Miller, 1989) 0, 2500, 5000, 10000 and 20000 ppm/diet (≈ 0, 197, 382, 761 and 1547 mg/kg bw/day for M; 0, 233, 454 and 875 mg/kg bw/day for F)	28 days	30	300	≥ 197 (M)- 233 (F) mg/kg bw/day: ↑ liver weights, hepatocellular	Changes in liver weight, no evidence of organ dysfunction (to the extent examined by clinical chemistry) Dose finding study, some deviations (limited histopathology at ≥ 10000 ppm only), supplementary information only
Rat- oral (Thomas et al., 1998) 0, 250, 750 and 2500 ppm/diet (≈ 0, 21, 65 and 215 mg/kg bw/day for M and F)	90 days	10	100	≥ 65 mg/kg bw/day centrilobular hepatocyte enlargement (minimal at 65 mg/kg bw/day, slight at 215 mg/kd bw/d)	No evidence of organ dysfunction (from haematology, clinical chemistry and urinalysis)
Mouse- oral (Gill and Wagner, 1990) 0, 100, 300, 1000, 3000 and 10000 ppm/diet (≈ 0, 19, 54, 178, 560 and 1919 mg/kg bw/day for M; 0, 24, 70, 235, 743 and 2996 mg/kg bw/day for F)	90 days	10	100	≥ 19 (M)-24 (F) mg/kg bw/day: hepatocellular hypertrophy, necrosis, inflammation, anisokaryosis - ≥ 54 (M)-70 (F) mg/kg bw/day: ↑ liver weight, vacuolisation, bilary hyperplasia Liver lesions were minimal at 100 and 300 ppm	Liver lesions of minimal severity at 100 and 300 ppm - No conclusion on organ dysfunction can be drawn Dose range finding study for the oncogenicity study, limited investigations (no haematology, clinical chemistry or urinanalysis, histopathology on 10 animals of the control and 10000 ppm group), Supplementary information only
Dog- oral (diet) (Leuschner,2002) Escalating doses in 2 M and 2 F: 30, 60, 75 and 90 mg/kg bw/day, (each dose administered on 3 consecutive	28 days	30*	300*	- ≥ 60 mg/kg bw/day: reduced motility, clonic convulsions, increased respiratory rate, emesis - ≥ 75 mg/kg bw/day: tonoclonic convulsions, mydriasis,	The intensity/ severity of the symptoms of the fixed dose group declined with time and had almost disappeared towards the end of the 4-week treatment. All the effects were observed starting

days followed by 2 wash-out days) Fixed doses (2 M and 2F/each dose): 75 and 90 mg/kg bw/day, 28 days Dose finding study, no histopathologic examination				inflated stomach, slight tremor - 90 mg/kg bw/day ataxia, salivation, abdominal/lateral position, pale gingiva; moribund condition of 1 female (of the escalating dose group): 4 h post adm. shaking of the head, lateral position, difficulty in breathing - no pathological (macroscopic) findings after necropsy	from 20-60 min after administration and lasted up to 6 hours. Moribund condition (dosing stopped after first administration) of 90 mg/kg of the escalating dose group which started with 90 mg/kg): already covered by acute toxicity classification
Dog- oral (diet) (Neumann, 1980, 1991, Leuschner, 2009) 0, 20, 60 and 90 mg/kg bw/day Re-evaluation of histopathological findings in the testes Leuschner, 2009	26 weeks	5*	50*	- ≥ 60 mg/kg bw/day: **diffuse atrophy pf the prostate (4/6, 2/6)**, **moderate atrophy of the testes (1/6), mild to moderate focal atrophy of the germinative epithelium (3/6),** parasitic granuloma in mesenteric lymph nodes - 90 mg/kg bw/day: mild to moderate diffuse atrophy of the germinative epithelium, follicular hyperplasia mesenteric Lymph nodes	**Severe organ damage (prostata and testis)**
Dog- oral (diet) (Leuscher, 2003) 0, 10, 30 and 90 mg/kg bw/day Re-evaluation of Histopathological findings in the testes, (Leuschner and Drommer, 2009)	52 weeks	2.5*	25*	- ≥ 30 mg/kg bw/day: **mortality (1 M and 1F at 30 mg, 1 F at 90 mg/kg), days 260-322** ≥ 30 mg/kg bw/day **Prostate mild atrophy 1/4M, 3/4M** **Testes: minimal to moderate atrophy/ degeneration in 2/4M at 30 mg/kg** and mild to marked diffuse atrophy and/or degeneration of	Incidence and severity of clinical signs at 90 mg/kg declined from study week 19 onwards. No other changes in histopathology. **Mortality** **Severe organ damage (testes/prostate)**

				the germinative epithelium in 4/4 M at 90 mg/kg - 90 mg/kg bw: ataxia, reduced motility, emesis, tremor, twitching, salivation -, transient hearing loss, ↑AP	
Dog oral (Gauvin, 2010) 0, 1, 3.5 and 15 mg/kg bw/day	52 weeks	2.5*	25*	≤ 15 mg/kg bw/day: no effects	No clinical macroscopic or microscopic signs (no information on the list of organs examined)
Rat oral (diet) (Jones, Finn, Mullee, 2003) 0,100, 500, 2500 ppm (0, 8, 39, 185 mg/kg/d)	90 days	10	100	2500 ppm: In 1 female, loss of hind limb function together with an increased respiratory rate	OECD TG 242 (Neurotoxicity) study
Rat** oral (diet) (Gauvin, 2010) 0, 250, 750 and 2500 ppm	90 days	10*	100*	2500 ppm: 1 death in 1/10 males Day 7 liver weight changes (M) and microscopic hepatocellular centrilobular hypertrophy (M+F)	Liver effects OECD TG 242 (Neurotoxicity) study
Rabbit- oral (gavage) (Neeper-Bradley, 1990a)	Developmental (12 days of dosing)	30	300	Dams: 100 mg/kg bw: 1/5 tremor, 1/5 hypoactive and death	No gross lesions observed. No dose response relationship to mortality after administration of repeated doses

*values for experimental animals as described in 1272/2007. The DS noted in the CLH Dossier: for guidance values in dog studies, the only available document is ECBI/64/06 "Dose limits for classification with R48 based on dogs studies", 2006. In this document it was proposed that the cut off values for dog studies should be below the limit dose for the rat.
** This study was mentioned in Table 43 (CLH report) as a dog study, but no study report on dogs was found in the CLH report. It is assumed that the data are from a combined toxicity and neurotoxicity study in Sprague-Dawley rats of the author, Gauvin (2010).

As has been the practice for other substances, RAC proposes to use the guidance values of the rodent studies also for the dog studies (unless there is a rationale to deviate from this practice). For the oral route, the guidance values to assist in Category 2 classification are 10 < dose ≤ 100 mg/kg bw/day based on 90-day studies (values adjusted for study duration in the Table above).

- As mortalities and testes/prostate atrophy/degeneration were seen in the repeated dose studies at dose levels above the guidance values, classification as STOT RE is not warranted.

Mortalities in repeated dose studies should be considered only if related to repeated dosing within the range of guidance values:

- In the 28-day study, the moribund status seen in one female at 90 mg/kg bw/day (clinical symptoms appeared 4 h after administration, the animal was killed 6 h after administration) is considered as an acute toxic effect.

- The neurological signs in rats that received doses of 30 mg/kg bw/day for 3 days (after two days of wash-out the next highest dose was applied) were reduced motility/convulsions/ataxia. These symptoms are also interpreted as neurotoxic effects (at lethal and non-lethal doses) of an acute nature.

- The interpretation of its acute nature is supported by the observations from the second part of the study (with a fixed dose regime). Similar neurological signs were reported to occur starting from 20-60 min after administration and these lasted up to 6 h.

In the 52-week dog study, the mortalities that occurred (2 at 30 mg/kg bw/day, 1 at 90 mg/kg bw/day, on days 260-322) were clearly unrelated to acute toxicity, but doses were above the guidance value for classification for STOT-RE and no dose-response relationship was seen.

Pneumonia may have contributed to the deaths. The female dogs that died prematurely (1 at 30 mg/kg bw/day and 1 at 90 mg/kg bw/day) showed moderate interstitial pneumonia and bronchopneumonia which were considered in the monograph as contributing to the deaths. Although the study authors regarded the deaths as substance-related, no other obvious cause of death could be determined.

- Diffuse atrophy of the prostate and atrophy of the testes were considered as serious organ damage that could be considered for classification if occurring within the range of guidance values.

As these adverse effects occurred at doses above the guidance value of 50 mg/kg bw/day for a 26 week study (25 mg/kg bw/day for 52 week), at 60 mg/kg bw/day in the 26 week study and at ≥ 30 mg/kg bw/day in the 52 week study, classification as STOT RE is not warranted. In addition, severity grades were minimal to mild in dogs at 10 and 30 mg/kg bw/day (except the one that died earlier with moderate testis toxicity) in the 52-week study and minimal to mild at 60 mg/kg bw/day in the 26-week study.

- No clear dose-response was observed for the diffuse prostate atrophy (4/6 males at 60 mg/kg bw/day, 2/6 males at 90 mg/kg bw/day) in the 26 week-study (see Table 31, CLH Report).

However, it is to be noted that the leading effect is the testes atrophy (with prostate atrophy as a secondary effect that could be more sensitive). While small foci of focal atrophy may occasionally occur in control animals, no clear increase in the fraction of animals affected but an increased mean severity of focal atrophy was seen at 60 mg/kg bw/day (see re-evaluated findings in Table 31, CLH report).

Increased rates of bilateral diffuse atrophy of the testes are the strongest evidence of a treatment-relationship (compared to indirect effects on accessory glands and focal testis atrophy). If observed at higher incidences than in controls (rarely seen as a spontaneous lesion), this lesion can be assumed to be treatment related. Four out of 6 male dogs at 90

mg/kg bw/day (26 week study, Leuschner 2009) showed diffuse bilateral testes atrophy with at elevated severity scores. This dose was clearly above the guidance value.

The low severity of focal testes atrophy (0.17 in Table 31, CLH report) given for 90 mg/kg bw/day in the 26 week study may be misleading. If 4 animals show a diffuse atrophy, the incidence of 1 animal with focal atrophy cannot be divided by the total number of males in the 90 mg/kg bw/day group. Animals presenting diffuse atrophy cannot show focal atrophy at the same time.

In the 52 week study, mild atrophy of the prostate in 1/4 males and minimal to moderate atrophy/degeneration of the testes in 2/4 males were seen at 30 mg/kg (Table 37, CLH report). Although the incidences/severity increased at 90 mg/kg bw/day, this effect does not warrant classification for STOT RE as 30 mg/kg bw/day and higher doses are above the guidance values (see also section 'Additional key elements' on reproductive toxicity).

- Several repeated dose toxicity studies in rats showed spinal injuries such as haemorrhage and necrosis of the spinal cord and most of these animals were found in moribund state with hind limb paralysis/paresis. These were explained as secondary traumatic lesions to the clinical signs of tremor and convulsions and were not considered as relevant for STOT RE classification.

In conclusion, the combination of minimum to mild testis/prostate toxicity and some mortalities with unclear relationship to the treatment at doses above the guidance values **do not warrant classification for STOT RE**.

The effects on testis toxicity were taken into consideration for the endpoint reproductive toxicity.

Supplemental information - In depth analyses by RAC

Rat - oral, 28 days, dose-range finding study (van Miller, 1989)

Continuous treatment of rats with metaldehyde at dietary concentrations of 2500 ppm or more over 28 days caused systemic toxicity as shown by increased liver weight and hepatocellular hypertrophy. At higher dose levels (10000 ppm for females and 20000 ppm for both sexes), mortality and spinal injuries, presumably as a result of convulsions, occurred. Changes in haematology (decrease in erythrocytes) were found in males at 10000 ppm and above.

Rat - oral, 90 days, Japanese MAFF Guidelines for Toxicological Studies 59 No 4200, 1985 (Thomas et al., 1998)

One female found dead (2500 ppm) on day 11 showed pale kidneys and spleen together with normally expected post-mortem changes. No other findings in haematology, clinical chemistry, urinanalysis and histopathology investigations in more than 30 tissues.

Mouse - oral, 90 days (dose-range finding study) (Gill and Wagner, 1990)

Mortality occurred in 5 males and 1 female of the 10000 ppm group: 3 males on study day 1, one female on study day 2, one male on study day 4 and one male on study day 8. A

female from the 3000 ppm treatment group was found dead on study day 24. Dose-related increases (mostly statistically significant) in absolute and relative liver weights were observed in both sexes at dose levels of 300 ppm and above. The percent increase for absolute liver weights compared to controls ranged from 6-181 % for males and 12-104 % for females. Decreases of 15-17 % in absolute and relative kidney weights were observed in males in the 10000 ppm treatment group.

Hepatic lesions were observed in all treatment groups and included hepatocellular necrosis, hypertrophy and/or hyperplasia, inflammation, anisokaryosis, hepatocellular vacuolisation, cholestasis and biliary hyperplasia. The number of the lesions observed for individual animals, the number of animals affected within a treatment group, and the severity of individual lesions increased in a dose-related manner for animals in all treatment groups. The severity of lesions were minimal for the 100 and 300 ppm groups, minimal and/or moderate for the 1000 and 3000 ppm groups, and in the range of minimal to severe for animals in the 10000 ppm group.

D*og - oral, 4 weeks dose-range finding study (Leuschner, 2002)*

Severe clinical symptoms occurred at 90 mg/kg in the escalating dose experiment such that one female dog was sacrificed in moribund condition (6 h after administration). The study was continued starting with a new lowest dose level of 30 mg/kg bw/day.

During the escalating dose period no behavioural changes were noted following the 3-day administration of 30 mg/kg bw/day. Reduced motility was observed in both male and female animals and clonic convulsions in one male and two females on the first day after administering 60 mg/kg bw/day. In addition, increased respiratory rate and emesis were noted in one female animal. The 3-day treatment with 75 mg/kg bw/day led to reduced motility in one male and one female animal on two or all three administration days and to tonoclonic convulsions in one male and one female animal on two or all three administration days. Emesis was also observed in one female on all three administration days. A further increase in the dose to 90 mg/kg bw/day led to ataxia in all animals and to occasional or regular slight tremor in one male and the two females during the treatment days. In addition, tonoclonic convulsion and emesis were observed in all female animals and salivation in one female animal during the application period. Abdominal position was noted in both females on the first administration day. None of the animals of the escalating dose phase starting at 30 mg/kg bw/day died.

In the fixed dose experiment, 75 and 90 mg/kg bw/day led to clinical symptoms but no mortality occurred. Treatment with 75 mg/kg bw/day led to slight tremor, inflated stomach, emesis, mydriasis and increased respiratory rate of both male and female dogs. All the effects were observed starting 1-2 hours after administration and lasted up to 6 hours. The intensity of the symptoms subsided with time. No signs of toxicity were observed from day 7 onwards. Treatment with 90 mg/kg bw/day led to slight tremor, clonic and tonoclonic convulsions, inflated stomach, emesis, increased respiratory rate, slight ataxia, lateral position, abdominal position, moderate salivation, pale gingival and mydriasis of both male and females starting on day 1 onwards. All the effects were observed starting from 20-60 min after administration and lasted up to 6 hours. Overall, the severity of all symptoms declined with time and the signs of toxicity had almost disappeared towards the end of the 4-week treatment. No mortality occurred in the fixed dose level experiment.

No treatment related significant effects on body weight, food and water consumption, electrocardiography, haematology, clinical biochemistry were reported and there were no data on histopathology.

Dog - oral (diet), 26 weeks, non-guideline study (Neumann, 1980, 1991)

Testes: Diffuse atrophy of the testes was found only in the mid (2/6 males) and high dose group (4/6 males). The atrophy seen in one control animal was a consequence of cryptorchism. One animal from the low dose group had very slight focal atrophy of the tubules. This was considered a spontaneous finding because it also occurred in one untreated control animal. The dose level of 20 mg/kg bw/day was therefore considered the NOAEL for the testes effects.
A microscopical re-examination of haematoxylin and eosin stained paraffin and PAS-stained sections of the testes of all 24 male dogs of the 26-week toxicity study was performed in 2009 with the objective to score findings using a current severity grading system.
The results were: Cryptorchism and moderate atrophy of the testes (1 of 6 animals) and mild to moderate focal atrophy of the germinative epithelium (3 of 6 animals) were noted for the animals of the 60 mg/kg bw/day dose group. The changes in the 90 mg/kg bw/day dose group consisted of a mild to moderate diffuse atrophy/degeneration of the germinative epithelium. Four of 6 animals were affected, the mean severity grade of the high dose animals for diffuse atrophy of the left and right testes were 1.67 and 1.83, respectively, compared to 0.00 and 0.00 in the control. The changes are morphologically clearly distinct from the spontaneous findings in the 20 mg/kg bw/day dose group and in the control group and therefore attributable to the treatment with the test item. Prostate: Diffuse atrophy of the prostate was found in the mid dose group (4/6 males) and the high dose group (2/6 males). One male from the high dose group also showed focal atrophy. No such effects were seen in the control or low dose group.

Dog- oral (diet), 52 weeks (Leuscher,2003, Leuschner and Drommer, 2006)

Clinical signs of toxicity (ataxia, reduced motility, emesis, tremor, twitching, salivation) were observed at the high dose group (90 mg/kg bw/day) from study week 1 onwards with incidence and severity declining over time from week 19 onwards. 1 male and 1 female animal from the mid dose group (30 mg/kg bw/day) and 1 female from the high dose group were found dead between study day 260 and 322. As no obvious cause of death could be determined, the deaths were considered by the study authors to be related to treatment (NB. two dogs had pneumonia, see above.) A reduction in body weight gain was noted in high dose animals but without statistical significance. Also in the high dose group, 1 male and 1 female animal did not react to the noise test in week 13 but were not affected in week 26 and 52. Red blood cell parameters (increased erythrocytes, haemoglobin and hematocrit) were affected predominantely in females at the highest dose group. The effects were strongest at week 13 but declined until week 26 and were no longer seen at week 52. A similar situation was observed for bilirubin and triglyceride values in females of the high dose group, which were increased at week 13 but returned to normal until the end of the study. In contrast, elevated levels of alkaline phosphatase were observed in males and females at weeks 13, 26 and 52. Relative and absolute liver weights were increased in both sexes of the high dose group. In histopathology, in one animal of the mid dose group and 3 animals of the high dose group, mild atrophy of the prostate was observed.

The expert statement (Leuschner and Drommer, 2006) documented individual data on clinical signs and of the re-examination of the testes. Clinical signs were only observed in

the highest dose group (90 mg/kg bw/day) from study week 1 onwards. The following symptoms were noted: ataxia, reduced motility, emesis, tremor, twitching and salivation. Incidence and severity appeared to decline from study week 19 onwards with none to two animals only affected.

This re-examination of the testes revealed that the histological changes of testes seen at the highest dose (90 mg/kg bw/day), where all animals were affected, consisted of mainly moderate to marked diffuse atrophy and/or degeneration of the germinative epithelium. At the two lower dose levels (10 and 30 mg/kg bw/day), findings were more focal in nature and of minimal to mild severity. Concerning the "moderate severity" finding in one mid dose (30 mg/kg bw/day) animal (No. 18), which died prematurely), it was explained that the finding was limited to only one testis (the other testis showed only minimal focal atrophy) and was therefore regarded to be a spontaneous change. Furthermore, it is stated in the position paper that the histopathological findings seen in testes at 10 and 30 mg/kg bw/day are of spontaneous nature and morphologically distinct from the treatment related findings seen at 90 mg/kg bw/day.

None of the lesions reported in treated dogs were seen in the control animals of this study.

Ninety day repeated dose oral (dietary) neurotoxicity study in the rat (OECD TG 424) (Jones, Finn, Mullee, 2003)

A 90 day neurotoxicity study was conducted in Sprague-Dawley Crl:CD rats. No evidence of neurotoxicity was observed following subchronic administration of metaldehyde at any dose level tested (100, 500 and 2500 ppm). At the high dose level of 2500 ppm (185 mg/kg bw/day), one female showed loss of hind limb function together with an increased respiratory rate. Loss of the hind limb function was considered to result from spinal cord injury, even though the lesion was not histopathologically examined. In the females receiving 2500 ppm, a slight and transient reduction in bodyweight gain was detected during the first week of treatment. The NOAEL for both systemic toxicity and neurotoxicity is considered to be 500 ppm (equivalent to 39 mg/kg bw/day) based on the finding of reduced body weight gain and the loss of hind limb function in one female at 2500 ppm.

Metaldehyde: A 90-day dietary combined toxicity and neurotoxicity study in Sprague-Dawley rats (Gauvin, 2010)

Metaldehyde exposure of up to 2500 ppm in food and mixtures did not produce any significant neurotoxicity in male or female rats on this study. A NOEL for neurotoxicity of 2500 ppm metaldehyde has been established by this study. Structurally, a daily dose exposure of 2500 ppm metaldehyde for 13 weeks was found to produce significant changes in liver weights and incidences of hepatocellular centrilobular hypertrophy. Changes in the 750 ppm treatment group were minimal and not considered adverse. For these reasons the NOAEL for metaldehyde-induced systemic toxicity in the present study is set at 750 ppm (39.3 and 46.85 mg/kg bw/day for male and female rats, respectively).

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

Table 44: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
In vitro studies			
Reverse mutation assay (S. typhimurium TA 98, TA 100, TA 1535, TA 1537 and E. coli WP2uvrA$^-$)	0, 50, 150, 500, 1500 and 5000 µg/plate suspended in DMSO **Negative** (+/- S-9 mix)	-	Thompson, P.; 1998
Reverse mutation assay (S. typhimurium TA 98, TA 100, TA 1535, TA 1537 and TA 1538)	0, 0.26, 1.28, 6.4, 32 and 160 µg/plate (1st experiment) 0, 4, 6, 16 and 32 µg/plate (2nd experiment) dissolved in DMSO **Negative** (+/- S-9 mix)	Supplementary information only	Friederich, U., Wuergler, F.; 1981
Reverse mutation assay (S. typhimurium TA 98, TA 100, TA 1535, TA 1537 and E. coli WP2uvrA$^-$)	0, 50, 150, 500, 1500 and 5000 µg/plate suspended in DMSO **Negative** (+/- S-9 mix)	-	Thompson, P.; 1998
Gene mutation assay in L5178Y mouse lymphoma cells	0, 20, 50, 100 and 200 µg/ml (- S-9 mix) 0, 20, 50, 100 and 167 µg/ml (+ S-9 mix) dissolved in HEPES-buffered cell culture medium **Negative** (+/- S-9 mix)	-	Debets, F., Enninga, I.; 1986
Chromosome aberration test in CHO cells	0, 20, 50, 100 and 200 µg/ml (- S-9 mix) 0, 20, 50, 100 and 167 µg/ml (+ S-9 mix) dissolved in HEPES-buffered cell culture medium **Negative** (+/- S-9 mix)	-	Debets, F.; 1986
Lethal DNA damage in Escherichia coli (WP2, WP67, CM871)	0, 100, 316, 1000, 3160 and 10000 µg/ml suspended in 0.15 % aqueous agar **Negative** (+/- S-9 mix)	-	May, K.; 1992
In vivo studies			
Oral micronucleus test in BKW mice	0, 25, 50 and 100 mg/kg bw suspended in arachis oil **Negative**	-	Jenkinson, P.; 1990

4.9.2 Human information

Not available.

4.9.3 Other relevant information

Not available.

4.9.4 Summary and discussion of mutagenicity

Metaldehyde was tested in a battery of *in vitro* genotoxicity testings including gene mutation in bacterial strains and L5178Y mouse lymphoma cells, chromosomal aberration in CHO cells and lethal DNA damage in Escherichia coli. None of these *in vitro* tests indicated genotoxicity of metaldehyde. In addition, an *in vivo* micronucleus assay in mice showed no genotoxic potential of metaldehyde. In conclusion, there was no indication that metaldehyde was genotoxic *in vitro* or *in vivo*.

4.9.5 Comparison with criteria

Metaldehyde was tested negative for genotoxicity in a battery of *in vitro* and one *in vivo* test.

4.9.6 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: no classification proposed

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS came to the conclusion that a proposal to classify metaldehyde for mutagenicity is not justified on the basis of the available genotoxicity data.

***In vitro* tests**

Metaldehyde was tested in a battery of *in vitro* genotoxicity testings including bacterial gene mutation tests (Ames tests), testing on induction of DNA damage in *Escherichia coli*, a mouse lymphoma assay (L5178Y mouse lymphoma cells) and a chromosomal aberration test (CHO cells). None of these *in vitro* tests indicated genotoxicity of metaldehyde.

***In vivo* test**

In addition, an *in vivo* micronucleus assay in mice showed no genotoxic potential of metaldehyde.

In conclusion, there was no indication that metaldehyde was genotoxic *in vitro* or *in vivo*. Therefore, the DS proposed that no classification as germ cell mutagen is required.

Comments received during public consultation

One MSCA agreed with the proposal for no classification for metaldehyde.

Assessment and comparison with the classification criteria

RAC concludes in agreement with the proposal of the DS that no classification for germ cell mutagenicity is warranted.

The available *in vitro* genotoxicity tests are negative. Based on the negative *in vivo* study (micronucleus test) no mutagenicity was induced in somatic cells (criterion for classification as Category 2). Taking into account its systemic availability metaldehyde is considered to be non-mutagenic *in vivo*. Information on induction of germ cell mutagenicity (criterion for classification as Category 1B) is not available.

RAC considers that metaldehyde does not meet the criteria for classification for mutagenicity as defined in the Regulation (EC) No. 1272/2008. Accordingly, **no classification as germ cell mutagen is warranted**.

4.10 Carcinogenicity

Table 45: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Chronic toxicity / Oncogenicity study in Sprague Dawley CD rats	0, 50, 1000 and 5000 ppm/diet (equivalent to 0, 2, 44 and 224 mg/kg bw/d for males; 0, 3, 60 and 314 mg/kg bw/d for females) NOAEL = 50 ppm Effects at LOAEL: -decreased body weight and body weight gain -increased serum cholesterol -hepatocellular hypertrophy Increase of hepatocellular adenomas in females of the 5000 ppm group was within the historical control range	-	Gill, M., Wagner, C.; 1992
Chronic toxicity study in Wistar rats	0, 200, 1000 and 5000 ppm/diet (no information on actual test substance intake is presented in the publication) NOAEL Could not be determined LOAEL = 200 ppm based on: - posterior paralysis - lordosis	This study is of limited validity.	Verschuuren, H. et al.; 1975
Oncogenicity study in CD-1 mice	0, 25, 100 and 300 ppm/diet (equivalent to 0, 4, 16 and 49 mg/kg bw/d for males; 0, 5, 20 and 60 mg/kg bw/d for females) NOAEL = 100 ppm Effects at LOAEL: - hepatocellular hypertrophy	-	Chun, J., Wagner, C.; 1993
Oncogenicity study in CD-1 mice	0 and 1000 ppm/diet (equivalent to 0 and 135 mg/kg bw/d for males; 0 and 163 mg/kg bw/d for females) LOAEL = 1000 ppm based on: - increased liver weight - hepatocellular toxicity - benign hepatocellular adenoma	Follow-up study to the oncogenicity study of Chun and Wagner, 1993.	Beyrouty, P.; 1998

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Rat

Reference:	**Chronic dietary toxicity / oncogenicity study with metaldehyde in rat**
Author(s), year:	Gill M. and Wagner C., 1992
Report/Doc. number:	Doc.No. 537-002, Lonza Report No. 1550 Conducting laboratory: Bushy Run Research Center, Pennsylvania, USA
Guideline(s):	US EPA Guideline 83-5 (1984), OECD Guideline 453 (1981)
GLP:	Yes
Deviations:	No
Validity:	Yes

Material and Methods:

60 male and 60 female Sprague Dawley CD rats per group received 0, 50, 1000 or 5000 ppm metaldehyde (batch no. 5448, purity 99 %) via the diet. The animals (source: Charles River Breeding Laboratories, Portage, MI, US) were approximately 8 weeks of age at the first dose. Two untreated control goups were included in this study. These groups were treated as independent entities for all activities performed during the study. The purpose was to collect data that would provide some information regarding the range of normal or control values for the parameters evaluated in this study. It was not considered appropriate to combine the data from the two control groups for the purposes of comparing the combined control data to those from the treated groups.

Table 46: Combined chronic toxicity / carcinogenicity study in Sprague Dawley CD rats; Experimental design and test substance intake

Group	**Number of animals per group**	**Concentration in the diet (ppm)**	**Test substance intake (mg/kg bw/d)**	
			males	**females**
Control 1	60 m. / 60 f.	0	0	0
Control 2	60 m. / 60 f.	0	0	0
Low	60 m. / 60 f.	50	2	3
Mid	60 m. / 60 f.	1000	44	60
High	60 m. / 60 f.	5000	224	314

A 28-day dose range finding study was performed with rats being exposed to 0, 2500, 5000, 10000 and 20000 ppm in the diet. Mortality and traumatic injury to the back and spinal cord were observed for male and female rats at the 20000 ppm level and for female rats also at the 10000 ppm level. Hepatocellular hypertrophy was observed in most animals that survived to sacrifice. The severity of the hepatocellular hypertrophy was dose-related. In addition, sporadic foci of individual hepatocellular degeneration were noted in some animals from all treatment groups. Associated with hepatocellular hypertrophy there was a dose-related increase in liver weight for all treated male rats, and for female rats from the 5000 and 10000 ppm groups. Based on this dose finding study, dose levels of 50, 1000 and 5000 ppm were selected for the long term study.
Test diets were prepared weekly from a concentrated premix by appropriate dilutions. Homogeneity, concentration and stability were checked by gas chromatography analysis.

In-life observations: During the treatment period, observations for mortality were made twice daily. Detailed clinical observations including palpations were performed once a week. Observations of overt clinical signs were made once daily during the treatment period except on days with detailed clinical observations. Body weight and food consumption data were collected for all animals weekly for the first 14 weeks of the study and every second week thereafter. Ophthalmoscopic

examinations were performed for all animals prior to the start of the study and prior to final sacrifice.

Clinical Pathology: Hematology and clinical chemistry evaluations were conducted on 15 fasted animals/sex/group at 26, 52, 78 and 104 weeks of the study. Blood was collected from the retroorbital sinus. Whenever possible, urinalysis was conducted on the same 15 animals/sex/group during study weeks 25, 51, 77 and 103. For urine collection the rats were placed in metabolism cages for 24 hours.

Hematology: erythrocytes, haemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count

Clinical chemistry: glucose, urea nitrogen, creatinine, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), creatine kinase (CK), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (AP), total protein, total cholesterol, albumin, globulin (calculated), A/G ratio (calculated), total bilirubin, direct bilirubin, indirect bilirubin (calculated), calcium, phosphorus, sodium, potassium and chloride

Urinalysis: color, appearance, specific gravity, total volume, pH, protein, glucose, ketone, bilirubin, blood, urobilinogen and microscopic elements

Pathology: Following the 104-week treatment period, terminal necropsy of all animals was undertaken. A complete necropsy was performed on all animals sacrificed at study termination, found dead or sacrificed moribund. Organ weights were determined for all animals sacrificed at termination: liver, kidneys, spleen, heart, brain with stem, adrenal glands, testes and ovaries. Complete histopathology was performed for all animals in both control groups and the high dose group and included gross lesions, spinal cord (cervical, midthoracic, lumbar), brain (cerebral cortex, cerebellar cortex, medulla/pons), pituitary, thyroid (with parathyroid), thymic region, trachea, lungs (with mainstem bronchi), heart, salivary gland (mandibular), liver, spleen, kidneys, adrenals, pancreas, testes, epididymis, prostate, seminal vesicles, ovaries, uterus (corpus and cervix), vagina, mammary gland, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, skin, representative lymph nodes (mesenteric, submandibular), peripheral nerve (sciatic), sternum (including marrow), femur (including articular surface), thigh musculature, eyes and aorta. In the low and mid dose groups, only gross lesions, liver, lungs and kidneys were examined microscopically.

Findings:

Mortality and clinical signs: There were no treatment-related effects on the incidence of mortality observed. The mortality rates for male rats (including those sacrificed moribund / sacrificed moribund due to enlarged mass, but excluding procedural and accidental deaths) were 65 %, 58 %, 68 %, 60 % and 65 % for control 1, control 2, low, mid and high dose groups, respecitvely. For the females, mortality rates were 50 %, 45 %, 50 %, 60 % and 58 %, respectively. The tendency for increased mortality for females of the mid and high dose groups resulted from an increase in the number of animals in these groups that died during the last weeks of the study and was not considered to reflect a direct response to treatment. This conclusion was supported by the fact that the mean survival times for animals in the mid and high dose groups were slightly greater than those for the control and low dose groups. No significant alterations in clinical signs were noted throughout the study. However, with respect to the results of a published study described later in this section (Verschuuren H., 1975), the clinical observations regarding behaviour/CNS are described in the table below.

Table 47: Combined chronic toxicity / carcinogenicity study in Sprague Dawley CD rats; Mortality rates

	Dose group level (ppm)									
	Males					**Females**				
	01	**02**	**50**	**1000**	**5000**	**01**	**02**	**50**	**1000**	**5000**
Total number of animals	60	60	60	60	60	60	60	60	60	60
Number sacrificed	20	24	17	22	20	30	31	30	24	25
Number found dead	24	22	32	26	24	10	9	12	17	19
Number sacrificed moribund	14	11	8	6	13	8	9	16	15	12
Number sacrificed due to enlarged mass	1	2	1	4	2	9	6	2	1	1
Number sacrificed due to ulcerated mass	0	0	1	2	1	3	3	0	3	3
Number cage accidents	1	0	0	0	0	0	2	0	0	0
Number procedural deaths	0	1	1	0	0	0	0	0	0	0
Mean survival time (days)	532	536	548	525	520	530	527	531	541	546

[1] Control group 1; [2] Control group 2

Table 48: Combined chronic toxicity / carcinogenicity study in Sprague Dawley CD rats; Clinical signs on behaviour / CNS (number of animals affected; earliest to latest day a finding was observed)

	Dose group level (ppm)				
	01	**02**	**50**	**1000**	**5000**
Males					
Hyperactive	2 (477-696)	1 (407-408)	-	1 (624)	1 (400)
Hypoactive	16 295-728)	4 (477-722)	8 (393-710)	13 (557-713)	11 (283-718)
Aggressive	-	1 (646)	-	1 (631-634)	1 (630)
Paresis - leg-hind both - leg-hind left - leg-hind right	 4 (666-728) 2 (708-722) 1 (666-673)	 1 (407-408) - 1 (715-722)	 3 (575-728) 1 (666-673) -	 3 (609-722) 1 (636) -	 3 (565-728) - -
Paralysis - leg-hind both - leg-hind right	 - -	 - 1 (708)	 1 (708-722) -	 - -	 - -
Ataxia	4 (516-691)	4 (407-722)	3 (554-702)	2 (568-660)	8 (489-715)
Tremor	3 (646-691)	1 (587)	1 (714)	2 (635-646)	4 (564-663)
Clonic convulsions	-	-	-	1 (630)	-
Tonic convulsions	-	-	-	1 (575)	-
Helicoptering	1 (604-610)	-	1 (708)	-	2 (393-505)

	Dose group level (ppm)				
	01	**02**	**50**	**1000**	**5000**
Circling	4 (484-728)	-	1 (631)	1 (610-631)	6 (456-718)
Prostration	6 (406-680)	4 (481-615)	4 (340-714)	2 (575-691)	7 (406-722)
Head tilt	8 (516-728)	-	4 (528-702)	2 (575-660)	6 (365-718)
Tucked posture	1 (604-610)	-	1 (645)	1 (568-571)	-
Exaggerated hind limb placement	1 (726-727)	1 (624-722)	3 (624-687)	1 (610-631)	-
Females					
Hyperactive	-	1 (463)	-	1 (484-498)	1 (491-503)
Hypoactive	7 (456-729)	13 (463-729)	11 (421-704)	11 (323-729)	9 (421-728)
Paresis - leg-hind both - paw-hind both	 - -	 - -	 - -	 1 (428-429) -	 3 (274-686) 1 (615-621)
Paralysis - leg-hind both	 -	 -	 -	 -	 1 (484-485)
Ataxia	6 (464-666)	5 (530-729)	9 (422-729)	8 (344-725)	11 (435-728)
Tremor	2 (358-582)	-	3 (574-680)	1 (565)	3 (609-678)
Clonic convulsions	-	-	1 (565)	-	-
Helicoptering	-	2 (463-576)	1 (652-708)	-	1 (603-666)
Circling	2 (421-593)	1 (468)	5 (446-652)	3 (477-666)	2 (435-624)
Prostration	3 (481-582)	5 (468-723)	11 (547-728)	8 (349-716)	3 (548-609)
Head tilt	6 (400-729)	8 (435-729)	11 (441-729)	10 (505-729)	4 (421-708)
Tucked posture	3 (505-616)	2 (533-631)	1 (446-470)	2 (505-554)	1 (611)
Exaggerated hind limb placement	2 (624-666)	5 (631-729)	5 (589-729)	5 (582-725)	3 (603-708)

[1] Control group 1; [2] Control group 2

[a] statistically significant different from control group 1 ($p<0.05$)

[b] statistically significant different from control group 2 ($p<0.05$)

Body weights: Body weights and/or body weight gain were significantly decreased in males of the high dose group during the study. The mean absolute body weight and body weight gain were decreased generally 2-4 % and 6-8 % throughout the first year of the study and generally 4-8 % and 7-12 % by week 78, respectively. After that time this effect was no longer apparent as mean body weights for the groups began to vary as the incidence of mortality increased. The mean body weight and body weight gain for the mid dose group of males was slightly lower than controls during the early part of the study. These differences were, however, not statistically significant except for body weight gain in study week 1. No effect on body weight was observed in low dose males.

Also in females, body weights and/or body weight gain were significantly decreased in the high dose group throughout the study until the last several measurement periods. The mean absolute body weight was generally 5-7 % lower than in controls and the mean body weight gain was generally 8-14 % lower than controls for most measurement periods. The mean body weight and body weight gain for the females from the mid dose group were lower than controls for approximately the first year of the study. The decrease in weight gain for the mid dose group was statistically significant for the first 10 weeks and intermittently through week 18. Statistically significant reductions in mean absolute body weight occurred for females in the mid dose group for some periods during the first 16 weeks of the study. There was no effect on body weight observed in the low dose group.

Table 49: Combined chronic toxicity / carcinogenicity study in Sprague Dawley CD rats; Body weight and body weight gains

	Dose group level (ppm)									
	Males					**Females**				
	01	**02**	**50**	**1000**	**5000**	**01**	**02**	**50**	**1000**	**5000**
Body weight (g)										
-week 0	306.4	303.7	306.6	305.6	305.5	205.1	202.0	203.2	203.9	203.2
-week 14	593.1	589.3	598.1	579.8	574.6	320.3	320.2	315.0	309.7a,	303.7a,
-week 26	669.1	662.3	680.4	652.5	645.6a	353.7	355.3	350.3	b	b
-week 52	765.1	770.7	782.9	751.7	736.0b	405.5	411.4	409.1	344.4	332.5a,
-week 78	823.0	794.9	832.7	801.7	761.2a	452.2	476.3	463.3	404.4	b
-week 104	713.1	699.7	747.2	725.7	742.6	486.3	437.7	479.9	457.9	386.1
									503.7	441.4
										473.6
Body weight gain (g)										
-week 0-1	49.5	49.3	49.9	43.0a,b	38.4a,b	23.2	21.3	20.0a	15.9a,b	14.0a,b
-week 0-14	286.7	285.2	291.5	274.2	269.1a,	115.2	118.2	111.8	105.7a,	100.4a,
-week 0-26	362.7	358.2	373.3	346.9	b	148.6	153.8	147.0	b	b
-week 0-52	459.0	466.8	475.3	445.8	340.1a	200.6	209.9	205.9	140.4a,	129.2a,
-week 0-78	517.7	492.0	526.0	495.2	430.2a,	248.1	274.8	259.8	b	b
-week 0-104	405.4	441.6	398.2	441.9	b	281.9	237.2	277.1	200.6	182.8
					454.9b				254.4	237.9
					422.5				299.7	270.3

[1] Control group 1; [2] Control group 2

[a] statistically significant different from control group 1 ($p<0.05$)

[b] statistically significant different from control group 2 ($p<0.05$)

Food consumption: Some statistically significant increases and decreases of food consumption in males and females were considered incidental and not related to treatment.

Ophthalmoscopic examination: Corneal crystals, keratitis and cataracts were the most common ophthalmic abnormalities noted in this study. The distribution across dosage groups indicated no test substance related effects.

Hematology: There were no differences in the mean values which were considered to be treatment-related. Female rats in the 5000 ppm group had a statistically significant decrease in MCV and MCH at week 26 and 78 while no statistical significance was reached at week 78 and 104. As the decreases were not consistent throughout the study and did not demonstrate a pathologic process, they were not considered to be biologically significant.

Table 50: Combined chronic toxicity / carcinogenicity study in Sprague Dawley CD rats; Hematology findings

	Dose group level (ppm)									
	Males					Females				
	01	02	50	1000	5000	01	02	50	1000	5000
MCV (µm³)										
- week 26	49.8	51.5	50.7	50.8	50.4	54.6	54.6	55.9	54.3	52.8a,b
-week 52	50.9	53.1a	51.9	51.6b	51.3b	55.9	56.0	56.5	55.3	54.2
-week 78	52.4	53.6	51.9	52.9	51.4	56.0	56.2	56.4	55.6	54.0a,b
-week 104	52.5	53.1	54.2	52.8	53.7	57.5	56.8	56.1	56.0	56.2
MCH (pg)										
-week 26	18.4	19.2	18.8	18.9a	18.7b	20.5	20.7	20.9	20.4	19.9a,b
-week 52	19.0	19.9	19.4	19.2	19.2	20.6	20.5	20.9	20.5	20.0
-week 78	19.3	19.9	19.2	19.6	19.2	21.1	21.2	21.5	20.9	20.4a,b
-week 104	17.7	18.0	18.3	17.8	18.1	19.5	19.5	19.2	18.9	19.1

[1] Control group 1; [2] Control group 2

[a] statistically significant different from control group 1 ($p<0.05$)

[b] statistically significant different from control group 2 ($p<0.05$)

Clinical chemistry: In males, no treatment-related effects were observed. Female rats developed an apparent treatment-related effect on cholesterol (increased levels) at all measurement periods in the 1000 and 5000 ppm groups. Evidence of probable treatment-related increases in total protein, globulin and corresponding decrease in the A/G ratio were also present in the 5000 ppm females at week 26. The increase in globulins and corresponding decrease in the A/G ratio was persistent up to week 52 and 78. A shift in mean values at week 78, relative to week 52, reflect a change in methodology. All other statistically significant differences between mean values for control and treated animals occurred in a random fashion and were not supported by treatment-related trends and therefore not considered to be related to treatment.

Table 51: Combined chronic toxicity / carcinogenicity study in Sprague Dawley CD rats; Clinical chemistry findings in females

	Dose group level (ppm)				
	Females				
	01	02	50	1000	5000
Cholesterol (g/L)					
- week 26	1.09	1.13	1.13	1.28a	1.57a,b
-week 52	1.15	1.23	1.32	1.60a,b	1.76a,b
-week 78	0.96	1.07	1.05	1.50a,b	1.55a,b
-week 104	1.25	1.24	1.18	1.48	1.67
Total protein (g/L)					
- week 26	69	70	66b	69	73a
-week 52	68	70	70	70	71
-week 78	74	77	78	77	79
-week 104	70	72	72	70	71
Globulin (g/L)					
- week 26	32	33	32	33	36a,b
-week 52	32	33	34	34	36a,b
-week 78	34	35	36	38a	38a
-week 104	31	33	34	32	33
A/G Ratio					
-week 26	1.14	1.11	1.08	1.08	1.01a,b
-week 52	1.14	1.13	1.06	1.04	0.98a,b
-week 78	1.24	1.19	1.19	1.06a	1.07a
-week 104	1.32	1.26	1.17	1.22	1.20

[1] Control group 1; [2] Control group 2

[a] statistically significant different from control group 1 ($p<0.05$)

[b] statistically significant different from control group 2 ($p<0.05$)

Urinalysis: There were no statistically significant differences between control and treatment groups for male or female rats at weeks 25, 51 or 103. At week 77, female rats in the 1000 and 5000 ppm groups had statistically significant increases in urine total volume. Since these increases were not noted again at study termination and there was no supporting evidence for kidney functional changes, they were not considered treatment-related.

Organ weights: Mean liver weights (absolute and relative to body weight or brain weight) were increased in males (10-16 %) and females (21-32 %) in the 5000 ppm treatment groups. These increases were statistically significant for females. No other effects on organ weights were noted.

Table 52: Combined chronic toxicity / carcinogenicity study in Sprague Dawley CD rats; Liver weights

	Dose group level (ppm)									
	Males					**Females**				
	01	**02**	**50**	**1000**	**5000**	**01**	**02**	**50**	**1000**	**5000**
Liver weights (g)										
-absolute weight	19.6	20.3	20.4	20.9	22.9	13.1	12.1	12.4	13.9	15.9a,b
-% body weight	2.95	2.82	3.15	3.04	3.25	2.85	2.97	2.79	2.95	3.58a,b
-% brain weight	847	861	873	895	971	630	599	608	680	772a,b

[1] Control group 1; [2] Control group 2

[a] statistically significant different from control group 1 ($p<0.05$)

[b] statistically significant different from control group 2 ($p<0.05$)

Gross pathology: Females had more liver masses and nodules in the high dose group but only at the scheduled sacrifice at week 104.

Histopathology: Based upon the preliminary findings for this study by the designated study pathologist that identified the liver as a target organ, the Sponsor requested an internal peer review of the livers by a second pathologist. Following completion of the peer review, both the designated and the second pathologist discussed the discrepancies between their diagnoses. The designated pathologist then made edits he deemed appropriate to his original diagnoses. The results which are described and discussed here reflect the final diagnoses. For reasons of transparency, also histopathological diagnoses from the initial liver microscopy and from the internal peer review of liver microscopy are presented in an additional table.

Non-neoplastic lesions: Dose related increases in the incidence and severity of hepatocellular hypertrophy were observed for male and female rats in the 1000 and 5000 ppm treatment groups. This effect was statistically significant for males at 1000 and 5000 ppm and females at 5000 ppm. The severity generally ranged from minimal to mild. Hepatocellular hypertrophy was usually centrilobular in distribution especially in those animals exposed to metaldehyde. In control animals and a few treated animals it tended to be periportal in distribution. No effect was noted on hepatocellular hyperplasia. Foci of cellular alteration were observed in control and treated animals. No clear relation to dose was observed though values were statistically significant for mid dose males found dead and for high dose males sacrificed at week 104. As part of the peer review, the altered cell foci were tabulated by cell type, i.e. vacuolated, clear, mixed, eosinophilic or basophilic. Again, there were no clear treatment-related differences.

Neoplastic lesions: Hepatocellular adenomas were observed in 6/60 females of the high dose group compared with incidences of 1/60 and 0/60 in the control groups, being statistically significant when compared to the second control group. In males, no hepatocellular adenomas were observed in metaldehyde treated animals. Hepatocellular carcinomas were observed for some males and

females in treated and control groups. The incidences were not dose-related. In males of the mid dose group, incidences of carcinomas reached statistical significance in animals sacrificed in study week 104. When the combined incidences of hepatocellular adenomas and carcinomas were analysed statistically, females of the high dose group had a significantly higher incidence of tumors when compared to the second control group. Historical control data were supplied from two studies conducted at the same laboratory (Bushy Run Research Center, Report Numbers 53-543 and 53-566), using the same study design and source of animals. In study no. 1 incidences of hepatocellular carcinomas and adenomas were in the same range as in the present study while in study no. 2 the incidences were somewhat lower.

In the present study, hepatocellular adenomas and carcinomas were usually apparent upon gross examination but were never very large or widespread within the organ. They were never identified as cause of death. These observations suggest that hepatocellular neoplasms developed late in the lives of the animals. The earliest to appear was a carcinoma in a male rat in the low dose group at 86 weeks of age.

There were no other neoplasms which occurred with significantly greater incidence in treated animals than in controls. Tumors of the pituitary were by far the most frequently occurring neoplasms, affecting 49 % of the males and 85 % of the females. A variety of tumors was reported in mammary glands of females. Numerous other tumors occurred in control and treated animals with similar frequencies and were expected in a study of this nature. There were no rare or unusual tumors which occurred in such a distribution as to suggest a relationship to treatment.

Table 53: Combined chronic toxicity / carcinogenicity study in Sprague Dawley CD rats; Histopathology findings in the liver

	Dose group level (ppm)									
	Males					**Females**				
	01	**02**	**50**	**1000**	**5000**	**01**	**02**	**50**	**1000**	**5000**
Non-neoplastic lesions										
Hepatocellular hypertrophy										
-sacrificed at week 104	-	2	3	11a,b	20a,b	1	3	-	5	20a,b
-found dead / sacrificed moribund	-	-	3	9a,b	18a,b	4	2	-	6	16a,b
-all animals on study	-	2	6a	20a,b	38a,b	5	5	-b	11	36a,b
Hepatocellular hyperplasia										
-sacrificed at week 104	1	-	-	-	-	-	-	2	1	2
-found dead / sacrificed moribund	-	1	-	2	1	2	1	2	-	1
-all animals on study	1	1	-	2	1	2	1	4	1	3
Focus of cellular alteration										
-sacrificed at week 104	10	18	9	10	18a	13	13	17	17	12
-found dead / sacrificed moribund	7	8	6	16a	9	5	8	5	10	8
-all animals on study	17	26	15	26	27	18	21	27	20	21
Neoplastic lesions										
Hepatocellular adenoma										
-sacrificed at week 104	1	-	-	-	-	1	-	-	-	5b
-found dead / sacrificed moribund	-	-	-	-	-	-	-	1	-	1
-all animals on study	1	-	-	-	-	1	-	1	-	6b

	Dose group level (ppm)									
	Males					Females				
	01	02	50	1000	5000	01	02	50	1000	5000
Hepatocellular carcinoma										
-sacrificed at week 104	1	-	1	4a	-	-	-	1	-	1
-found dead / sacrificed moribund	1	-	3	-	2	1	-	-	-	-
-all animals on study	2	-	4	4	2	1	-	1	-	1
Hepatocellular adenomas + carcinomas	**3**	**-**	**4**	**4**	**2**	**2**	**-**	**2**	**-**	**7b**

Historical control data (from conducting laboratory)				
	Control 1 (males)	**Control 2 (males)**	**Control 1 (females)**	**Control 2 (females)**
Study 1				
-adenomas	1	7	6	6
-carcinomas	-	1	-	-
-adenomas+carcinomas	1	8	6	6
Study 2				
-adenomas	3	3	1	-
-carcinomas	-	-	-	-
-adenomas+carcinomas	3	3	1	-

[1] Control group 1; [2] Control group 2

[a] statistically significant different from control group 1 ($p<0.05$)

[b] statistically significant different from control group 2 ($p<0.05$)

Table 54: Combined chronic toxicity / carcinogenicity study in Sprague Dawley CD rats; Initial and peer review liver histopathology for adenomas and carcinomas

	Dose group level (ppm)									
	Males					Females				
	01	02	50	1000	5000	01	02	50	1000	5000
INITIAL liver histopathology										
-adenoma	3	1	4	3	5	1	-	1	2	7
-carcinoma	-	-	-	2	1	1	-	1	-	-
-adenoma + carcinoma	3	1	4	5	6	2	-	2	2	7
PEER REVIEW liver histopathology										
-adenoma	2	-	-	-	-	1	-	1	-	5
-carcinoma	1	-	4	4	3	1	-	1	-	3
-adenoma + carcinoma	3	-	4	4	3	2	-	2	-	8

[1] Control group 1; [2] Control group 2

Conclusion:

Administration of metaldehyde in the diet for 104 weeks did not result in toxicologically significant alterations in mortality, clinical signs of toxicity, palpable masses or food consumption. Body weight and body weight gain were reduced throughout the study period in males of the 5000 ppm group and females of the 1000 and 5000 ppm groups. Occasionally decreased MCV and MCH values in females of the 5000 ppm dose group remained of questionable toxicological significance. A clear treatment-related effect was observed in clinical chemistry evaluation when increased cholesterol levels were observed in females of the 1000 and 5000 ppm groups. Additionally, some alterations in total protein, globulin and albumin / globulin ratio values were found in females of the 5000 ppm group. Pathology findings revealed the liver to be the target organ of metaldehyde. Increased liver weights (absolute and relative to body and brain weight) were found in the 5000 ppm dose group as well as an increased rate of liver masses and nodules. The incidence and severity of hepatocellular hypertrophy increased with dose. Statistical significance was reached in males already at a dose of 50 ppm, but this finding did not correlate with other liver findings.

Hepatocellular tumors were found in males and females in treatment and control groups. No dose relation was observed in male animals. In females, statistical significance was reached for the 5000 ppm group when compared to one of the two concurrent control groups. However, when tumor incidences were compared with historical control data, a relation to treatment with metaldehyde was found to be questionable. In conclusion, a NOAEL of 50 ppm (2 and 3 mg/kg bw/d for males and females, respectively) is defined based on slightly lower body weight and body weight gain during the first year of the study for females, increased serum cholesterol for females, and hepatocellular hypertrophy for both sexes. No carcinogenic potential of metaldehyde was assumed based on the results of this study.

In the valid long term toxicity / oncogenicity study in Sprague Dawley CD rats (Gill, M., Wagner, C.; 1992- see above), the main target organ identified was the liver, as it was observed also in the short term toxicity studies. Body weight and body weight gain were decreased in the mid dose (1000 ppm, females only) and high dose groups (5000 ppm, both sexes). Increased absolute and relative liver weights were noted in rats of the high dose group. The incidence and severity of hepatocellular hypertrophy increased with dose in both sexes of the mid and high dose groups. At the same dose levels, blood cholesterol levels were increased in females. Additionally, some alterations in total protein, globulin and albumin/globulin ratio were found in females of the high dose group. Hepatocellular tumors were found in males and females in treatment and control groups. While no dose relation was observed in males, statistical significance against one of the two control groups was reached in females of the high dose group regarding hepatocellular adenomas alone and the sum of hepatocellular adenomas and carcinomas. As the incidences were within the historical background range of the conducting laboratory, a relation to treatment with metaldehyde was not assumed. No effects were noted at the low dose level of 50 ppm.

The second study in Wistar rats (Verschuuren, H. et al.; 1975) was of limited validity due to study design and reporting. The dose levels tested (200, 1000 and 5000 ppm) were comparable to the other study regarding the mid and high dose level, however, the outcome of the study was different. Effects on the liver were only found at the 5000 ppm level in form of increased liver weights reaching statistical significance in males only. No treatment-related changes in liver histopathology were reported. On the other hand, clinical signs of posterior paralysis were observed in 1/25 males from the 200 ppm group (first sign at day 569), 1/25 males (first sign at day 657) and 1/25 females of the 1000 ppm group (first sign at day 652) and 5/25 females from the 5000 ppm group (first sign at day 19, 641, 625, 659 and 559). The clinical signs were reflected in histopathology where transverse lesions of the spinal cord were observed in 3 of the females showing posterior paralysis. In the 3 animals with posterior paralysis receiving 200 and 1000 ppm, only lordosis was detected. The histological investigations showed that the lesions were caused by trauma i.e. a “sudden kink” in the vertebral column leading to a fracture or distortion of the vertebrae and a subsequent compression of the spinal cord. This effect is similar as described in the 28-day study in rats, when animals receiving very high doses (10000 and 20000 ppm) developed hind limb paralysis/paresis. No NOAEL could be derived. The LOAEL of this very limited study is 200 ppm.

Mouse

Reference:	**Chronic dietary oncogenicity study with metaldehyde in mice**
Author(s), year:	Chun J. and Wagner C., 1993
Report/Doc. number:	Bushy Run Research Center, Pennsylvania, USA Lonza Report No. 1549
Guideline(s):	US EPA Guideline 83-2 (1984), OECD Guideline 451 (1981)
GLP:	Yes
Deviations:	No
Validity:	Yes

Material and Methods:

CD-1 mice were administered metaldehyde (batch no. 5448, purity 99.0 %) in the diet at concentrations of 0, 25, 100 or 300 ppm for at least 78 weeks. The doses corresponded to approximately 0, 4, 16 and 49 mg/kg bw/d for males and 0, 5, 20 or 60 mg/kg bw/d for females. Two control groups were included in this study. These groups were treated as independent entities for all activities performed during the study. The purpose was to collect data that would provide some information regarding the range of normal or control values for the parameters evaluated in this study. The animals (source: Charles River Laboratories, Portage, MI, USA) were approximately 8 weeks old and weighed 25.7-33.1 g (males) or 20.2-26.9 g (females).

Table 55: Carcinogenicity study in CD-1 mice; Experimental design and test substance intake

Group	**Number of animals per group**	**Concentration in the diet (ppm)**	**Test substance intake (mg/kg bw/d)**	
			males	**females**
Control 1	60 m. / 60 f.	0	0	0
Control 2	60 m. / 60 f.	0	0	0
Low	60 m. / 60 f.	25	4	5
Mid	60 m. / 60 f.	100	16	20
High	60 m. / 60 f.	300	49	60

Test diets were prepared weekly from a concentrated premix by appropriate dilutions. Homogeneity, concentration and stability were checked by gas chromatography analysis.

In-life observations: During the treatment period, observations for mortality were made twice daily. Detailed clinical observations including palpations were performed once a week. Observations of overt clinical signs were made once daily during the treatment period except on days with detailed clinical observations. Body weight and food consumption data were collected for all animals weekly for the first 14 weeks of the study and every second week thereafter.

Clinical Pathology: Hematological investigations were conducted for 10 non-fasted animals/sex from the high dose and control groups at study week 52, and for 10 animals/sex from all groups prior to the final sacrifice. No clinical chemistry evaluations or urinalysis were performed. Hematology parameters included erythrocytes, haemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, total leukocyte count and differential leukocyte count.

Pathology: A complete necropsy was performed on all animals (surviving, found dead or sacrificed moribund). Organ weights were determined for all animals sacrificed at termination: liver, kidneys, spleen, heart, brain with stem and testes. Complete histopathology was performed for all animals in

both control groups and the high dose group and included gross lesions, lungs (with mainstem bronchi), brain (cerebral cortex, cerebellar cortex, medulla/pons), pituitary, thyroid (with parathyroid), thymic region, trachea, heart, sternum (including marrow), salivary gland (mandibular), liver (3 lobes), spleen, kidneys, adrenals, pancreas, testes, epididymis, prostate, seminal vesicles, ovaries, uterus (corpus and cervix), vagina, aorta, skin, esophagus, stomach, gall bladder, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, lymph nodes (mesenteric, submandibular), mammary gland, peripheral nerve (sciatic), femur (including articular surface), thigh musculature, eyes and spinal cord (cervical, mid thoracic, lumbar). In the low and mid dose groups, only gross lesions, liver, lungs and kidneys were examined microscopically.

Findings:

Mortality and clinical signs: There were no treatment-related effects on the incidence of mortality observed. The survival rates for male mice (including those sacrificed moribund) were 73 %, 83 %, 75 %, 70 % and 77 % for control 1, control 2, low, mid and high dose groups, respecitvely. The corresponding values for females were 70 %, 70 %, 67 %, 75 % and 82 %, respectively. The mean survival time ranged from 514-536 days for males and 527-541 days for females.

There were no treatment-related clinical signs or increases in the incidence of animals with palpable masses observed in any group, especially with respect to behaviour / CNS.

Table 56: Carcinogenicity study in CD-1 mice; Mortality rates

	Dose group level (ppm)									
	Males					**Females**				
	0[1]	**0[2]**	**25**	**100**	**300**	**0[1]**	**0[2]**	**25**	**100**	**300**
Total number of animals	60	60	60	60	60	60	60	60	60	60
Number sacrificed at week 79	44	50	45	42	46	42	42	39	45	45
Number found dead	9	7	9	14	11	9	6	13	10	3
Number sacrificed moribund	7	3	6	4	3	9	12	6	5	7
Number sacrificed due to enlarged mass	-	-	-	-	-	-	-	2	-	4
Number sacrificed due to trauma	-	-	-	-	-	-	-	-	-	1
Mean survival time (days)	514	536	528	522	520	530	527	530	541	531

[1] Control group 1; [2] Control group 2

Body weights and food consumption: There were no effects on absolute body weights or body weight gain that were attributed to treatment with metaldehyde for male and female mice of any dose group. Occasionally statistically significant increases or decreases were not considered to be treatment-related due to the lack of a consistent pattern, the lack of dose-response, and/or a small magnitude of changes. The same applies for food consumption.

Hematology: No treatment-related changes were observed for male and female mice.

Organ weights: Liver weights (absolute and relative to body weight and brain weight) was slightly but not significantly increased in metaldehyde treated males. No other effects on organ weights were noted in any of the treatment groups.

Table 57: Carcinogenicity study in CD-1 mice; Liver weights

	Dose group level (ppm)									
	Males					**Females**				
	0[1]	**0[2]**	**25**	**100**	**300**	**0[1]**	**0[2]**	**25**	**100**	**300**
Liver weights (g)										
-absolute weight	2.382	2.543	2.565	2.630	2.668	2.114	2.201	2.209	2.158	2.145
-% body weight	6.024	6.105	6.413	6.654	6.689	5.833	5.930	6.098	5.908	5.8851
-% brain weight	468	496	505	508	534	409	422	433	409	414

[1] Control group 1; [2] Control group 2

Gross pathology: No gross lesions attributed to treatment were noted at necropsy.

Histopathology:

Non-neoplastic lesions: The only change which was attributed to treatment was an increase in the incidence of hepatocellular hypertrophy in males and females of the highest dose group. Hypertrophy was graded minimal to mild in most animals and was primarily centrilobular in location. Hepatocellular hypertrophy was observed in some mice which were found dead or sacrificed moribund during the study, but the incidence was low in these animals, possibly because early autolytic changes or degeneration of the tissues due to disease may have made it more difficult to diagnose.

Neoplastic lesions: Hepatocellular adenomas appeared in 8/60 male mice in both control groups, in 4/60 males at 25 ppm, 9/60 males at 100 ppm and 15/60 males at 300 ppm. In females, the incidences were low with only one adenoma found in the second control and the 25 and 100 ppm groups. Most of the adenomas were large, well differentiated tumours involving much of the affected lobe. Hepatocellular carcinomas occurred less frequently than adenomas. The incidences were 1/60 and 3/60 in the control groups, 6/60 in the 25 ppm group and 3/60 in the 100 and 300 ppm group for male mice. In females, only one hepatocellular carcinoma was observed (100 ppm). Most carcinomas were small in comparison to the size of adenomas, and most developed as an anplastic component of a preexisting adenoma. In those cases in which both benign and malignant components were identified within the same mass, the more malignant designation was assigned. Two males (1 of the second control group and 1 of the 300 ppm group) were found to have an adenoma and a carcinoma in different lobes, both of which are listed separately in the tables.

Though there is a nominal increase of hepatocellular adenomas in the highest dose group, this difference is statistically not significant and is not considered toxicologically relevant.

Table 58: Carcinogenicity study in CD-1 mice; Histopathology findings in the liver

	Dose group level (ppm)									
	Males					**Females**				
	0[1]	**0[2]**	**25**	**100**	**300**	**0[1]**	**0[2]**	**25**	**100**	**300**
Non-neoplastic lesions										
Hepatocellular hypertrophy										
-sacrificed at week 79	16	20	8[b]	20	34[a,b]	7	2	5	6	15[b]
-found dead / sacrificed moribund	2	-	1	1	3	2	2	-	-	3
-all animals on study	18	20	9b	21	37[a,b]	9	4	5	6	18[b]

	Dose group level (ppm)									
	Males					**Females**				
	0[1]	**0[2]**	**25**	**100**	**300**	**0[1]**	**0[2]**	**25**	**100**	**300**
Neoplastic lesions										
Hepatocellular adenoma										
-sacrificed at week 79	7	8	3	8	13	-	1	1	-	-
-found dead / sacrificed moribund	1	-	1	1	2	-	-	-	1	-
-all animals on study	8	8	4	9	15	-	1	1	1	-
Hepatocellular carcinoma										
-sacrificed at week 79	-	2	6a	3	1	-	-	-	-	-
-found dead / sacrificed moribund	1	1	-	-	2	-	-	-	1	-
-all animals on study	1	3	6	3	3	-	-	-	1	-
Hepatocellular adenomas + carcinomas	9	10[3]	10	12	17[3]	-	1	1	2	-

[1] Control group 1; [2] Control group 2
[3] including one mice with adenoma and carcinoma
[a] statistically significant different from control group 1 ($p<0.05$)
[b] statistically significant different from control group 2 ($p<0.05$)

Conclusion:

In a 79 week oncogenicity study in CD-1 mice, no changes in mortality, clinical signs, body weight, food consumption, hematology or necropsy findings were noted. Liver weight (absolute and relative) was slightly but not significantly increased in the high dose males. In histopathology, a significant increase in the incidence of hepatocellular hypertrophy was noted in both sexes of the high dose group. An increase of hepatocellular adenomas in high dose males was statistically not significant. In conclusion, a NOAEL of 100 ppm (16 and 20 mg/kg bw/d for males and females, respectively) is considered based on the increased incidence of hepatocellular hypertrophy in both sexes at 300 ppm. No carcinogenic potential of metaldehyde was assumed based on the results of this study.

Reference:	**A chronic dietary oncogenicity study with metaldehyde in mice**
Author(s), year:	Beyrouty P., 1998
Report/Doc. number:	ClinTrials BioResearch Ltd., Senneville, Quebec H9X 3R3, Canada Laboratory Project I.D. 87013; Lonza Report No. 2976, Doc. No.: 555-002
Guideline(s):	Follow up study to 90-day study and 18-month oncogenicity study in mice
GLP:	Yes
Deviations:	Not applicable: Follow up study to 90-day study and 18-month oncogenicity study in mice
Validity:	Yes

Purpose of the study:

This study was conducted as a follow-up to a 90-day mouse dietary dose range-finding study (*Gill M. and Wagner C., 1990*) and an 18-month mouse dietary oncogenicity study (*Chun J. and Wagner C., 1993*) conducted by Lonza Inc. at Bushy run Research Center (Export, PA, USA). In the 90-day study, metaldehyde was mixed in the diet at concentrations of 0, 100, 300, 1000, 3000 and

10000 ppm. Mortality was observed at dietary concentrations of 3000 and 10000 ppm and dose-related increases in liver weight and hepatic lesions were observed for male and female mice following ≥ 300 ppm. Based on the results of the 90-day study, a maximum dietary concentration of 300 ppm was selected for the initial 18-month mouse dietary study. In the 18-month study, treatment related effects were limited to hepatocellular hypertrophy for male and female mice in the 300 ppm treatment group. Therefore, the supplementary study was conducted at a metaldehyde concentration of 1000 ppm in order to satisfy the regulatory requirements for a maximum tolerated dose. Animals used in this study were the same strain and were from the same supplier and source as animals used in the initial oncogenicity study. In addition, the study design and the procedures used for the microscopic diagnoses of liver lesions were the same as those used for the initial oncogenicity study.

Material and methods:

Test material	Metaldehyde
Lot/Batch	Batch no. 5448
Purity	101%
Vehicle	Diet
Species	Mouse
Strain	Swiss Crl:CD-1 (ICR)BR strain
Age	55-57 days
Weight at dosing	Males: 28.6-39.5 g, Females: 22.8-33.7 g
Source	Charles River Breeding Laboratories, Portage, Michigan

Metaldehyde was mixed in the diet and administered to the CD-1 mice (60 mice/sex/group) for 18 months. Animals were assigned to two separate control groups and one treatment group receiving 1000 ppm metaldehyde.

Table 59: Supplementary Oncogenicity Study in CD-1 mice; Study design

Group	**Number of animals per group**	**Concentration in the diet (ppm)**	**Actual test substance intake (mg/kg bw/d)**	
			males	**females**
Control 1	60 m. / 60 f.	0	0	0
Control 2	60 m. / 60 f.	0	0	0
Treatment group	60 m. / 60 f.	1000	135	163

Test diets were prepared weekly from a concentrated premix by appropriate dilution. Homogeneity, concentration and stability were checked weekly throughout the study.

In-life observations: During the treatment period, observations for clinical signs and mortality were made twice daily. Detailed clinical observations including palpations were performed once a week. Body weight and food consumption data were collected for all animals weekly for the first 14 weeks of the study and every second week thereafter.

Clinical Pathology: Hematological investigations were conducted for 10 non-fasted animals/sex from each group at 12 and 18 months of treatment. No clinical chemistry evaluations or urinalysis were performed. Hematology parameters included erythrocytes, haemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelet count, total leukocyte count and differential leukocyte count.

Pathology: A complete necropsy was performed on all animals (surviving, found dead or sacrificed moribund). Organ weights were determined for all animals sacrificed at termination: liver, kidneys,

spleen, heart, brain with stem and testes. Complete histopathology was performed for all animals in both control groups and the treatment group and included gross lesions, lungs (with mainstem bronchi), brain (cerebral cortex, cerebellar cortex, medulla/pons), pituitary, thyroid (with parathyroid), thymic region, trachea, heart, sternum (including marrow), salivary gland (mandibular), liver (left lobe, ventral portion of right lobe and right portion of median lobe), spleen, kidneys, adrenals, pancreas, testes, epididymides, prostate, seminal vesicles, ovaries, uterus (horns, body and cervix), vagina, aorta (thoracic), skin (inguinal), esophagus, stomach, gall bladder, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, lymph nodes (mesenteric, submandibular), mammary gland (inguinal, females), peripheral nerve (sciatic), femur (including articular surface), thigh musculature, eyes and spinal cord (cervical, mid thoracic, lumbar).

Findings:

Mortality and clinical signs: There were no treatment-related effects on the clinical appearance or the incidence of mortality observed. The survival rates for male mice (including those sacrificed moribund) in study week 79 were 85 %, 73 %, 83 % for control 1, control 2, and metaldehyde group, respectively. The corresponding values for females were 75 %, 77 % and 72 %, respectively. The mean survival time ranged from 514-536 days for males and 527-541 days for females.

There were no treatment-related clinical signs or increases in the incidence of animals with palpable masses observed in any group, especially with respect to behaviour / CNS.

Table 60: Supplementary Oncogenicity Study in CD-1 mice; Mortality rates

	Dose group level (ppm)					
	Males			**Females**		
	0[1]	**0[2]**	**1000**	**0[1]**	**0[2]**	**1000**
Total number of animals	60	60	60	60	60	60
Number sacrificed at week 79	51	44	50	45	46	43
Number found dead	5	6	8	12	9	12
Number sacrificed preterminally	4	10	2	3	5	5
Survival rate	85%	73%	83%	75%	77%	72%

[1] Control group 1; [2] Control group 2

Body weights: There were no effects on absolute body weight or body weight gain that were attributed to treatment with metaldehyde for males and females of the 1000 ppm dose group.

Food consumption: Statistically significant increases in food consumption were observed for metaldehyde-treated mice, when compared to one or both control groups. Statistically significant effects for females were generally only observed when compared to one control group. For males and females, the magnitude of the difference from the control group(s) was small (generally around 3 to 8 %). Given the direction of the change (i.e. increase), the changes were considered not to be toxicologically significant.

Haematology: No treatment-related changes were observed for male and female mice.

Gross pathology: An increased incidence of metaldehyde-treated male mice with liver masses (15/60) was seen compared to the control groups (6/60 and 9/60, resp.). Similarly, an increase in the incidence of metaldehyde-treated male mice with enlarged liver (7/60) was seen compared to controls (0/60 and 1/60, resp.). There were no treatment-related gross pathology findings for female mice or additional treatment-related findings for male mice.

Organ weights: Both metaldehyde-treated male and female mice showed significant increases in liver weight (absolute, relative to body weight and relative to brain weight). The average magnitude

of the increase in absolute liver weight was approximately 46 % for males and 14 % for females compared to the control groups. Other statistically significant organ weight changes occasionally observed between groups were considered incidental.

Table 61: Supplementary Oncogenicity Study in CD-1 mice; Liver weights

	Dose group level (ppm)					
	Males			**Females**		
	0[1]	**0[2]**	**1000**	**0[1]**	**0[2]**	**1000**
Liver weight absolute (mg)	1630	1676	2378 [a,b]	1555	1474	1770 [a,b]
Liver weight relative to body weight (g %)	4255	4429	6302 [a,b]	4425	4201	5066 [a,b]
Liver weights relative to brain weight (g %)	311531	322061	455841 [a,b]	287691	279856	323812 [a,b]

[1] Control group 1; [2] Control group 2
[a] Statistically significantly different from control 1
[b] Statistically significantly different from control 2

Histopathology:

Treatment-related histopathological lesions in the liver for male mice and, to lesser extent, for female mice were observed. Histopathologic lesions included hepatocellular hypertrophy in male and female mice and single cell necrosis, focal or multifocal necrosis, pigment accumulation, sinusoidal histiocytosis and benign hepatocellular adenoma in male mice. It should be noted that in 3 males, both hepatocellular adenoma and carcinoma were found concurrently. A small increase in the incidence of female mice in the metaldehyde treatment group with hepatocellular eosinophilic cell foci or hepatocellular adenoma was observed. Hepatocellular hypertrophy, eosinophilic cell foci and hepatocellular adenoma observed in this study often shared similar cellular morphology and were considered to represent a continuum of changes over time. These changes are consistent with an adaptive hypertrophic response of the liver to an increase in metabolic demand with subsequent development of proliferative changes and hepatocellular toxicity.

Other non-neoplastic or neoplastic findings seen at histopathology were spontaneous in nature and generally within the limits reported for aging CD-1 mice and therefore considered to be incidental and unrelated to treatment.

Table 62: Supplementary Oncogenicity Study in CD-1 mice; Non-neoplastic and neoplastic lesions in the liver

	Dose group level (ppm)					
	Males			**Females**		
	0[1]	**0[2]**	**1000**	**0[1]**	**0[2]**	**1000**
Non-neoplastic lesions						
Necrosis, single cell	15	12	43[a,b]	4	9	8
Necrosis	7	7	17[a,b]	5	6	9
Pigment accumulation	10	7	26[a,b]	21	25	16
Hypertrophy, hepatocellular	9	15	55[a,b]	3	3	17[a,b]
Histiocytosis, sinusoidal	1	7	18[a,b]	3	4	6
Eosinophilic cell focus	1	3	3	0	0	5[a,b]
Neoplastic lesions						
Hepatocellular adenoma (benign)	4	5	14[a,b]	1	0	5[a,b]
Hepatocellular carcinoma (malign)	2	2	4	0	0	0

[1] Control group 1; [2] Control group 2
[a] statistically significant different from control group 1 ($p<0.05$)
[b] statistically significant different from control group 2 ($p<0.05$)

Conclusion:

Treatment of male and female mice with metaldehyde at 1000 ppm in the diet for 18 months resulted in changes in the liver of male, and to a lesser extent of female mice. These changes included increased liver weight and an increase in the incidence of animals with hepatocellular toxicity and benign hepatocellular adenoma. The nature of these findings is consistent with an adaptive hypertrophic response of the liver to an increase in metabolic demand with subsequent development of proliferative changes and hepatocellular toxicity. Historical control data from two other studies conducted at the same laboratory show that hepatocellular adenomas have been reported in control group mice of the same strain, sex and source (Charles River Laboratories, Portage, MI) in incidences ranging from 6/60 to 13/60 (Chun and Wagner, 1993; Doc.No. 537-001, Appendix 3, p.9).

Reference:	**Review of carcinogenicity studies with Metaldehyde**
Author(s), year:	Harder V., Roth T., Hofer M., 2010
Report/Doc. number:	SCC Scientific Consulting Company, Wendelsheim, Germany SCC Project No. 262-004; Report date 2010-04-22, Doc. No.: 581-009
Guideline(s):	Not applicable.
GLP:	Not applicable.
Deviations:	Not applicable.
Validity:	Not applicable. Position Paper

In the **Position Paper from the notifier**, the tumour incidences of **male mice only** are discussed and historical control values for males from 2 studies conducted at the same laboratory (BRRC Project Report Number 53-515 and 53-528, see table below) were cited. The following arguments were provided by the notifier in the Position Paper:

- Hepatocellular adenomas are known to be common findings in male mice of this strain and have been reported in control animals from other studies in incidences ranging from 6/60 to 13/60.
- Furthermore, hepatocellular adenomas are considered to be of a questionable biological significance in mice as they rarely have an adverse effect on the animal´s health or contribute to its death.
- No dose dependent increase was observed for hepatocellular carcinomas. There were no instances of hepatocellular carcinomas metastasizing to any other organ.
- PSD evaluated the studies/publications in 1996 and concluded that there is no evidence for a carcinogenic potential of Metaldehyde (PSD, 1996).

Alltogether, the notifier concluded that Metaldehyde showed no evidence of a carcinogenic potential in mice.

For reasons of clarity, a table on the incidences of hepatocellular adenomas and carcinomas from both carcinogenicity studies in mice (Chun and Wagner, 1993, Beyrouty, 1998) together with the respective control values is presented in the Position paper. Additionally to the notifier´s Position paper, also values for female animals are presented here.

Table 63: Overview on hepatocellular tumour incidences and historical control values in CD-1 mice

Hepatocellular Neoplasms	**Tumour incidence**

		Males	Females
Hepatocellular ADENOMA			
Study of Chun and Wagner, 1993			
Concurrent Control Group 1	0 ppm	8/60	0/60
Concurrent Control Group 2	0 ppm	8/60	1/60
Low Dose Group	25 ppm	4/60	1/60
Mid Dose Group	100 ppm	9/60	1/60
High Dose Group	300 ppm	15/60	0/60
Study 1#: Historical Control Group 1	0 ppm	6/60	No information available #
Study 2#: Historical Control Group 2	0 ppm	13/60	No information available #
Study of Beyrouty, 1998			
Concurrent Control Group 1	0 ppm	4/60	1/60
Concurrent Control Group 2	0 ppm	5/60	0/60
Single Dose Group	1000 ppm	**14/60**[1,2]	**5/60**[2]
Hepatocelluar CARCINOMA			
Study of Chun and Wagner, 1993			
Concurrent Control Group 1	0 ppm	1/60	0/60
Concurrent Control Group 2	0 ppm	3/60	0/60
Low Dose Group	25 ppm	6/60	0/60
Mid Dose Group	100 ppm	3/60	1/60
High Dose Group	300 ppm	3/60	0/60
Study of Beyrouty, 1998			
Concurrent Control Group 1	0 ppm	2/60	0/60
Concurrent Control Group 2	0 ppm	2/60	0/60
Single Dose Group	1000 ppm	4/60	0/60

[1] statistically significantly different from control group 1, $p<0.05$
[2] statistically significantly different from control group 2, $p<0.05$
Historical control data for Studies 1 and 2 were derived from the original study report which was submitted for DAR evaluation (Chun and Wager, 1993, "Chronic Dietary Oncogenicity study with Metaldehyde in Mice", Appendix 3, Anatomic Pathology Report, page 9-10). Only values for male animals are presented there. In the Pathology Report of the carcinogenicity study, Study 1 and 2 are described as two studies conducted with animals of the same strain, sex and source at a comparable time period (BRRC Project Report Numbers 53-515 and 53-528). Historical control data for male mice from both studies (53-515 and 53-528) were cited also in the Position Paper submitted by the notifier in April 2010.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

Not available.

4.10.3 Other relevant information

Not available.

4.10.4 Summary and discussion of carcinogenicity

Metaldehyde showed no carcinogenic potential in rats. In the first mouse study (Chun and Wagner, 1993), there was a dose related increased incidence of hepatocellular adenomas in male CD-1 mice, but no statistical significance. The incidence was slightly exceeding HCD at the high dose of 300 ppm. In the second mouse study (Beyrouty, 1998), for both male and female CD-1 mice there was a statistically significant increase in hepatocellular adenomas at 1000 ppm (single dose study) compared to the concurrent control groups. No HCD is available for the second mouse study. At the PRAPeR 79 (Pesticide Risk Assessment Peer Review) expert meeting in July 2010 the experts decided not to propose classification for metaldehyde with R40 based on the hepatocellular adenomas found in CD-1 mice (majority after vote).

4.10.5 Comparison with criteria

The issue of a possible carcinogenic potential of metaldehyde has been discussed at the **PRAPeR 79 (Pesticide Risk Assessment Peer Review) expert meeting in July 2010**:

For hepatocellular carcinomas in CD-1 mice, there is no dose relation or statistical significance for males. For females, there was only one incidence of carcinoma at mid dose without statistical significance. The experts agreed that the hepatocellular carcinomas are of no concern.

In the first mouse study (Chun and Wagner, 1993), there was a dose related increased incidence of hepatocellular adenomas in males, but no statistical significance. Incidence was slightly above HCD at the high dose of 300 ppm. No HCD is available for females.

In the second mouse study (Beyrouty, 1998), for both males and females there is a statistically significant increase in hepatocellular adenomas at 1000 ppm (single dose study) compared to the concurrent control groups. No HCD is available for the second mouse study. Furthermore, it was mentioned that males may have exceeded the tolerable limit in the Beyrouty study since in the 90-day preliminary mouse study (Gill and Wagner, 1990) liver toxicity was observed at all doses ≥ 100 ppm (19 mg/kg bw/day and above). This liver toxicity may have contributed to the incidence of adenomas observed in the long term carcinogenicity studies.

The experts considered whether the hepatocellular adenomas in CD-1 mice are enough to trigger classification based on the above data. Finally, the experts voted on whether classification with R40 should be proposed: **The majority of experts voted not to propose classification for carcinogenic properties of metaldehyde.**

Metaldehyde showed no carcinogenic potential in rats.

Based on the arguments above the dossier submitter agrees with the conclusion, that the results of both mouse studies are too weak to justify classification and labelling for carcinogenicity.

4.10.6 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: no classification proposed

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

In a Chronic toxicity/oncogenicity study (Gill and Wagner; 1992) in Sprague Dawley CD rats, which was compliant with OECD TG 453, the increase in hepatocellular adenomas was within the HCR.

In another oncogenicity study in CD-1 mice (Chun and Wagner, 1993), compliant with OECD Guideline 451, no carcinogenic potential of metaldehyde was assumed based on the results. Also, in the follow-up GLP compliant study (Beyrouty, 1998), toxicity and an increase in benign hepatocellular adenoma was observed but again no carcinogenic potential of metaldehyde was assumed.

Based on the studies mentioned above, the Dossier Submitter concluded that metaldehyde demonstrated no carcinogenic potential.

NB. No effects on survival, clinical signs, body weights, food consumption, organ weights, gross and microscopic pathology were reported unless indicated here as being treatment-related.

In a 104-week OECD TG 453 (diet) study on Sprague-Dawley (SD) rats (Gill and Wagner, 1992), absolute and relative liver weights were increased in female rats at the high dose of 5000 ppm. Dose related increases in the incidence and severity of hepatocellular hypertrophy were observed for male and female rats in the 1000 and 5000 ppm treatment groups. Hepatocellular adenomas were observed in 6/60 females (*please note that the table below indicates 7 adenomas in the initial liver histopathology*) of the high dose group compared with incidences of 1/60 and 0/60 in the two control groups, being statistically significant when compared to the second control group. In males, no hepatocellular adenomas were observed in metaldehyde treated animals. Hepatocellular carcinomas were observed for some males and females in treated and control groups. The incidences were not dose-related. In males of the mid dose group, incidences of carcinomas reached statistical significance in the animals sacrificed in study week 104. When the combined incidences of hepatocellular adenomas and carcinomas were analysed statistically, females of the high dose group had a significantly higher incidence of tumours when compared to the second control group. Historical control data were supplied from two studies conducted at the same laboratory, using the same study design and source of animals. In study no. 1 incidences of hepatocellular carcinomas and adenomas were in the same range as in the present study while in study no. 2 the incidences were somewhat lower.

The results from initial and peer reviewed liver histopathology were summarised as shown in the table below:

Table: Initial and peer reviewed liver histopathology findings in SD rats (corresponds to Table 54, CLH Report)

	Dose group level ppm (mg/kg bw/day)									
	Males					Females				
	01* (0)	02** (0)	50 (2)	1000 (44)	5000 (224)	01 (0)	02 (0)	50 (3)	1000 (60)	5000 (314)
Initial liver histhopathology#										
Adenoma	3	1	4	3	5	1	-	1	2	7
Carcinoma	-	-	-	2	1	1	-	1	-	-
Adenoma + carcinoma	3	1	4	5	6	2	-	2	2	7
Peer review liver histopathology										
Adenoma	2	-	-	-	-	1	-	1	-	5
Carcinoma	1	-	4	4	3	1	-	1	-	3
Adenoma + carcinoma	3	-	4	4	3	2	-	2	-	8

* Control group 1, ** Control group 2
# The incidences differ from the incidences in Table 53 of the CLH Dossier.

Some data on chronic toxic effects (increased relative liver weights in high dose male rats, lordosis and traumatic lesions in the spinal cord secondary to posterior paralysis in 5/25 high dose female rats, 1/25 male and 1/25 female of the mid dose groups, 1/25 low dose male), but no information on tumour data were presented from the second chronic study in rats with test concentrations of 200, 1000 and 5000 ppm in the diet over 107 weeks which was judged as of limited validity (Verschuuren *et al.*, 1975). Increased relative liver weights were observed in males (10-16 %) and females (21-32 %) in the 5000 ppm treatment groups.

In the first mouse study (Chun and Wagner, 1993), there was a seemingly dose related increase incidence of hepatocellular adenomas in male CD-1 mice (receiving metaldehyde in diet at concentrations of 0, 25, 100 or 300 ppm), but without statistical significance. The incidence slightly exceeded the HCD at the high dose of 300 ppm. No carcinogenic potential of metaldehyde was assumed based on the results of this study.
In this study, liver weights (absolute and relative to body weight and brain weight) was slightly but not significantly increased in metaldehyde treated males. The only non-neoplastic change which was attributed to treatment was an increase in the incidence of hepatocellular hypertrophy in males and females of the highest dose group.

Table: Carcinogenicity study in CD-1 mice; Histopathology findings in the liver (see Table 58, CLH Report)

	Dose group level ppm (mg/kg bw/day)									
	Males					Females				
	01* (0)	02** (0)	25 (4)	100 (16)	300 (49)	01 (0)	02 (0)	25 5)	100 (20)	300 (60)
Liver histopathology										
Adenoma	8	8	4	9	15	0	1	1	0	0
Carcinoma	1	3	6	3	3	0	0	0	1	0
Adenoma + carcinoma	9	10#	10	12	17#	0	1	1	2	0

* Control group 1; ** Control group 2
#Including one mouse with adenoma and carcinoma

In the second (supplementary) 18-month mouse study (Beyrouty, 1998), in both male and female CD-1 mice (from the same supplier) there was a statistically significant increase in hepatocellular adenomas at 1000 ppm (single dose study) compared to the concurrent control groups. No HCD is available for the second mouse study.

In this study, an increased incidence of metaldehyde-treated male mice with liver masses (15/60) was observed, compared to the control groups (6/60 and 9/60, respectively). Similarly, an increase in the incidence of metaldehyde-treated male mice with enlarged liver (7/60) was seen compared to controls (0/60 and 1/60, respectively). Both metaldehyde-treated male and female mice showed significant increases in liver weight (absolute, relative to body weight and relative to brain weight). The average magnitude of the increase in absolute liver weight was approximately 46 % for males and 14 % for females compared to the control groups.

Treatment-related histopathological lesions in the liver were observed for male mice, and to a lesser extent for female mice. Histopathological lesions included hepatocellular hypertrophy in male and female mice and single cell necrosis, focal or multifocal necrosis, pigment accumulation, sinusoidal histiocytosis and benign hepatocellular adenoma in male mice. It should be noted that in 3 males, both hepatocellular adenoma and carcinoma were found concurrently. A small increase in the incidence of female mice in the metaldehyde treatment group with hepatocellular eosinophilic cell foci or hepatocellular adenoma was observed. Hepatocellular hypertrophy, eosinophilic cell foci and hepatocellular adenoma observed in this study often shared similar cellular morphology and were considered to represent a continuum of changes over time. These changes are consistent with an adaptive hypertrophic response of the liver to an increase in metabolic demand, with subsequent development of proliferative changes and hepatocellular toxicity. Historical control data from two other studies conducted at the same laboratory show that hepatocellular adenomas have been reported in control group mice of the same strain, sex and source (Charles River Laboratories, Portage, MI) in incidences ranging from 6/60 to 13/60.

Table: Supplementary Oncogenicity Study in CD-1 mice; Non-neoplastic and neoplastic lesions in the liver (see Table 62, CLH Report)

	Dose group level ppm (mg/kg bw/day)					
	Males			Females		
	01* (0)	02** (0)	1000 (135)	01 (0)	02 (0)	1000 (163)
Non-neoplastic lesions						
Necrosis, single cell	15	12	43a,b	4	9	8
Necrosis	7	7	17a,b	5	6	9
Pigment accumulation	10	7	26a,b	21	25	16
Hypertrophy, hepatocellular	9	15	55a,b	3	3	17a,b
Histiocytosis, sinusoidal	1	7	55a,b	3	4	6
Eosinophilic cell focus	1	3	3	0	0	5a,b
Neoplastic lesions						
Hepatocellular adenoma (benign)	4	5	14a,b	1	0	5a,b
Hepatocellular Carcinoma (malign)	2	3	4	0	0	0

* Control group 1; ** Control group 2

a statistically significant difference relative to control group 1 ($p<0.05$)
b statistically significant difference relative to control group 2 ($p<0.05$)

The CLH Report referred to the PRAPeR 79 (Pesticide Risk Assessment Peer Review) expert meeting (July, 2010), where the experts decided not to propose classification for metaldehyde with the risk phrase 'R40' (limited evidence of a carcinogenic effect) based on the hepatocellular adenomas found in CD-1 mice (majority after vote). A summary of a Position Paper from Herder *et al.* (2010) interpreted hepatocellular adenomas as a common finding in male mice of this strain, referred to the incidence range in the control mice, the lack of dose-dependence and questioned the biological relevance of this tumour type in mice.

Comments received during public consultation

One MSCA supported no classification and addressed the lack of mechanistic data.

Assessment and comparison with the classification criteria

Two chronic studies in CD-1 mice (from the same supplier) revealed increased incidences of hepatocellular adenomas in male mice. Statistical significance was not gained at 300 ppm (49 mg/kg bw/day) and was only seen in male mice at 1000 ppm (135 mg/kg bw/day) in a supplementary study of the same design. The study control groups showed lower incidences in this supplementary study (4/60 and 5/60) than in the first mouse study (8/60 in both control groups). The incidences in the study controls of the supplementary study were slightly below the given historical range (6/60 to 13/60) and thus the statistical significance may be explained by the rather low control incidences.

However, it was not documented whether the historical control ranges came from contemporary studies.

Based on the CLP Guidance, liver tumours in B6C3F1 mice were generally accepted as being an example of a mouse strain with high spontaneous tumour rates that limit the biological significance of substance-related increased incidences in carcinogenicity studies. The reported historical control range of 6/60 to 13/60 corresponds to findings in published data on CD-1 mice. From a total of 49 carcinogenicity studies from the 1990's to more recently conducted studies (until 2012) Charles River Laboratories reported spontaneous rates in CD-1 mice at 15% and about 3% in males females, respectively[1]. The same breeder reported in 1995 (close to the time of study on metaldehyde) ranges of 6-12% for males and 0-2% for females[2]. Chandra and Frith (1992) reported spontaneous incidences of 11% in 725 male mice and 1.8% in 725 female mice. These data show that at least a moderately high level of spontaneous liver adenomas are known for the CD-1 male mouse.

Increased incidences of hepatocellular adenomas in female CD-1 mice were statistically significantly increased at 1000 ppm only (5/60 in comparison to 0/60 and 1/60 in control groups), but no dose-related effect was seen at concentrations up to 300 ppm and the absolute incidence of adenoma at the high dose was low. Although information on the

[1] http://www.criver.com/files/pdfs/rms/cd1/act_2012_cd1_mice_carcinogenicity_study_data.aspx

[2] http://www.criver.com/files/pdfs/rms/cd1/cd1-mouse-tox-data-1995.aspx

spontaneous incidences in female mice at the source is not given, it remains uncertain whether 5/60 adenomas in female CD-1 mice at 1000 ppm should be considered to be treatment-related.

In female rats (see Table "Initial and peer reviewed liver histopathology findings in SD rats", above), there was a slightly higher incidence of liver cell adenomas in the high dose group in comparison to the internal control groups (Gill and Wagner, 1992). However, the incidence was only significantly different from one of the two control groups and did not show dose-dependence. A non-significantly increased incidence of liver cell carcinomas was seen in male rats from 50 ppm onwards. Tumour data were not presented in the CLH report from the second rat study (Verschuuren *et al*., 1975), which was of limited validity (low number of animals/group, summarised information only from a publication). According to the original publication, no tumours were observed in the livers of control and treated rats in this study.

No metaldehyde-related increase of hepatocellular carcinomas or on other types of tumours in the liver or elsewhere were observed in studies on mice. The slight increase in liver carcinomas in all dose groups in male rats were not significantly different from controls .In addition, no dose-response relationship was obvious, nor a clear increase in incidence over a rather large dose-range (one hundred-fold difference between the lowest and the highest dose). Thus, it may be considered questionable whether this is a treatment-related effect.

Few mechanistic data on the tumourgenesis of hepatocellular adenomas in CD-mice are available. Based on this information, it is assumed that metaldehyde is not acting via a genotoxic mechanism. Increased incidences of necrosis and single cell necrosis, as observed in the second supplementary mouse study, may be indicative of a regenerative proliferative response (however no hepatocellular hyperplasia was reported). The pigment accumulation and histiocytosis could be interpreted as secondary to cytotoxic effects. Verschurren *et al.* (1975) found increased activities of liver enzymes aniline hydroxylase ((AH) and aminopyrine demethylase (APDM).

A statistically significant increase in liver adenomas in CD-1 mice was observed. However, uncertainties about the causality to metaldehyde treatment remain as it cannot be excluded that the increase in liver adenomas in male mice resulted from low incidences in the laboratory control groups and whether the marginal increase in female mice were treatment-related at all. Hepatocellular adenomas did not progress to increased rates of malignant liver tumours in the concentrations tested up to 1000 ppm in CD-1 mice. These considerations do not support a firm conclusion that metaldehyde is carcinogenic in this species. The higher incidence in male mice than the marginal increase in female mice corresponds to the sex differences seen in untreated mice from the same breeder.

As to the rat data, RAC takes note of the overall limited data and uncertainties from the available studies. The significant increase in liver cell adenomas as seen in female rats at 5000 ppm in comparison to one of the two control groups together with the increased incidences of liver cell carcinomas in male rats could potentially justify a classification as a carcinogen. RAC is in particular concerned about the increased numbers of liver cell carcinomas in the male rat without any mechanistic explanation. Although no clear dose response relationship was seen in these dose groups (despite a very large dose range), their incidences were clearly (but not statistically significantly) above the control incidence. The information on historical control data given in the CLH report was not documented in sufficient detail to decide whether the observed control incidences were within the expected ranges. The CLH report referred to control data from two studies (60 rats/sex/group)

conducted at the laboratory using the same source of animals and concluded that the elevated incidences seen in female rats were within the historical background range. The timely relationship to the Gill and Wagner study (1992) is unknown and the overall amount of historical control animals (2 studies only, documented in Table 53 of the CLH report) was too small as a basis for comparison.

Moreover, RAC observes that the marked differences between the initial liver histopathology findings and the findings after internal peer review (Table 54 in the CLH report) created notable uncertainties. In addition, the incidences of liver tumours in Tables 53 and 54 of the CLH report showed inconsistencies. Definitions on the diagnostic terms and justifications why the differences occurred were missing.

The increased incidences and severity of liver cell hypertrophy in the mid and high dose groups did not give any hint on the mode of action. RAC notes the insufficient considerations and the lack of additional investigations on the mode of action.

The overall impression was that the reliability of the rat study and its documentation is limited. Whereas this leaves some concern on the slight increase in liver tumours, RAC in the end considers that due to the identified uncertainties and inconsistencies, the low reliability of the rat data does not allow a firm conclusion that metaldehyde has a carcinogenic potential in rats. Combining this with the similar conclusion for mice, RAC concludes that **no classification for carcinogenicity** is appropriate.

4.11 Toxicity for reproduction

Table 64: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Two generation study in CD rats	0, 50, 1000 and 2000 ppm/diet (equivalent to 0, 3.2, 65 and 134 mg/kg bw/d for males; 0, 4.0, 81 and 160 mg/kg bw/d for females) Reproduction NOAEL: 2000 ppm Parental NOAEL: 50 ppm Offspring NOAEL: 1000 ppm Parental and offspring toxicity effects at LOAEL: - reduction of bw and bw gain	-	Chun, J., Neeper-Bradley, T.; 1993
Two generation study in Wistar rats	0, 200, 1000 and 5000 ppm/diet (no information on actual test substance intake is presented in the publication)	No NOAELs are defined due to the limited reporting and the limited validity of this study.	Verschuuren, H. et al.; 1975
Developmental toxicity study in Sprague Dawley CD rats	0, 25, 75 and 150 mg/kg bw/d oral gavage Maternal NOAEL: 75 mg/kg bw/d Fetal NOAEL: 150 mg/kg bw/d Maternal effects at LOAEL: – mortality and clinical signs – reduced bw gain	-	Neeper-Bradley, T., Chun, J.; 1990
Dose range finding study in NZW rabbits	0, 50, 100, 200, 350 and 500 mg/kg bw/d oral gavage Clinical signs of maternal toxicity leading to premature death were already observed at the dose level of 100 mg/kg	This is a dose finding study. No NOAELs were defined due to limited fetal examinations.	Neeper-Bradley, T.; 1990
Developmental toxicity study in NZW rabbits	0, 10, 40 and 80 mg/kg bw/d oral gavage Maternal NOAEL: 80 mg/kg bw/d Fetal NOAEL: 80 mg/kg bw/d	-	Neeper-Bradley, T.; 1990

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Two two-generation studies were submitted. The newer study (*Chun J. and Neeper-Bradley T., 1993*) was conducted according to international guidelines and GLP and is considered valid. The older study (*Verschuuren H. et al., 1975*) was not conducted according to guidelines or GLP and is presented in the form of a publication. It shows several limitations like short reporting, missing individual numbers on several study parameters and limited statistical evaluations which do not allow to set sound and scientifically based NOAELs. Furthermore, this study was conducted with a test material from a different supplier and obviously not from the notifier. Therefore, the results from this study are of limited validity.

Reference:	**Two generation reproduction study in CD rats with metaldehyde administered in the diet**
Author(s), year:	Chun J. and Neeper-Bradley T., 1993
Report/Doc. number:	Bushy Run Research Center, Pennsylvania, USA Lonza Report No. 1544, Doc.No. 543-001
Guideline(s):	US EPA Guideline 83-4, OECD Guideline 416 (1983)
GLP:	Yes
Deviations:	No
Validity:	Valid

Material and Methods:

Groups of 28 male and 28 female outbred albino CD rats (source: Charles River Laboratories, Portage, MI, USA) received metaldehyde (batch no. 5448, purity > 99 %) in dietary concentrations of 0, 50, 1000 and 2000 ppm. The mean body weight range of F0 animals on the day of first treatment was 227.0 – 277.7 g for males and 164.3 – 165.3 g for females. The F0 animals were exposed to the diet for a prebreed period of 10 weeks. Following the prebreed period the F0 animals were randomly paired within dose groups for a 21-day period to produce the F1 generation. Exposure to the test diets continued through mating, gestation, parturition and lactation. After the F1 pups were weaned, the F0 females were sacrificed. F0 males were necropsied after delivery of the F1 litters. At weaning, 28 F1 weanlings/sex/group were randomly selected as parents of the next generation. The F1 weanlings were exposed to the same dietary concentrations of metaldehyde as their parents for at least 10 weeks. In addition, 10 F1 pups/sex/group were randomly selected for necropsy. After the prebreed period, the F1 animals were paired as described above to produce the F2 offspring. One week after weaning of F2 pups, 10 pups/sex/group were randomly selected for necropsy and the remaining F2 offspring were examined for gross external abnormalities, euthanized and discarded.

In-life evaluations: Parental animals were examined twice daily for mortality and overt signs of toxicity and once daily for any clinical signs of toxicity. Detailed clinical examination was conducted weekly. Body weights and food consumption were measured weekly. All pups from the F1 and F2 generations were examined as soon as possible on the day of birth (day 0) to determine the number of viable and stillborn pups per litter. Litters were evaluated twice daily for survival. On day 4 after birth, the size of each litter was adjusted by eliminating extra pups to four males and four females in each litter. Culled pups were examined externally, sacrificed and discarded. All pups were examined for abnormalities at birth and throughout the lactational period. All pups dying during lactation were necropsied when possible to investigate the cause of death.

Necropsy: Animals that died or were sacrificed prior to scheduled sacrifice were subjected to full necropsy as soon as possible after they were found. Adult males surviving the treatment period were examined externally and sacrificed after parturition of the litters. All surviving females were similarly examined and sacrificed after weaning of their offspring. Necropsy included examination of the external surfaces, all orifices, cranial cavity, carcass, external surfaces of the brain and spinal cord, the thoracic, abdominal and pelvic cavities and their viscera including reproductive organs, and cervical tissues and organs. In addition, spinal cords of adult animals that died or were sacrificed in a moribund condition were retained.

Histopathology: The following tissues from F0 and F1 parental animals from the control and high dose groups were examined histopathologically: spinal cord, vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, and tissues with gross lesions.

Dose finding study: The dose levels selected for this study were based on the results of a reproductive toxicity dose range finding study in CD rats conducted at dietary concentrations of 0,

625, 1250, 2500 and 5000 ppm metaldehyde. No treatment-related clinical signs were observed in males. One female from the high dose group was observed with hind limb paresis on day 10 and was sacrificed. Necropsy of this animal indicated luxation of the spine between the 9th and 10th thoracic vertebrae. Body weight and weight gain as well as food consumption tended to be reduced during prebreed period for all treated males and females. During gestation/lactation, six additional females from the high dose group were sacrificed due to hind limb paralysis/paresis. Necropsy of this animals indicated vertebral fractures/luxations and perivertebral hemorrhaging in all animals. In the last week of lactation, five females from the 2500 ppm group were observed with hind limb paralysis/paresis. These animals were also observed with vertebral fractures/luxations and/or perivertebral hemorrhaging. Pup body weights were reduced in the last two weeks of lactation in the 2500 and 5000 ppm groups presumably resulting from direct intake of the test diets.

Findings:

In-life observations: No treatment-related mortality or clinical signs of toxicity were observed in F0 and F1 males during the entire study period. In F0 females, no clinical signs were noted during prebreed, mating and gestation. During lactation on days 16-18, three females from the 2000 ppm group were sacrificed due to bilateral hind limb paralysis. In F1 females, no treatment-related mortality was observed. One F1 female (no. 13923) of the 50 ppm group that showed paresis on day 43 had lymphosarcoma and was sacrificed. One non-gravid female from the 2000 ppm dose group (no. 13973) showed prostration, tremors, abdominal breathing and rapid respiration on day 102 but survived to scheduled sacrifice on day 134.

Table 65: Reproductive toxicity study in CD rats; Clinical signs on behaviour / CNS (number of animals affected; earliest to latest day a finding was observed)

	Dose group level (ppm)			
	0	**50**	**1000**	**2000**
F0 females				
Paralysis (leg-hind-both)	-	-	-	3 (110-113)
Twitch (entire body)	-	-	-	1 (113)
F1 females				
Paresis (leg-hind-left)	-	1 (43)	-	-
Ataxia	-	-	-	1 (94)
Tremor, Prostration	-	-	-	1* (102)

* non-gravid female no. 13973

Reproduction parameters: No reproductive parameters including mating, fertility and gestational indices as well as gestational length were affected by treatment in both the F0 and F1 generation.

Table 66: Reproductive toxicity study in CD rats; Reproductive parameters

	Dose group level (ppm)			
	0	**50**	**1000**	**2000**
F0 generation				
No. F0 pairs	28	28	28	28
No. males impregnating females	28	27	26	28
No. plug / sperm-positive females	28	27	27	28
No. pregnant	25	26	25	27
No. males siring litters	25	25	24	26

	Dose group level (ppm)			
	0	**50**	**1000**	**2000**
No. live litters on postnatal day 0	25	25	24	27
Gestational length (days)	22.0 ± 0.4	22.1 ± 0.6	22.0 ± 0.6	22.0 ± 0.4
Mating index males[1)]	100	96.4	92.9	100
Mating index females[2)]	100	96.4	96.4	100
Fertility index males3[)]	89.3	92.6	92.3	92.9
Fertility index females[4)]	89.3	96.3	92.6	96.4
Gestation index[5)]	100	96.2	96.0	100
F1 generation				
No. F0 pairs	28	27[a)]	28	28
No. males impregnating females	28	26	27	27
No. plug / sperm-positive females	28	27	28	28
No. pregnant	27	24	23	24
No. males siring litters	27	22	23	23
No. live litters on postnatal day 0	27	22	23	22
Gestational length	22.0 ± 0.5	22.0 ± 0.7	22.0 ± 0.5	22.0 ± 0.6
Mating index males[1)]	100	96.3	96.4	96.4
Mating index females[2)]	100	100	100	100
Fertility index males[3)]	96.4	84.6	85.2	85.2
Fertility index females[4)]	96.4	88.9	82.1	85.7
Gestation index[5)]	100	91.7	100	91.7

[1)] Number of males impregnating females x 100 / Total number of males paired
[2)] Number of plug-/sperm-positive females x 100 / Total number of females paired
[3)] Number of males siring litters x 100 / Number of males impregnating females
[4)] Number of pregnant females x 100 / Number of plug-/sperm-positive females
[5)] Number of females with live litters x 100 / Number of females pregnant
[a)] One female was sacrificed on day 43 prior to pairing

F1 and F2 offspring: There were no treatment-related effects on litter size or sex ratio for the F1 and F2 pups. Pup body weight of the treated groups was similar to control values through the first four days of lactation. From lactation day 7-28, mean body weights were slightly reduced in the 2000 ppm groups in F1 and F2 pups. These reductions corresponded to a decreased body weight gain in this period of time. A single statistically significant decrease in pup weight gain (lactational day 7-14) in F2 pups receiving 50 or 1000 ppm was not considered to be biologically significant due to the small magnitude of effects and due to the fact that absolute body weight was not changed. There was no effect on pup viability and survival in F1 and F2 pups. While the number of dead pups appeared to be increased for the 2000 ppm dose group on day 21, all 24 of the dead pups were sacrificed due to concurrent maternal sacrifices on lactational days 16, 17 or 18. At necropsy, there were no gross lesions observed that were attributed to treatment.

Table 67: Reproductive toxicity study in CD rats; Pup body weight and body weight gain (g)

	Dose group level (ppm)							
	Males				**Females**			
	0	**50**	**1000**	**2000**	**0**	**50**	**1000**	**2000**
F1 pups								
Body weight								
Lactation day 1	7.27	7.50	7.39	7.34	6.85	7.00	6.99	6.84
day 7	17.78	17.88	17.59	17.10	16.90	16.69	16.82	16.03
day 14	36.51	36.44	37.07	34.52	34.97	34.40	35.70	33.04
day 21	59.67	59.41	60.37	56.61	56.76	55.56	57.60	53.79
day 28	104.19	104.40	105.25	99.90	94.89	93.39	95.24	90.56

	Dose group level (ppm)							
	Males				Females			
	0	50	1000	2000	0	50	1000	2000
Body weight gain								
Lactation day 1-4	3.65	3.75	3.59	3.57	3.50	3.54	3.48	3.39
day 4-7	6.86	6.62	6.60	6.19	6.54	6.17	6.35	5.80**
day 7-14	18.73	18.56	19.49	17.42	18.07	17.71	18.88	17.01
day 14-21	23.16	22.97	23.30	21.88	21.79	21.16	21.90	20.48
day 21-28	44.51	44.99	44.88	43.29	38.13	37.83	37.63	36.80
F2 pups								
Body weight								
Lactation day 1	7.52	7.75	7.21	7.03	7.15	7.14	6.77	6.74
day 7	17.57	17.98	17.34	16.95	17.08	17.19	16.55	16.21
day 14	37.16	36.62	35.59	34.47	36.61	35.04	34.49	33.23**
day 21	60.54	59.56	58.16	56.24**	58.63	56.49	55.94	53.93**
day 28	100.89	100.51	99.56	95.91	93.50	91.51	90.04	88.25
Body weight gain								
Lactation day 1-4	3.23	3.47	3.81	3.24	6.82	6.76	6.32	6.66
day 4-7	3.22	3.36	3.63	3.11	6.71	6.57	6.16	6.36
day 7-14	19.59	18.63	18.26	17.52	19.23	17.85*	17.94*	17.02**
day 14-21	23.38	22.94	22.56	21.77	22.32	21.45	21.45	20.71
day 21-28	40.35	40.95	41.40	39.29	34.87	35.03	34.10	34.31

* Significantly different from control group ($p < 0.05$)
** Significantly different from control group ($p < 0.01$

Necropsy and histopathology of F0 parental animals: There were no treatment-related necropsy findings for F0 males and females surviving to scheduled sacrifice. Necropsy of F0 females from the 2000 ppm dose group that were sacrificed due to hind limb paralysis indicated vertebral fractures or spinal cord luxations for two of the three females. Additional gross findings associated with spinal cord injury included dilation / distention of the urinary bladder and/or blood in the bladder. Microscopic examination for all three F0 females with hindlimb paralysis from the 2000 ppm group showed hemorrhage and/or necrosis of the spinal cord. In addition, minimal spinal cord hemorrhage was also observed microscopically for one female from the 2000 ppm group that survived to scheduled sacrifice without previous clinical signs of injury. There were no other treatment-related microscopic findings for F0 animals that survived to scheduled sacrifice.
Organ weights, necropsy and histopathology of F1 parental animals: In F1 males, relative liver weight was increased at the 2000 ppm dose level. In F1 females, both absolute and relative liver weights were increased for the 2000 ppm dose group. No necropsy or microscopic findings observed in F1 animals of either sex or any dose level were attributed to treatment with metaldehyde.

Two F1 females from the 50 ppm group were sacrificed due to clinical signs or found dead during the study.
Female (13920) showed no clinical signs but was found dead. Histopathology for this animal exhibited among other findings: meningitis, myelitis and hemorrhage of the spinal cord. However, these effects were in all probability a result of infection with septic emboli to various organs.

Female (13923) showed paresis and was sacrificed due to severe clinical signs on day 43. However, histopathology of this animal exhibited lymphosarcoma but no adverse histopathological findings of the spinal cord.

Table 68: Reproductive toxicity study in CD rats; Histopathology findings

	Dose group level (ppm)			
	Females			
	0	50	1000	2000
F0 females				

	Dose group level (ppm)			
	Females			
	0	**50**	**1000**	**2000**
Necropsy Paralysis Spinal cord: consistency change, shape/contour change, color change	 - -	 - -	 - -	 3/28[1)] 1/28[1)]
Histopathology Spinal cord: hemorrhage Spinal cord: necrosis	 - -	 - -	 - -	 3[1)] 2[1)]
F1 females				
Histopathology Spinal cord: hemorrhage, meningitis, myelitis Lymphosarcoma	 - -	 1[2)] 1[3)]	 - -	 - -

[1)] found in the three females that were sacrificed due to clinical signs of paralysis (leg-hind-both)
[2)] died of infection with septic emboli to various organs
[3)] the female was sacrificed due to clinical signs of paresis (leg-hind-left); autopsy revealed lymphosarcoma

Table 69: Reproductive toxicity study in CD rats; Histopathology findings in female adults sacrificed at week 19

	Dose group level (ppm)			
	Females			
	0	**50**	**1000**	**2000**
F0 females				
Histopathology Spinal cord: hemorrhage Spinal cord: vacuolization Spinal cord: neuronal vacuolization	 1 1 1	 - - -	 - - -	 1 0 2
F1 females				
Histopathology Spinal cord: hemorrhage	 1	 -	 -	 -

Conclusion:

No effects on reproduction were observed in this study. The NOAEL for reproductive toxicity therefore is larger than 2000 ppm (equivalent to 134 mg/kg bw/d in males and 160 mg/kg bw/d in females).

Systemic parental toxicity was evident in the high dose level (2000 ppm) when three females of the F0 generation developed paralysis (both hind legs) and were sacrificed moribund. Histopathology showed hemorrhage and necrosis of the spinal cord. One F1 female of the low dose group (50 ppm) showed signs of paresis of the left hind leg and was sacrificed. Autopsy revealed lymphosarcoma and hemorrhage, meningitis and myelitis. Though a connection to treatment cannot be excluded, this single case of paresis in the F1 females was not considered relevant for setting the NOAEL as no effects were observed at the two higher dose levels and the severity of the effect (paresis) was lower than in F0 females (paralysis). Body weight and body weight gain was affected only in F1 females. Statistically significant changes were observed at 1000 ppm and above during preebreeding, gestation and lactation. Absolute and/or relative liver weights were increased at the high dose level in both sexes. Offspring toxicity was demonstrated at the high dose level as body weight and body weight gain were decreased predominantely in F2 pups. In conclusion, the NOAEL for parental systemic toxicity was 50 ppm (equivalent to 3.2 mg/kg bw/d in males and 4.0 mg/kg bw/d in females) based on reduction of body weight. For offspring toxicity, the NOAEL was

1000 ppm (equivalent to 65 mg/kg bw/d in males and 81 mg/kg bw/d in females), again based on reduction of body weight and body weight gain.

4.11.1.2 Human information

Not available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Developmental toxicity studies according to GLP and international guidelines were conducted in rats and rabbits.

Reference:	**Developmental toxicity evaluation of metaldehyde administered by gavage to CD (Sprague Dawley) rats**
Author(s), year:	Neeper-Bradley T. and Chun J., 1990
Report/Doc. number:	Doc.No. 551-003, Lonza Report No. 1545 Conducting laboratory: Bushy Run Research Center, Pennsylvania, USA
Guideline(s):	US EPA Guideline 83-3 (1982), OECD Guideline 414 (1981)
GLP:	Yes
Deviations:	No
Validity:	Valid

Material and Methods:

Groups of 25 presumed pregnant rats (strain: CD of Sprague Dawley origin; source: Charles River Breeding Laboratories, Michigan, USA) received metaldehyde (batch no 5448; purity 99.0 %; suspended in corn oil) from day 6 to 15 of pregnancy by oral gavage at a constant dosing volume of 5 ml/kg bw. Dose levels were 0 (vehicle control), 25, 75 and 150 mg/kg bw/d. These dose levels were selected on the results of a preliminary study (reported in summary form only) with dose levels of 0, 50, 100, 150, 200 and 250 mg/kg bw/d given to groups of 6 pregnant rats. In this dose-range finding study, maternal toxicity including mortality was observed at dose levels ≥ 200 mg/kg bw/d. At 150 mg/kg bw/d, treatment-related clinical signs and reductions in body weight and food consumption were observed. There were no indications of developmental toxicity at any treatment level in this preliminary study.

Observations for mortality and clinical signs were made at least daily. Body weights were recorded on days 0, 6, 9, 12, 15, 18 and 21 of gestation, food consumption was measured at three-day intervals throughout gestation. On gestation day 21, females were subjected to a post-mortem macroscopic examination. The reproductive tract and abdominal and thoracic organs were examined grossly. In addition, maternal liver and uterine weights were determined. Further investigations included the number of corpora lutea/ovary, implantation and resorption sites, number and distribution of live and dead fetuses, weight and sex of each fetus and external malformed fetuses. Half of the fetuses from each litter were processed for the examination of visceral (thoracic and abdominal) abnormalities. These fetuses were then decapitated and their heads examined for abnormalities of craniofacial structures. The remaining half of fetuses were processed for skeletal staining and examined for skeletal malformations and variations. Dosing suspensions were prepared three times during the main study and were analyzed for test substance content prior to use.

Findings:

Maternal toxicity: Following 1 - 2 days of treatment, 6/25 dams at 150 mg/kg were found dead, which was considered related to treatment (all 6 dams were pregnant). In addition, at this dose level, treatment-related clinical signs of toxicity occured in the surviving rats during treatment period and included ataxia, tremor and rapid respiration. One of the six dams also showed paresis of both hind legs. No clinical signs were seen in the other dose groups. Maternal body weight was significantly reduced at 150 mg/kg on gestation day 9. A statistically significant reduction in mean body weight gain at 150 mg/kg was observed for the same period of time on gestation days 6-9 and 6-15. Also maternal food consumption was reduced in this group for the first three days of treatment (days 6-9), but increased subsequent to treatment (days 15-21) which can be considered a compensatory effect. No dams (at any dose level) aborted, delivered early or were removed from the study. At scheduled laparotomy, one dam each at 0, 25 and 150 mg/kg and three dams at 75 mg/kg were not pregnant. All remaining pregnant dams had one or more live fetuses at scheduled sacrifice (no litters were fully resorbed). 18 litters were available for examination at 150 mg/kg and 22-24 litters were available for examination at each of the other dose groups.

At necropsy, the pathological findings in the 6 dams which died before termination included perioral and perinasal wetness and encrustation, colour changes in the lungs, ulceration in the glandular and nonglandular portions of the stomach, blood and colour changes of the urinary bladder and distended intestines. Pathological findings in females of the high dose group considered to be related to treatment comprised hydronephrosis and dilated renal pelvis (3 dams) and paravertebral hemorrhages in the thoracic (2 dams) and lumbar region (1 dam). There were no significant treatment-related effects on uterine and liver weight at any dose level.

Table 70: Death, behaviour/CNS effects in dams at 150 mg/kg bw

Dam No	Treatment day	Gestation day	Behavirour/ CNS	Time of death (gestation day)	Lesion (probably CNS related)- gross examination
24533	1	6	Twitch, ataxia	Scheduled sacrifice	-
24599	1	6	Ataxia, twitch, tremor	7	Paravertebral hemorrhage thoracic area
24588	1	6	Tremor	Scheduled sacrifice	-
24591	2	7	Ataxia, hyperactive	8	-
24497	2	7	Ataxia	8	-
24466	1	6	Tremor, ataxia	7	-
24524	1	6	Twitch	Scheduled sacrifice	Paravertebral hemorrhage thoracic and lumbar areas
24472	2	7	-	7	-
24480	2	7	Ataxia, prostration, paresis	8	-

Litter data/fetal parameters: There were no significant effects of treatment on any gestational parameter. The number of corpora lutea, total implantations, pre- and postimplantation losses, sex ratio, and mean fetal and placental weights gave no indication of any response to treatment in any dose group. Examination of the fetuses at necropsy showed no treatment-related increases in individual external, visceral or skeletal malformations. The only finding in fetuses showing statistical significance was an increased incidence of bilobed centrum of the 12th thoracic vertebra - considered a skeletal variation - at 25 mg/kg dose group (39/158) when compared with controls (24/157), but not in the 75 (23/145) and 150 mg/kg dose groups (25/150). The incidence of all other variations (external, visceral or skeletal) were not significantly altered by the administration of metaldehyde.

Significant findings of this study are given in table 71.

Table 71: Rat oral developmental toxicity (teratogenicity) study; Summary of substantial study findings (group mean values)

Parameter	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg
No. on study	25	25	25	25
No. that died	0	0	0	6
No. examined at laparotomy	25	25	25	19
No. pregnant at laparotomy	24	24	22	18
Maternal body weight gain (g)				
day 0 - 6	25.97	26.98	29.93	26.23
day 6 - 15	34.53	35.88	31.31	25.41**
day 15 - 21	88.27	86.49	88.67	93.82
Food consumption (g/animal/day)				
day 0 - 6	20.94	21.19	21.60	21.02
day 6 - 15	19.40	19.57	19.10	16.91**
day 15 - 21	25.52	25.65	27.40*	27.33*
Total implants	14.5	14.3	15.3	14.0
No. of live fetuses (% males)	13.7 (46.4 %)	13.6 (48.7 %)	13.8 (54.4 %)	13.3 (46.4 %)
No. of early resorptions	0.9	0.7	1.5	0.7
No. of late resorptions	0	0	0	0.1
No. of dead fetuses	0	0	0	0
Fetal body weight per litter (g)	5.130	5.093	5.208	5.295
Total number of fetuses with malformations	10	10	12	10

** ($p \leq 0.01$), * ($p \leq 0.05$); significantly different

Conclusion:

The maternal NOAEL in this study can considered to be 75 mg/kg bw/d based on mortality and clinical signs, but also on slight reduction in body weight gain and food consumption during the treatment period at 150 mg/kg bw/d. Fetal survival and growth was not affected in any dose group. In addition, there was no teratogenic effect observed. Therefore the fetal NOAEL can be set at 150 mg/kg bw/d.

Reference:	**Developmental toxicity dose range-finding study of metaldehyde administered by gavage to New Zealand White rabbits**
Author(s), year:	Neeper-Bradley T., 1990a
Report/Doc. number:	Doc.No. 551-001, Lonza Report No. 1503 Conducting laboratory: Bushy Run Research Center, Pennsylvania, USA
Guideline(s):	Dose range finding study according to US EPA Guideline 83-3 (1982) and OECD Guideline 414 (1981)
GLP:	Yes
Deviations:	low animal number and fetal examinations restricted to external malformations and variations
Validity:	Valid

Material and Methods:

The objective of this study was to determine the appropriate dose levels of metaldehyde for a subsequent definitive developmental toxicity study in New Zealand White rabbits. Five mated females per group (source: Hazelton-Dutchland Laboratories Inc., Denver, PA, USA) received metaldehyde (batch no. 5448, purity 99.0 %, suspended in corn oil) from gestation days 6 to 18 by oral gavage at a dosing volume of 2 ml/kg. The dose levels were 0 (vehicle control), 50, 100, 200,

350 and 500 mg/kg bw/d. Dosing suspensions were prepared twice times during the main study and were analyzed for test substance content prior to use.

Observations for mortality and clinical signs were made twice daily during the treatment period and daily throughout the rest of the study. Body weights were recorded on days 0, 6, 13, 19, 24 and 29 of gestation. All surviving females were sacrificed on gestation day 29 and subjected to a macroscopic examination. The reproductive tract and abdominal and thoracic organs were examined grossly. Maternal liver and uterine weights were determined. Further investigations included the number of corpora lutea, implantation and resorption sites, number and distribution of live and dead fetuses, fetal body weight per litter, external malformations and variations.

Findings:

Maternal toxicity: Following 1 - 2 days of treatment, 4/5 does at 500 mg/kg and 2/5 does at 350 and 200 mg/kg were found dead. 1/5 females receiving 100 mg/kg died on gestation day 12. All of those females were pregnant. The remaining females of the 200, 350 and 500 mg/kg dose groups were sacrificed on gestation day 8 due to the severity of clinical signs. All euthanized females were pregnant with the exception of one at 200 mg/kg. Characteristic clinical signs of these dose groups were ataxia, whole body twitching, tremors and rapid respiration. Rapid respiration was observed during treatment of does receiving 100 mg/kg. One doe of the 50 mg/kg group developed abdominal breathing and rapid respiration on gestation day 16 and aborted on gestation day 26. Of the 13 does surviving to the scheduled sacrifice, 2 does (one each at 0 and 100 mg/kg) were nonpregnant. Four litters were evaluated at 0 and 50 mg/kg and three litters were evaluated at 100 mg/kg.

There were no statistically significant effects on maternal body weight and body weight gain. However, food consumption was reduced from gestation days 6-7 in females receiving 100, 200 and 350 mg/kg. At necropsy, some of the does at 100, 200, 350 and 500 mg/kg which died prior to scheduled sacrifice exhibited broken vertebrae and gastrointestinal tract lesions, including color changes and ulcerations of the glandular stomach and distended intestines with no evidence of technical dosing error. In addition, color changes of the liver and ulcerations and color changes in the nonglandular portion of the stomach were observed at doses 100 mg/kg and above. Does surviving to scheduled sacrifice on gestation day 29 did not exhibit any treatment-related gross lesion. Maternal organ weights at scheduled sacrifice showed no statistically significant differences among groups. There were no apparent differences in corrected body weight or corrected gestational body weight change (corrected for gravid uterine weight). Relative liver weight was non statistically significant increased at 100 mg/kg.

Table 72: Death, behaviour/CNS effects in dams

Dam No	Dose group (mg/kg bw)	Treatment day	Gestation day	Behavirour/ CNS	Time of death (gestation day)	Lesion (probably CNS related)- gross examination
9896	100	3	9	Hypoactive	11	-
9888	100	1/ 9-10	6/ 15-16	Tremor (6), head tilt (15-16)	Scheduled sacrifice	-
9891	200	2	7	tremor	8 (euthanized, sponsor requested)	-
9892	200	2	7	Ataxia, twitch, paresis, tremor	8	Vertebral column broken lower lumbar region
9902	200	1	6	Tremor, ataxia	7	-
9889	350	2	7	Tremor, tonic convulsions,	7	Vertebral column broken

				prostration, apparent broken back		
9909	350	1	6	Tremor, ataxia,	7	-
9918	500	1	6	Tremor, twitch	7	Vertebral column broken near first lumbar vertebra
9913	500	1	6	Ataxia, tremor, twitch	7	Vertebral column broken near first lumbar vertebra
9887	500	1	6	Ataxia	7	-
9900	500	1/2	6/7	Tremor (6), ataxia (7)	7 (euthanized, sponsor requested)	-
9890	500	1	6	Twitch, ataxia	7	-

Litter data/fetal parameters: Gestational parameters for does at scheduled sacrifice were approximately equivalent across all groups from 0 to 100 mg/kg, with no statistically significant changes in the number of corpora lutea, live fetuses, early or late resorptions, dead fetuses, implantations, percent live fetuses per litter or sex ratio. While there were greater numbers of live fetuses at 50 and 100 mg/kg, due to the larger number of total implants, the number of non-viable implants was also increased. The apparent increase in the number of dead fetuses was most probably due to the greater number of total implants. Fetal body weights per litter were equivalent across groups. There were no external malformations or variations observed in any fetus of this study.

Significant findings of this study are given in table 73.

Table 73: Rabbit oral developmental dose finding study; Summary of substantial study findings (group mean values)

Parameter	**0 mg/kg**	**50 mg/kg**	**100 mg/kg**	**200 mg/kg**	**350 mg/kg**	**500 mg/kg**
No. on study	5	5	5	5	5	5
No. found dead	-	-	1	2[a)]	2[a)]	4[a)]
Abortions	-	1	-	-	-	-
No. examined at laparotomy	5	4	4	-	-	-
No. pregnant at laparotomy	4	4	3	-	-	-
Total implants	5.3	10.8	9.0	-	-	-
No. of live fetuses	5.0	9.5	7.7	-	-	-
No. of early resorptions	0	0.5	0.7			
No. of late resorptions	0	0	0	-	-	-
No. of dead fetuses	0.2	0.7	0.7			
Fetal body weight per litter (g)	45.00 (n=4)	40.18 (n=4)	40.21 (n=3)	-	-	-

[a)] the remaining females were sacrificed on gestation day 8

** ($p \leq 0.01$), * ($p \leq 0.05$); significantly different

Conclusion:

In this dose range finding study in NZW rabbits, severe maternal toxicity was observed at the high dose levels of 100 mg/kg and above. Clinical signs like ataxia, whole body twitching, tremors and rapid respiration were observed, leading to premature death of 1/5, 2/5, 2/5 and 4/5 females of the 100, 200, 350 and 500 mg/kg dose group. At necropsy, broken vertebrae and gastrointestinal tract lesions were found in some of these animals. The remaining females receiving 200, 350 or

500 mg/kg were euthanized due to clinical signs on gestation day 8. Treatment rapid respiration was also observed in one doe of the 50 mg/kg dose group, which aborted on gestation day 26.

There were no indications of fetal toxicity in this study. Also, no external malformations or variations were observed. No examination for visceral anomalies or detailed skeletal examination were performed.

Based on the effects indicative for maternal toxicity at 100 mg/kg bw/day (mortality and clinical signs) and the slight effect observed at 50 mg/kg (abdominal breathing and rapid respiration in one dam), the doses for the main study were selected as 0, 10, 40 and 80 mg/kg bw/d. No NOAELs are set for this dose finding study. Due to low animal number and missing detailed fetal investigations this study is of supplementary information only.

Reference:	**Developmental toxicity evaluation of metaldehyde administered by gavage to New Zealand White rabbits**
Author(s), year:	Neeper-Bradley T., 1990b
Report/Doc. number:	Doc.No. 551-002, Lonza Report No. 1504 Conducting laboratory: Bushy Run Research Center, Pennsylvania, USA
Guideline(s):	US EPA Guideline 83-3 (1982), OECD Guideline 414 (1981)
GLP:	Yes
Deviations:	No
Validity:	Valid

Material and Methods:

16 mated females per group (source: Hazelton-Dutchland Laboratories Inc., Denver, PA, USA) received metaldehyde (batch no. 5448, purity 99.0 %, suspended in corn oil) from gestation days 6 to 18 by oral gavage at a dosing volume of 2 ml/kg. The dose levels of 0 (vehicle control), 10, 40 and 80 mg/kg bw/d were selected on the basis of a dose finding study (Neeper-Bradley T., 1990a), when maternal mortality was observed at a dose level of 100 mg/kg. Dosing suspensions were prepared three times during the study and were analyzed for test substance content prior to use.

All females were thoroughly examined daily for clinical signs of toxicity (twice daily during dosing period). In addition, the animals were examined twice daily for mortality and morbidity. All females on study were weighed on gestation day 0, 6, 13, 19, 24 and 29. Food consumption was recorded daily. All surviving females were sacrificed on gestation day 29 and subjected to a macroscopic examination. The reproductive tract and abdominal and thoracic organs were examined grossly. Maternal liver and uterine weights were determined. Further investigations included the number of corpora lutea, implantation and resorption sites, number and distribution of live and dead fetuses and fetal body weight per litter. All fetuses were examined for external malformations and variations, visceral anomalies and skeletal malformations and variations. Half of the fetuses were also examined for soft tissue craniofacial structures.

Findings:

Maternal toxicity: Pregancy rate was approximately equivalent across all groups ranging from 75 – 100 %. One doe receiving 40 mg/kg was found dead on gestation day 17, however, no reason for this death is explained in the study report. Necropsy of this female showed color changes of liver and spleen and blood in the urinary bladder, while histopathology revealed no abnormalities. There were no clearly treatment-related clinical signs of toxicity observed in any of the treatment groups, though one female of the 80 mg/kg dose group developed rapid respiration during the treatment period. There were no statistically significant differences among groups for maternal body weight,

body weight gain or food consumption. Necropsy findings in does surviving to the scheduled sacrifice were not considered to be treatment-related. There were no apparent differences among groups in gravid uterine weight, corrected body weight or body weight change. While not statistically significant, there appeared to be dose-dependent increases in liver weight and relative liver weight. However, at the 80 mg/kg dose, liver weights were only increased by 6 %. Gestational parameters showed no statistically significant changes in the number of corpora lutea, live fetuses, early or late resorptions, dead fetuses or sex ratio.

Fetal examination: No statistical changes in fetal body weights per litter were observed. There were no treatment-related increases in the incidence of individual fetal malformations by category (external, visceral, skeletal) or of total malformations observed at any dose level in this study. There were also no treatment-related increases in the incidence of individual fetal variations, by category or total, observed.

Significant findings of this study are given in table 74.

Table 74: Rabbit oral developmental toxicity (teratogenicity) study; Summary of substantial study findings (group mean values)

Parameter	0 mg/kg	10 mg/kg	40 mg/kg	80 mg/kg
No. on study	16	16	16	16
No. that died	0	0	1	0
No. examined at laparotomy	16	16	15	16
No. pregnant at laparotomy	12	13	13	16
Maternal body weight gain (g)				
day 0 - 6	123	113	124	132
day 6 - 19	14	-103	-164	-1
day 19 - 29	279	312	340	326
Food consumption (g/animal/day)				
day 0 - 6	189	169	181	184
day 6 - 19	92	81	70	79
day 19 - 29	155	147	152	182
Liver weight				
-absolute	95	95	98	100
-relative to body weight	2.73	2.75	2.81	2.87
Total implants	10.8	9.8	9.4	10.4
No. of live fetuses	9.3	8.0	8.2	8.7
No. of early resorptions	0.2	1.2	0.3	0.7
No. of late resorptions	0.2	0.1	0.2	0.1
No. of dead fetuses	1.1	0.6	0.7	0.7
Fetal body weight per litter (g)	40.22	41.09	39.25	40.64
Total % fetuses with malformations (external, soft tissue, skeletal)	2.7	1.9	0.9	4.3
Total % fetuses with variations (external, soft tissue, skeletal)	99.1	99.0	100	99.3

** ($p \leq 0.01$), * ($p \leq 0.05$); significantly different

Conclusion:

In this study, no maternal toxicity was observed at the dose levels employed (0, 10, 40 and 80 mg/kg bw/d). The lack of maternal toxicity at least at the high dose level is in contrast to the dose range finding study, when clear toxicity resulting in the death of 1/5 females was observed at the dose level of 100 mg/kg. Rapid respiration, a characteristic sign of toxicity in the dose range finding study, was noted in one female at 80 mg/kg in the actual study, which suggests that the dose of 80 mg/kg was just below the level of clear maternal toxicity in the population of rabbits in the actual study. Obviously, metaldehyde has a very steep dose – effect relationship regarding acute

toxic effects including mortality, that is why the study is acceptable for evaluation of the teratogenic potential of this test substance even when no maternal toxicity was observed. In conclusion, the maternal NOAEL of this study was set at 80 mg/kg bw/d. Fetal survival and growth were not affected in any dose group. In addition, there was no teratogenic effect observed. Therefore the fetal NOAEL is greater than 80 mg/kg bw/d.

4.11.2.2 Human information

Not available.

4.11.3 Other relevant information

Not available.

4.11.4 Summary and discussion of reproductive toxicity

Two two-generation studies were submitted. The newer study (*Chun J. and Neeper-Bradley T., 1993*) was conducted according to international guidelines and GLP and is considered valid. The older study (*Verschuuren H. et al., 1975*) was not conducted according to guidelines or GLP and is presented in the form of a publication. It shows several limitations like short reporting, missing individual numbers on several study parameters and limited statistical evaluations which do not allow to set sound and scientifically based NOAELs. Furthermore, this study was conducted with a test material from a different supplier and obviously not from the notifier. Therefore, the results from this study are of limited validity.

In the newer two generation study conducted in CD rats, no effects on reproductive performance were noted throughout the study. The NOAEL for reproductive toxicity therefore is higher than the highest dose tested: > 2000 ppm (equivalent to 134 mg/kg bw/d in males and 160 mg/kg bw/d in females). Systemic parental toxicity was evident at the high dose level (2000 ppm) when three females of the F0 generation developed paralysis (both hind legs) and were sacrificed moribund. Histopathology showed hemorrhage and necrosis of the spinal cord. Such findings were also observed in the older two generation study, where 50-75% of the females of the 5000 ppm dose group and up to three females of the 1000 ppm dose group developed posterior paralysis with transverse lesions of the spinal cord. None of the males was affected in any generation in both studies. The finding of hind limb paralysis was also observed in repeated dose toxicity studies at high dose levels, however, the increased body weight of the pregnant females may have promoted this effect. Further signs of systemic toxicity were reduced body weight in F1 females receiving 1000 or 2000 ppm and increased liver weight in both sexes receiving 2000 ppm. Offspring toxicity was evident at 2000 ppm and resulted in decreased body weight and body weight gain. In conclusion, the NOAEL for parental systemic toxicity was 50 ppm (equivalent to 3.2 mg/kg bw/d in males and 4.0 mg/kg bw/d in females) based on reduction of body weight. For offspring toxicity, the NOAEL was 1000 ppm (equivalent to 65 mg/kg bw/d in males and 81 mg/kg bw/d in females), again based on reduction of body weight and body weight gain.

In the rat study, severe maternal toxicity was observed at the highest dose level of 150 mg/kg, including ataxia, tremor, rapid respiration, paresis of hind legs, and finally mortality. In these animals, necropsy revealed hydronephrosis, dilated renal pelvis and paravertebral hemorrhages. Also body weight and body weight gain as well as food consumption were slightly reduced in the dams of the high dose group. No effects regarding maternal toxicity were observed at the low and mid dose of 25 and 75 mg/kg. No effects on gestational parameters or on fetal toxicity were observed at any dose. Also, no evidence of teratogenicity was observed in this study. In conclusion,

the NOAEL for maternal toxicity in rats was 75 mg/kg bw/d and the NOAEL for fetal toxicity and teratogenicity was greater than 150 mg/kg bw/d.

The teratogenicity of metaldehyde in rabbits was investigated in one dose finding study and in the main study. In the dose finding study, severe maternal toxicity was observed at dose levels of 100 mg/kg and above, leading to ataxia, tremors, whole body twitching, rapid respiration and finally to death following 1-2 days of treatment. The remaining females of the high dose groups (200, 350 and 500 mg/kg) were sacrificed on gestation day 8 due to the severity of clinical signs. At necropsy, some of the high dose females showed broken vertebrae and gastrointestinal lesions, including color changes and ulcerations of the glandular stomach and distended intestines. All animals surviving to the scheduled sacrifice did not exhibit any treatment-related gross lesion. One female of the 50 mg/kg dose group of the dose finding study developed rapid respiration during dosing period, which was regarded to be related to treatment. There was no effect on body weight of the dams and the fetuses. Limited fetal examinations showed no external malformations or variations. Based on the findings of this study, dose levels of 10, 40 and 80 mg/kg bw/d were set for the main study. However, in the main study no maternal toxicity occurred. Rapid respiration, a characteristic sign of toxicity in the dose range finding study, was noted in one female at 80 mg/kg in the main study, which suggests that the dose of 80 mg/kg was just below the level of clear maternal toxicity in the population of rabbits in the main study. Obviously, metaldehyde has a very steep dose-effect-relationship regarding acute toxic effects including mortality. Therefore the study is acceptable for evaluation of the teratogenic potential of this test substance even if no maternal toxicity was observed. No effects on gestational parameters, fetal survival and growth were observed. There was no evidence of teratogenicity. In conclusion, the maternal and fetal NOAEL for developmental toxicity in rabbits was 80 mg/kg bw/d.

4.11.5 Comparison with criteria

In a valid two generation study conducted in CD rats, no effects on reproductive performance were noted throughout the study. Systemic parental toxicity was evident at the high dose level (2000 ppm) when three females of the F0 generation developed paralysis (both hind legs) due to transverse lesions of the spinal cord. Further signs of systemic toxicity were reduced body weight in F1 females in the mid and high dose group (receiving 1000 or 2000 ppm, respectively) and increased liver weight in both sexes in the high dose group. Offspring toxicity was evident only in the high dose group and resulted in decreased body weight and body weight gain. In conclusion, the NOAEL for reproductive toxicity is > 134 mg/kg bw/d, the NOAEL for parental systemic toxicity is 3.2 mg/kg bw/d (based on reduction of body weight) and the NOAEL for offspring toxicity is 65 mg/kg bw/d (based on reduction of body weight and body weight gain).

In the 26 and 52 week dog toxicity studies diffuse atrophy of the testes and/or degeneration of the germinative epithelium were observed from 60 mg/kg bw/d onwards. However as fertility was not affected in the two generation study this effect was not considered relevant for reproductive toxicity.

In the rat developmental toxicity study severe maternal toxicity was observed at the highest dose level of 150 mg/kg, including mortality, clinical signs and reduced body weight and body weight gain. No effects regarding maternal toxicity were observed at the low and mid dose. No effects on gestational parameters or on fetal toxicity were observed at any dose. In conclusion, the NOAEL for maternal toxicity in rats was 75 mg/kg bw/d and the NOAEL for fetal toxicity and teratogenicity was greater than 150 mg/kg bw/d. In the main developmental toxicity study in rabbits no maternal toxicity as well as no effects on gestational parameters and development were observed up to the highest dose tested of 80 mg/kg bw/d.

Taken together, no specific effects on fertility and no developmental toxicity was observed following metaldehyde administration to test animals. No classification is proposed.

4.11.6 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: no classification proposed

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on fertility

Two two-generation studies were submitted. The newer study (*Chun and Neeper-Bradley, 1993*) was conducted according to international guidelines and GLP and is considered valid. The older study (Verschuuren *et al., 1975*) was not conducted according to guidelines or GLP.

In the newer two generation study (OECD TG 416) conducted in CD rats, no effects on reproductive performance (see Table 66, CLH report) were noted throughout the study. The NOAEL for reproductive toxicity therefore is proposed to be higher than the highest dose tested: > 2000 ppm (equivalent to 134 mg/kg bw/day bw/day in males and 160 mg/kg bw/day in females). Systemic parental toxicity was evident at the high dose level (2000 ppm) when three females of the F0 generation developed paralysis (both hind legs) and were sacrificed moribund. Histopathology showed haemorrhage and necrosis of the spinal cord. Such findings were also observed in the older two generation study (Verschuuren *et al*., 1975), where 50-75% of the females of the 5000 ppm dose group and up to three females of the 1000 ppm dose group developed posterior paralysis with transverse lesions of the spinal cord. None of the males was affected in any generation in either study. The finding of hind limb paralysis was also observed in repeated dose toxicity studies at high dose levels, however, the increased body weight of the pregnant females may have contributed to this effect. Further signs of systemic toxicity were reduced body weight in F1 females receiving 1000 or 2000 ppm and increased liver weight in both sexes receiving 2000 ppm. Offspring toxicity was evident at 2000 ppm and resulted in decreased body weight (day 14 and day 21) and body weight gain (day 7-14). In conclusion, the NOAEL for parental systemic toxicity was 50 ppm (equivalent to 3.2 mg/kg bw/day in males and 4.0 mg/kg bw/day in females) based on reductions in body weight. For offspring toxicity, the NOAEL was 1000 ppm (equivalent to 65 mg/kg bw/day in males and 81 mg/kg bw/day in females), again based on the reductions in body weight and body weight gain.

In the 26 week (Neumann, 1980, 1991; Leuschner, 2009) and 52 week dog toxicity studies (Leuschner, 2002; Leuschner, Drommer, 2006), diffuse atrophy of the testes and/or degeneration of the germinative epithelium were observed from 60 mg/kg bw/day onwards. However as fertility was not affected in the two generation study in rats this effect in dogs was not considered relevant for reproductive toxicity, but should be covered by classification as STOT RE 2.

Developmental effects

In the rat developmental toxicity study (OECD TG 414; Neeper-Bradley, Chun, 1990) severe maternal toxicity was observed at the highest dose level of 150 mg/kg bw/day, including mortality, clinical signs and reduced body weight and body weight gain. No effects regarding maternal toxicity were observed at the low and mid dose. No effects on gestational parameters or on foetal toxicity were observed at any dose. In conclusion, the NOAEL for maternal toxicity in rats was 75 mg/kg bw/day and the NOAEL for foetal toxicity and teratogenicity was greater than 150 mg/kg bw/day.

In the main developmental toxicity study in rabbits (Neeper-Bradley, 1990b) no maternal toxicity or effects on gestational parameters and development were observed up to the highest dose tested of 80 mg/kg bw/day.

The DS concluded that no specific effects on fertility and no developmental toxicity was observed following metaldehyde administration to test animals. No classification was proposed.

Comments received during public consultation

One MSCA agreed with the proposal for no classification.

Additional key elements

Additonal information on the 52 week study in dogs:

The original monograph states that clinical signs of toxicity (ataxia, tremor, etc., declining after week 19) were only seen at 90 mg/kg in the dogs of the 52 week study. Mortalities (without any premortal signs) were observed at 30 mg/kg bw/day (1 F (with pneumonia) and 1 M) and at 90 mg/kg bw/day (1 F (with pneumonia) of 4 dogs) from week 37 onwards (on days 260-322).

Mortality and testis toxicity was observed in one dog (Nr. 18), while minimal to mild testis toxicity or atrophy of the prostate was observed (without lethality) in two other dogs (No. 20, No. 19). In conclusion, testis (or prostate) toxicity was observed in 2 of 4 treated males at 30 mg/kg without any evidence of systemic toxicity. A coincidence of mortality and testis toxicity was observed in another dog at this dose.

At 10 mg/kg bw/day 1 dog (Nr. 12) had a juvenile testis (considered as spontaneous lesion) and mild testis atrophy was seen another dog (Nr. 10). No testis effects were seen in the control dogs.

The overall incidences (sum of atrophy/degeneration in testis or prostate)increased with dose (1/4 at 10 mg/kg, 3/ at 30 mg/kg and 4/4 at 90 mg/kg). Indications of systemic toxicity were evident at 90 mg/kg bw/day, possibly the cause of one premature death at 30 mg/kg. The severity grades of testis effects were minimal to mild in dogs at 10 and 30 mg/kg bw/day, except the one that died earlier with moderate testis toxicity.

The CLH dossier referred to a position paper (Leuschner and Drommer, 2006) which after re-assessment of the findings came to the conclusion that the findings at 10 and 30 mg/kg

bw/day were of spontaneous nature and presented historical background information on studies from 2003-2006 from the laboratory (see the STOT RE section).

Assessment and comparison with the classification criteria

RAC agreed with the DS that no classification on developmental toxicity is warranted.

Regarding fertility, the concern from testicular toxicity in dogs has to be assessed together with the clinical signs of toxicity (ataxia, tremor) seen at 90 mg/kg and the late deaths at 30 and 90 mg/kg bw/day. While no mortality occurred in male dogs at 90 mg/kg during 52 weeks of exposure (a female dog at this dose died with pneumonia), one male dog at 30 mg/kg that died prematurely showed moderate testis toxicity. However, 2 other dogs with minimal to mild testes (prostate) toxicity did not show any other signs of toxicity. This indicates that testis toxicity was a direct effect of metaldehyde treatment.

The evidence for testis toxicity was supported by the findings of increased severity of focal testis atrophy at 60 mg/kg bw/day and increased severity and incidence of bilateral diffuse atrophy in 4 out of 6 male dogs at 90 mg/kg bw/day treated for 26 weeks. As no clinical symptoms or other signs of general toxicity were reported for doses up to 90 mg/kg bw/day, these effects were considered as direct effects of exposure to metaldehyde.

RAC discussed the data in the light of the given concern on fertility dysfunction and the overall data available that may warrant classification for reproductive toxicity, category 2, for effects on fertility or no classification. Since there was no evidence that the testis toxicity seen in dogs is less relevant than the (negative) data from rats, that did not show testis toxicity in the repeated dose studies and where no impact on fertility parameters were seen in the two-generation study, RAC considers that the testis toxicity seen in dogs is relevant and may pose a hazard to exposed humans. Toxicokinetic data that could be helpful to identify species differences and peculiarities were only available for the rat. Thus the testis toxicity observed in dogs in the absence of general toxicity supports the need for classification as toxic for reproduction, category 2, for effects on fertility.

RAC therefore concluded that **classification as Repr. 2; H361f is warranted**.

A specific concentration limit (SCL) was not proposed by the DS, nor was it discussed by RAC. As the effective dose levels (and their ED_{10}) could be estimated to be above 4 mg/kg bw/day, there was no need to further discuss a SCL.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Table 75: Summary table of relevant neurotoxicity studies

Method	Results	Remarks	Reference
Acute neurotoxicity study in rats	0, 75, 150 and 250 mg/kg bw by gavage NOAEL = 75 mg/kg bw Effects at LOAEL: transient clinical signs and findings in neurological screening	-	Haferkorn, J.; 2009
Acute neurotoxicity study in rats	0, 75, 150 and 250 mg/kg bw by gavage NOAEL = 75 mg/kg bw Effects at LOAEL: findings in neurological screening	-	Herberth, M.T., 2011
90-day repeated dose oral neurotoxicity study in rats	0, 100, 500 and 2500 ppm/diet (equivalent to 0, 8, 39 and 185 mg/kg bw/d)	-	Jones, L., Finn, J., Mullee, D.; 2003
90-day dietary combined toxicity and neurotoxicity study in Sprague-Dawley rats	0, 250, 750 and 2500 ppm/diet (equivalent to 0, 13.22, 39.3 and 132.62 mg/kg bw/day in male and 0, 15.23, 46.85 and 149.79 mg/kg bw/day in female)	-	Gauvin, G.V., 2010
Relevant aspects of neurological effects associated with exposure to Metaldehyde	Neurofunctional effects of metaldehyde are consequences of acute toxicity; no classical neurotoxin	Position paper	Harder, V., Roth, T., Hofer, M.; 2010

Reference:	**Acute neurotoxicity study in rats by oral administration of metaldehyde**
Author(s), year:	Haferkorn J., 2009
Report/Doc. number:	LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany; LPT Report No. 23443, Doc. No.: 541-002
Guideline(s):	EC method B.43; OECD Guideline 424; OPPTS 870.6200
GLP:	Yes
Deviations:	No
Validity:	Yes

Material and methods:

Test material	META Metaldehyde techn.
Lot/Batch	38337
Purity	99.6%
Vehicle	Corn oil
Species	Rat, males and females
Strain	CD, Crl:CD(SD)
Age	Males: 44-47 days, Females: 50-53 days
Weight at dosing	Males: 178-228 g; Females: 159-198 g
Source	Charles River Laboratories, Sulzfeld, Germany
Number of animals	40 males, 40 females (10 animals/sex/group)

Metaldehyde was administered orally by gavage as single administration. Three dose levels and one control group were tested. Dose level groups of 20 (10 male and 10 female) animals each were used for functional testing and detailed clinical observations. 10 (5 male and 5 female) animals from each dose level group were selected for perfusion in situ and neurohistopathology. In case animals showed neurotoxic effects those animals were included in the subgroup selected for perfusion in situ and neurohistopathology. At the start of the experiment the animals were weighted and assigned to the groups by randomization. The animals were kept singly in Makrolon cages.

Table 76: Acute neurotoxicity study: Study design

Group	Metaldehyde dose (mg/kg bw, p.o.)	Number and sex of animals
1	0 Control	10 m 10 f
2	75 (low dose)	10 m 10 f
3	150 (intermediate dose)	10 m 10 f
4	250 (high dose)	10 m 10 f

Test item stability, its concentration and homogeneity in the vehicle was shown to be adequate in a separate study (Flügge, 2009, Doc. No. 411-002).

Observations:

General health condition: All animals were checked at least once daily at the same time for clinical signs. Daily cage-side observations included skin/fur, eyes, mucous membranes, respiratory and circulatory systems, somatomor activity and behaviour patterns.

Mortality/Morbidity: All animals were checked at least twice daily for signs of morbidity or mortality. Premortal symptoms were recorded in detail; as soon as possible after exitus, a post mortem examination was performed.

Body weight: The weight of each rat was recorded on the day of treatment and weekly thereafter throughout the experimental period.

Food and water consumption: The quantity of food left by individual animals was recorded on a weekly basis. Drinking water consumption was monitored daily by visual appraisal throughout the study.

Detailed clinical observations were made before the administration, 2 and 8 hours after administration and during the 14 day recovery period at days 7 and 14 after dosing. These observations were made outside the home cage in a standard arena and at the same time. Signs noted included changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, pilo-erection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation, walking backwards) would have been recorded using the scoring systems that include criteria or scoring scales for each measurement in the observations.

Functional tests:

The functional tests were performed before dosing, at 2 and 8 hours after administration and 7 and 14 days after dosing in all animals.

Righting reflex

The animal was grasped by its tail and flipped in the air (approx. 60 cm) above the cart surface so that it turned head over heels. The normal animal should land squarely on its feet in which case the score sheet space was given as '0'. If it landed on its side, 1 point was scored; if it landed on its back, 2 points were scored. This test was repeated five times and the total scores were recorded.

Body temperature

An electronic probe thermometer (with a blunt probe) was used to take a rectal temperature, being allowed to equilibrate for 30 seconds before the reading was recorded.

Salivation

Discharge of clear fluid from mouth, most frequently seen as beads of moisture on lips in rats. Normal state was to see none, in which case the score sheet space was given as '0'. If present, a plus sign was recorded.

Startle response

With the animal on the cart, the metal cage was struck with the blunt probe. The normal animal should exhibit a marked but short-lasting response, in which case the space on the scoring sheet was given as '0'. If present, a plus sign was entered.

Respiration

While at rest on the cart, the animal's respiration cycle was observed and evaluated in terms of a scale from 1 (reduced) to 5 (increased), with 3 being normal.

Mouth breathing

Rats are normally obligatory nose-breathers. Each animal was observed, whether it was breathing through its mouth or not. In the normal case a '0' was given. If present, a plus sign was recorded.

Urination

When an animal was removed from its cage, the pan beneath the animal's cage was examined while returning the animal to its cage. The signs of urination were evaluated on a scale of 0 (lacking) to 5 (polyurea).

Convulsions

If clonic or tonic convulsions were observed to occur, they were graded on intensity (from 1, minor, to 5, marked) and the type and intensity were recorded. In the normal case a '0' was given.

Pilo-erection

The fur of the animal's back was observed, whether it was raised or elevated. In the normal case (no pilo-erection) a '0' was given. If pilo-erection was present, a plus sign was entered.

Diarrhea

In examining the pan beneath an animal's cage, it was noted if there were any signs of loose or liquid stools. Normal state was for there to be none ('0'), in case of diarrhea the intensity was recorded on a scale of 1 (slight) to 5 (much increased).

Pupil size

The pupils were determined if they were constricted or dilated and were graded on a scale of 1 (constricted) to 5 (dilated), with 3 being normal.

Pupil response

The beam of light from the pen light was played across the eyes of the animal and the changes in pupil size were noted. In the normal animal, the pupil is constricted when the beam is on it and then dilate back to normal when the light is removed ('+' = normal). It was noted if there was no response (in which case a minus sign was recorded in the blank space).

Lacrimation

The animal was observed for the secretion and discharge of tears. In rats the tears contain a reddish pigment. No discharge is normal and in this case the box was given as '0'. If the discharge was present, a plus sign was entered.

Impaired gait

The occurrence of abnormal gait was evaluated. The most frequent impairments were waddling (W), hunched gait (H), or ataxia (A, the inability of all the muscles to act in unison). The extent of any impairment was recorded on a scale of 1 (slight) to 5 (marked). The normal reaction was given as '0'.

Stereotypy

Each animal was evaluated for stereotypic behaviour (isolated motor acts or partial sequences of more complex behavioural patterns, occurring out of context and with an abnormal high frequency). These were graded on a scale of 0 (= normal) to 5 if such signs were present.

Toe pinch

The blunt probe was used to bring pressure to bear on one of the digits of the hindlimb. This should evoke a response from the normal animal, graded on a scale from 1 (absent) to 5 (exaggerated); 3 = normal.

Tail pinch

The procedure detailed above was utilised with the animal's tail instead of its hindlimb and was graded on the same scale with 1 (absent) to 5 (exaggerated); 3 = normal.

Wire maneuver

The animal was placed on the metal rod suspended parallel to the cart approx. 60 cm above it. Its ability to move along the rod was evaluated. If impaired, a score of 1 (slightly impaired) to 5 (unable to stay on wire) was recorded. The normal reaction was given as '0'.

Hind leg splay

Using an ink pad, the hind paws were marked with ink. The rat was then held 30 cm above a sheet of blotting paper on the cart. The animal was dropped and the distance between the prints of the two hind paws was measured.

Positional passivity

When placed in an awkward position (such as on the edge of the top of the wire bottomed cage) on the cart surface, it was examined whether the animal immediately moved into a more normal position ('0'). If not, a score was recorded on a scale of 1 (slightly impaired) to 5 (cataleptic).

Tremors

Periods of continued fine movements, usually starting in the limbs (and perhaps limited to them). The normal case is to have none, in which case a '0' was recorded. If present, they were graded on a scale of 1 (slight and infrequent) to 5 (continuous and marked).

Positive geotropism

The animal was placed on the inclined (at an angle of approx. 30°) top surface of the wire cage with its head facing downward. It should turn 180° and face 'uphill', in which case a '0' was given. If this did not occur, a negative sign was recorded.

Limb rotation

One of the animal's hindlimbs was taken and moved through its normal plane of rotation. In the normal state, it should rotate readily but there should be some resistance. The variations from normal were from no resistance (1) to markedly increased resistance or rigidity (5), with 3 being normal.

Auditory function

Each animal was placed into a container and observed for Preyer's reflex (twitching of the pinna) in response to a high frequency sound stimulus. The stimulus was repeated, if necessary, up to 3 times. A normal reaction was given as '+' in the blank, a lacking reaction was indicated by ‘0’.

Grip strength

Prior to testing, the gauge (Chatillon, Model DPP - 1.0 kg) was calibrated with a set of known weights and the apparatus adjusted for the size of the animal (about 1 cm clearance on both sides of the animal). After the strain gauge was zeroed and set in the record mode, the animal was placed into the trough with the forepaws inside the triangular grasping ring. Using one hand, the animal was grasped about 2.5 cm of the way up toward the base of the tail and steadily pulled (approx. 2.5 cm/sec) away from the ring until the grip was broken. The animal continued to be pulled along the trough until the hindlimbs grasped the T-bar. The trial was completed when grip of the hindlimbs was broken. Three successive readings were taken for each animal with an intertrial interval long enough to record the data and zero both meters for the next trial.

Locomotor activity

The rats were individually placed into a motility system with microprocessor control and automatic statistical evaluation. Each unit had 2 individually adjustable channels so that slight static movements and active moving could be differentiated and separately scored (TSE Systems, Bad Homburg). The motility meter created a magnetic field, movement of the animals interfered with the magnetic field. Two separate magnetic fields allowed two sensitivity levels to be chosen to distinguish between two types of movements:

-High sensitivity: Stereotype, static movement, (static movements) (e.g. grooming, a stationary movement of the animal without leaving its own position)

-Low sensitivity: Active locomotion (active moving)

<u>Necropsy and histopathology:</u>

The nervous system from 5 animals/sex/group was fixed in situ according to standard perfusion procedure for neurohistopathological examination. The following organs or parts of organs of all animals were fixed in 7% buffered formalin except the eyes which were preserved in Davidson’s solution for optimum fixation: center of cerebrum (incl. hippocampus, midbrain, cerebellum, pons, medulla oblongata), cauda equina, dorsal root ganglia, dorsal and ventral root fibres, eye with optic

nerve and retina (2), forebrain, muscle, skeletal, calf, sciatic nerve (proximal), spinal cord (cervical and lumbal swellings), tibial nerve (proximal, knee) and tibial nerve (calf muscle branches).

The afore-listed organs of 5 animals/sex of groups 1 and 4 were examined neurohistologically after preparation of sufficient paraffin sections with haematoxylin-eosin staining. The spinal cord and peripheral nerve sections included both cross or transverse and longitudinal sections. Attention was paid to the vasculature of the nervous system. In addition, to identify the dorsal root ganglia that were not present in the original slide, several H&E stained step sections seperated by approximately 100 µm of the cervical and/or lumbar spinal cord (transverse and longitudinal section) were prepared in a number of animals (nos. 1, 2, 3, 6, 7, 11, 12, 16, 17, 18, 61, 62, 63, 69, 70, 73, 75, 78 and 80) and also examined microscopically. The frequency and severity of each lesion were recorded.

Findings:

Mortality: 5 of 10 female rats treated with 250 mg/kg died prematurely within 24 hours after the administration.

Clinical signs: None of the male and female rats treated with 75 mg/kg revealed any changes of behaviour or external appearance. Oral treatment with 150 mg/kg caused slight tremor in females. The high dose of 250 mg/kg caused death (5/10), reduced motility, ataxia, tremor, reduced muscle tone, tonic convulsions, piloerection and diarrhea in all females on test day 1. All high dosed males revealed tremor and diarrhea on test day 1. All casualities and clinical symptoms started 1 to 6 hours after administration and were limited to the day of administration.

Food and drinking water consumption: Food consumption was slightly decreased in the 5 surviving females of the high dose group during the experimental phase. Drinking water consumption was not changed.

Body weights and body weight gain: There was no treatment-related effect observed at any dose level 8 and 14 days after administration.

Table 77: Mortality, clinical signs, body weight and food consumption

Symptoms/ Criteria	Control		75 mg/kg bw		150 mg/kg bw		250 mg/kg bw	
	m (n=10)	f (n=10)	m (n=10)	f (n=10)	m (n=10)	f (n=10)	m (n=10)	f (n=10)
Clinical signs								
Reduced motility	none	none	none	none	none	none	none	**+ to ++ 3 to 6h (10)**
Ataxia	none	none	none	none	none	none	none	**+ 6h (10)**
Tremor	none	none	none	none	none	**+ 6h (10)**	**+ 3 to 6h (10)**	**+ to ++ 3 to 6h (10)**
Reduced muscle tone	none	none	none	none	none	none	none	**++ 6h (10)**
Tonic convulsions	none	none	none	none	none	none	none	**+ 6h (10)**
Pilo-erection	none	none	none	none	none	none	none	**+ 3 to 6h**

Symptoms/ Criteria	Control		75 mg/kg bw		150 mg/kg bw		250 mg/kg bw	
	m (n=10)	f (n=10)	m (n=10)	f (n=10)	m (n=10)	f (n=10)	m (n=10)	f (n=10)
								(8)
Diarrhea	none	none	none	none	none	none	+ **1 to 3h (10)**	+ **1 to 3h (10)**
Mortality								
Within 6h	0	0	0	0	0	0	0	0
Within 24h	0	0	0	0	0	0	0	**5**
Within 7d	0	0	0	0	0	0	0	**5**
Within 14d	0	0	0	0	0	0	0	**5**
Mean body weight [g] / (Mean body weight gain [%])								
Before admin.	196.2	173.7	204.5	177.7	203.3	180.2	193.7	170.7
Test day 8	265.7 (+35.5)	203.2 (+17.1)	279.8 (+37.2)	208.3 (+17.3)	277.5 (+36.9)	213.9 (+18.8)	262.9 (35.9)	198.6 (+15.1)
Test day 15	309.3 (+57.9)	219.6 (+26.5)	314.3 (+54.2)	214.8 (+20.9)	310.7 (+53.3)	224.6 (+24.7)	306.3 (+58.3)	207.8 (+20.4)
Food consumption [g/week/rat]								
Testweek 1	173.6	134.3	189.5	135.9	184.7	139.6	172.8	**110.0**
Testweek 2	149.5	118.5	170.0	139.5	159.3	131.7	153.5	**104.4**

h: hours *p.a.*; d: days *p.a.*; +: slight/observed; ++: moderate

Neurological screening: No treatment-related effects were observed after administration of 75 mg/kg. Oral treatment with 150 and 250 mg/kg caused changes in the appearance, behaviour and reactivity of male and female rats 2 and 8 hours after administration. No changes were observed in control animals except for the extreme low values for "hind-leg splay" 8 hours and 7 days after treatment compared to the other observation time points.

Pilo-erection, diarrhea and impaired ability for wire maneuver were noted in all male rats treated orally with 150 mg/kg. The female rats revealed the same symptoms and, in addition, impaired gait, tremor and decreased resistance during limb rotation. These findings were observed 8 hours after treatment and subsided 7 days after administration.

The high dose of 250 mg/kg metaldehyde revealed further signs of toxicity in form of tremor in the male rats and decreased hindleg splay in the female rats 2 hours after administration. Furthermore, significantly increased body temperature was observed in both sexes and a reduced righting reflex, convulsions, impaired gait, a reduced toe/tail pinch response and a decreased resistance during limb rotation were noted in the female rats 8 hours after administration. A significantly increased hindleg splay was noted for the females 8 hours after administration but as the values of the controls were extremely low compared to other scoring time points, it is considered to be spontaneous.

All clinical changes had sibsided on test day 2 and all neurological changes on the next examination time point for neurological screening on test day 7.

The study authors considered all symptoms observed, especially in the 250 mg/kg dose group with a mortality rate of 50 % in females, to be related to the overall toxicity of metaldehyde at lethal or close-to-lethal doses and not signs of neurological toxicity.

Table 78: Grip strength and locomotor activity

Symptoms/ Time point	Control		75 mg/kg bw		150 mg/kg bw		250 mg/kg bw	
	m (n=10)	f (n=10)	m (n=10)	f (n=10)	m (n=10)	f (n=10)	m (n=10)	f (n=10)
Grip strength forelimb [g]								
pre-dose	306.0	297.6	320.1	297.0	328.4	278.9	322.7	300.8
2 hours *p.a.*	303.6	298.3	320.4	295.7	333.7	298.2	317.4	290.8
8 hours *p.a.*	334.2	293.0	323.9	288.9	318.2	277.3	244.6	nd
7 days *p.a.*	390.2	386.4	374.9	342.5	389.1	348.0	404.2	393.7
14 days *p.a.*	399.2	348.9	356.5	327.8	383.6	326.7	387.4	298.8
Grip strength hindlimb [g]								
pre-dose	138.7	137.6	134.9	129.6	139.8	132.3	141.1	144.2
2 hours *p.a.*	148.9	138.3	139.7	123.0	148.0	134.5	132.1	137.4
8 hours *p.a.*	148.0	134.9	140.0	131.4	133.1	113.3	104.3	nd
7 days *p.a.*	274.2	250.9	136.4	128.2	145.0	130.7	307.2	263.7
14 days *p.a.*	158.1	139.3	142.1	138.8	147.7	134.3	145.5	131.1
Spontaneous motility – high sensitivity (slight movements) [number of movements/10 min]								
pre-dose	685.2	687.0	697.3	860.8	894.4	800.5	815.3	832.8
2 hours *p.a.*	320.4	281.2	526.7	706.0	349.7	612.2	549.8	617.1
8 hours *p.a.*	398.4	307.5	453.0	545.0	590.9	652.4	531.4	555.8
7 days *p.a.*	503.7	570.2	570.0	811.7	561.3	998.6	630.0	930.6
14 days *p.a.*	340.7	597.0	516.7	778.1	706.4	858.6	591.2	763.2
Spontaneous motility – low sensitivity (active moving) [number of movements/10 min]								
pre-dose	140.5	106.4	119.2	145.8	178.9	141.8	154.0	131.9
2 hours *p.a.*	36.1	29.5	68.1	82.5	45.4	69.6	60.3	70.9
8 hours *p.a.*	53.1	33.5	69.6	82.3	87.9	79.4	71.5	73.3
7 days *p.a.*	103.2	93.5	109.1	146.3	97.0	216.5	117.4	163.0
14 days *p.a.*	63.5	111.9	92.7	133.1	139.0	155.0	108.6	104.6

nd: not determined; **: stat. sign. with $p \leq 0.01$

Table 79: Neurological screening, functional observations

Parameter	Control		75 mg/kg bw		150 mg/kg bw		250 mg/kg bw	
	m (n=10)	f (n=10)	m (n=10)	f (n=10)	m (n=10)	f (n=10)	m (n=10)	f (n=10)
Predose / 2h after dosing / 8h after dosing / 7d after dosing / 14d after dosing								
Rightning reflex	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/**1.0**/-/-
Body temperature [°C]	36.7/37.0/36.9/ 37.3/37.6	37.0/37.1/37.0/ 37.4/37.7	37.0/36.9/37.1/ 37.5/37.8	36.8/37.0/37.0/ 37.5/37.8	37.0/37.1/37.3/ 37.5/37.8	36.9/37.1/37.3/ 37.6/37.8	37.1/37.2/**37.5*/** 37.3/37.8	37.0/37.2/**38.0*/** 37.2/37.7
Salivation	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-
Startle response	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-
Respiration	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-
Mouth breathing	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-
Urination	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-
Convulsion	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/**2.0**/-/-
Pilo-erection	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/+/-/-	-/-/+/-/-	-/-/+/-/-	-/+/+/-/-
Diarrhea	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/**1.0**/-/-	-/-/**1.0**/-/-	-/-/**1.0**/-/-	-/-/**1.0**/-/-
Lacrimation	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-
Impaired gait	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/**1.0**/-/-	-/-/-/-/-	-/**2.0**/**3.0**/-/-
Stereotypy	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-
Toe pinch	-/-/-/2.9/3.0	-/-/3.0/3.2/-	-/-/-/3.0/3.0	-/-/3.0/3.0/-	-/-/-/3.0/3.0	-/-/3.0/3.0/-	-/-/-/3.1/3.0	-/-/**2.0**/3.4/-
Tail pinch	-/-/-/2.9/3.0	-/-/3.0/2.9/-	-/-/-/3.0/3.0	-/-/3.0/3.0/-	-/-/-/3.0/3.0	-/-/3.0/3.0/-	-/-/-/2.9/3.0	-/-/**2.0**/3.0/-
Wire maneuver	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/1.0/-/-	-/-/2.0/-/-	-/-/1.0/-/-	-/-/-/-/-
Hind leg splay	6.5/6.9/6.1/6.5/ 6.9	5.2/5.2/4.9/4.9/ 5.4	6.4/6.7/6.7/6.8/ 7.0	5.2/5.2/5.1/6.1/ 5.4	6.7/6.7/6.3/6.9/ 7.0	5.3/5.5/5.4/6.0/ 5.5	6.6/6.6/6.7/6.6/ 6.9	5.6/4.9/**6.7*[1)]**/5. 1/ 5.3
Positional passivity	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-
Tremors	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/**1.0**/-/-	-/**1.0**/**1.0**/-/-	-/**1.0**/**3.0**/-/-
Positive geotropism	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/nd/-/-	-/-/nd/-/-	-/-/nd/-/-	-/nd/nd/-/-
Limb rotation	-/-/-/-/-	-/-/3.0/-/-	-/-/-/-/-	-/-/3.0/-/-	-/-/-/-/-	-/-/**2.0**/-/-	-/-/-/-/-	-/-/**1.0**/-/-
Auditory function	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-	-/-/-/-/-

-: no influence; +: slight/observed; ++:moderate; nd: not determined; *: stat. sign. with $p \leq 0.01$

1) considered to be spontaneous due to low control values at 8h after dosing

Necropsy and histopathology: Administration of 75, 150 and 250 mg/kg metaldehyde did not produce any macroscopic or microscopic findings considered to be related to treatment. There were no microscopic alterations recorded in the nervous tissues or in the vasculature of the nervous tissue.

Conclusion:

CD-1 rats were treated with a single dose of 75, 150 or 250 mg/kg metaldehyde orally by gavage. 5 of 10 female rats treated with 250 mg/kg died prematurely within 24 hours after administration. Oral treatment with 150 mg/kg and 250 mg/kg caused transient clinical signs and findings in the neurological screening with were recorded only on the administration day with females being more affected than males. The neurological screening revealed several changes like pilo-erection, tremor, diarrhea, increased body temperature, convulsions, impaired ability for wire maneuver, impaired gait, decreased resistance during limb rotation, increased hindleg splay, a reduced righting reflex and/or a reduced toe/tail pinch response. The histomorphological examination of the nervous system did not reveal any pathological evidence. The study author concluded that all neurological changes should be regarded related to the overall toxicity of metaldehyde at lethal or close-to-lethal doses and not to signs of neurological toxicity, as no histopathological changes were found. However, the RMS feels that the effects observed in the neurological screening at 150 and 250 mg/kg cannot be solely attributed to general acute toxicity, as they already appear at the non-lethal dose level of 150 mg/kg. Furthermore, even if these findings are acute toxic effects, they are also neurotoxic effects. The NOAEL of this study for both the systemic toxicity and the neurotoxicity is therefore set at 75 mg/kg bw metaldehyde, based on the findings in the neurological screening at the dose level of 150 mg/kg.

Reference:	**An oral (gavage) acute neurotoxicity study of metaldehyd in rats**
Author(s), year:	Herberth, M. T. (2011)
Report/Doc. number:	Report No. WIL-823002 Conducting laboratory: WIL Research Laboratories, LLC, Ashland, Ohio
Guideline(s):	OECD Guideline 424 (1997) US EPA, OPPTS 870.6200 (1998)
GLP:	Yes
Deviations:	No
Validity:	Yes

Material and Methods:

Test material	Metaldehyde
Lot/Batch	070605
Purity	100.0%
Vehicle	Corn oil
Species	Rat
Strain	CD, Crl:CD(SD)
Age	Approximately 6 weeks
Weight at dosing	Males: 146-213g; Females: 134-185g
Source	Charles River Laboratories, Inc., Raleigh, NC
Number of animals	40 males, 40 females

The vehicle and test substance formulations were administered as a single oral (gavage) dose via an appropriately sized flexible Teflon®-shafted, stainless steel ball-tipped dosing cannula (Natume, Japan). The dose volume for all groups was 5 mL/kg. Individual doses were based on the body weights recorded prior to dosing on study day 0. Animals were not fasted prior to dose administration to avoid potential confounding effects of inanition on behavior.

Table 80: An oral (gavage) acute neurotoxicity study of metaldehyd in rats – Study design

		Number of Animals	
Group Number	**Dose Level (mg/kg/day)**	**Male**	**Female**
1	0	10	10
2	75	10	10
3	150	10	10
4	250	10	10

General Observations: All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity.
Individual body weights were recorded at least weekly, beginning approximately 1 week prior to test substance administration (study day -7). Mean body weights and body weight changes were calculated for the corresponding intervals. When body weights could not be determined for an animal during a given interval (due to an unscheduled death), group mean values were calculated for that interval using the available data.

Clinical Observations: Clinical examinations were performed once daily on all animals. The absence or presence of findings was recorded for individual animals. On the days that the functional observational battery was conducted, no additional clinical findings were recorded, with the exception of unscheduled observations that occurred prior to the scheduled FOB on study day 0.

FOB (Functional Observational Battery): FOB findings were recorded for all animals prior to the initiation of dose administration (study day -6), at the time of peak effect (6 hours post-dosing) on study day 0, and on study days 7 and 14. The time of peak effect was considered to be 6 hours post-dosing based on the severity and incidence of FOB findings at 6 hours following a single dose of metaldehyde[1]. The FOB used at WIL Research is based on previously developed protocols (Gad, 1982[2]; Haggerty, 1989[3]; Irwin, 1968[4]; Moser *et al*., 1988[5]; Moser *et al*., 1991[6,7]; and O'Donoghue, 1989[8]). Testing was performed by the same biologists, to the extent possible, without knowledge of the animal's group assignment. The FOB was performed in a sound-attenuated room equipped with a white-noise generator set to operate at 70 ± 10 dB. All animals were observed for the following parameters as described below.

Home Cage Observations:
Posture, Convulsions/tremors, Feces consistency, Biting, Palpebral (eyelid) closure

Handling Observations:
Ease of removal from cage, Lacrimation/chromodacryorrhea, Piloerection, Palpebral closure, Eye prominence, Red/crusty deposits, Ease of handling animal in hand, Salivation, Fur appearance, Respiratory rate/character, Mucous membranes/eye/skin color, Muscle tone

Open field Observations:
Rearing, Convulsions/tremors, Grooming, Bizarre/stereotypic behavior, Time to first step (seconds), Gait, Arousal, Urination/defecation, Gait score, Backing

Sensory Observations:
Approach response, Startle response, Pupil response, Forelimb extension, Air righting reflex, Touch response, Tail pinch response, Eyeblink response, Hindlimb extension, Olfactory orientation

Neuromuscular Observations:
Hindlimb extensor strength, Hindlimb foot splay, Grip strength-hind and forelimb, Rotarod performance

Physiological Observations:
Catalepsy, Body temperature, Body weight

[1]Herberth, M.T. An Oral (Gavage) Dose Range-Finding Acute Neurotoxicity Study of Metaldehyde in Rats (Study No. WIL-823001). WIL Research Laboratories, LLC, Ashland, OH, **2011.**
[2]Gad, S.C. A neuromuscular screen for use in industrial toxicology. *Journal of Toxicology and Environmental Health* **1982,** *9(5-6),* 691-704.
[3]Haggerty, G.C. Development of tier I neurobehavioral testing capabilities for incorporation into pivotal rodent safety assessment studies. *Journal of the American College of Toxicology* **1989,** *8(1),* 53-69.
[4]Irwin, S. Comprehensive observational assessment: Ia. a systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia* **1968,** *13,* 222-257.
[5]Moser, V.C.; McCormick, J.P.; Creason, J.P.; MacPhail, R.C. Comparison of chlordimeform and carbaryl using a functional observational battery. *Fundamentals of Applied Toxicology* **1988,** *11(2),* 189-206.
[6]Moser, V.C. Applications of a neurobehavioral screening battery. *Journal of the American College of Toxicology* **1991a,** *10(6),* 661-669.
[7]Moser, V.C.; McDaniel, K.L.; Phillips, P.M. Rat strain and stock comparisons using a functional observational battery: baseline values and effects of amitraz. *Toxicology and Applied Pharmacology* **1991b,** *108(2),* 267-283.
[8]O'Donoghue, J.L. Screening for neurotoxicity using a neurologically based examination and neuropathology. *Journal of the American College of Toxicology* **1989,** *8(1),* 97-115.

Locomotor Activity: Locomotor activity was assessed for all animals prior to the initiation of dose administration (study day -6), at the time of peak effect (6 hours post-dosing) on study day 0, and on study days 7 and 14. Locomotor activity, recorded after completion of the FOB, was measured automatically using a personal computer-controlled system that utilizes a series of infrared photobeams surrounding an amber plastic, rectangular cage to quantify the motor activity of each animal. Four-sided black plastic enclosures were used to surround the transparent plastic boxes and decrease the potential for distraction from extraneous environmental stimuli or activity by technicians or adjacent animals. The black enclosures rested on top of the photobeam frame and did not interfere with the path of the beams. The locomotor activity assessment was performed in a sound-attenuated room equipped with a white-noise generator set to operate at 70 ± 10 dB. The testing of treatment groups was conducted according to replicate sequence. Each animal was tested separately. Data were collected in 5-minute epochs, and the test session duration was 60 minutes. These data were compiled as six 10-minute subintervals for tabulation. Data for ambulatory and total motor activity were tabulated. Total motor activity was defined as a combination of fine motor skills (*i.e.*, grooming, interruption of 1 photobeam) and ambulatory motor activity (interruption of 2 or more consecutive photobeams).

Necropsy: A complete necropsy was conducted on all animals found dead. The necropsy included examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera. Gross lesions were retained in 10% neutral-buffered formalin for possible future histopathological examination.
On study day 15, all surviving animals were anesthetized. The central and peripheral nervous system tissues were dissected and preserved. Fixed brain weight and brain dimensions (length [excluding olfactory bulbs] and width) were recorded. Any observable gross changes and abnormal coloration or lesions of the brain and spinal cord were recorded.

Histopathology: The following nerve tissues listed were prepared for a qualitative histopathological examination by embedding in paraffin (central nervous system tissues) or plastic (peripheral nervous system tissues), sectioning, and staining with hematoxylin and eosin:
Brain - olfactory bulbs, cerebral cortex (2 levels), hippocampus/dentate gyrus, basal ganglia, thalamus, hypothalamus, midbrain, cerebellum, pons, and medulla oblongata; Spinal cord - at cervical swellings C3-C7 and at lumbar swellings T13-L4; Trigeminal ganglia/nerves[a]; Lumbar dorsal root ganglia at T13-L4[b]; Lumbar dorsal root fibers at T13-L4[b]; Lumbar ventral root fibers at T13-L4[b]; Cervical dorsal root ganglia at C3-C7[b]; Cervical dorsal root fibers at C3-C7[b]; Cervical ventral root fibers at C3-C7[b]; Cervical spinal nerve; Lumbar spinal nerve; Sciatic nerves (mid-thigh region)(2)[c]; Sciatic nerves (at sciatic notch)(2)[c]; Sural nerves (2)[c]; Tibial nerves (2)[c]; Peroneal nerves (2)[c]; Optic nervesa; Eyesa; Skeletal muscle (gastrocnemius); Other sites (if deemed necessary)

[a] - Both processed and evaluated microscopically
[b] - Four to 6 tissues were collected at necropsy; 2 were evaluated microscopically.
[c] - One processed for microscopic examination.
(2) - Two sections (1 transverse and 1 longitudinal) of the tissue were evaluated from the right hind leg. The tissues from the left hind leg were collected and preserved for possible future evaluation.

Results:

Mortality: Test substance-related mortality was observed in the 250 mg/kg group as 1 male and 2 females in this group were found on study day 0. The male died 3 hours 27 minutes after test substance administration, but prior to the FOB evaluation at the time of peak effect (6 hours following dose administration). There were no clinical findings noted for this male at the daily examinations prior to dosing; however, slight tremors and tonic convulsions were noted approximately 10 minutes prior to death. One female was found dead during the 6-hour FOB evaluation (6 hours 16 minutes following dose administration), while the other female was found dead following the FOB and locomotor activity evaluations (9 hours 11 minutes following dose administration). Multiple signs of overt toxicity were noted for these females during the FOB evaluation. In addition, tonic and clonic convulsions were noted 6 hours following dose administration for the female which died 9 hours 11 minutes following dose administration.

Body Weight: Body weights were unaffected by test substance administration. There were no statistically significant differences when the control and test substance-treated groups were compared.

Clinical Observations: There were no test substance-related clinical findings observed during the daily clinical observations of all animals which survived until the scheduled euthanasia (study day 15). All findings were noted with similar incidence in the control group, were limited to single

animals, occurred in a non-dose-related manner, and/or were common findings for laboratory rats of this age and strain.

FOB (Functional Observational Battery):

Home cage observations (Table 81):

Test substance-related effects on home cage parameters were noted in the 150 mg/kg group females and 250 mg/kg group males and females at the time of peak effect (6 hours following dose administration) on study day 0. Clonic convulsions (tremors of the limbs and/or whole body tremors) and slight or markedly coarse tremors were noted for both females from the 250 mg/kg group that were found dead during or following the FOB evaluation. In addition, slight tremors were noted for 2 surviving males in the 250 mg/kg group and 1 and 2 surviving females in the 150 and 250 mg/kg groups, respectively. The differences from the control group were not statistically significant; tremors and convulsions were also noted in these groups during the open field observations. Therefore, these observations were attributed to test substance administration. No other test substance-related effects on home cage parameters were noted for the 150 mg/kg group females or 250 mg/kg group males and females at the time of peak effect on study day 0, and on study days 7 and 14. On study day 0, fewer males and more females in the 250 mg/kg group had feces when compared to the respective control groups; the difference in the males was significant ($p<0.05$). Consequently significantly ($p<0.05$) more males and fewer females in the 250 mg/kg group were noted without feces relative to the respective control groups. Because opposite trends were observed in each sex, the differences in the presence or absence of feces were not attributed to test substance administration. Significantly ($p<0.05$) fewer females in the 150 mg/kg group were asleep (lying on the side or curled up) compared to the control group. Because 9 of 10 females in the 150 mg/kg group were observed with normal home cage behavior (sitting/standing normally or alert/oriented toward the observer) and there was no dose-response apparent, the absence of females that were asleep was not considered test substance-related. In addition, significantly ($p<0.05$) more females in the 150 mg/kg group had feces on study day 14 when compared to the control group. An effect on feces consistency was not observed in the 250 mg/kg group on study day 14, therefore the difference observed at 150 mg/kg was not considered to be test substance-related. Home cage parameters were unaffected by test substance administration for the 75 mg/kg group males and females and the 150 mg/kg group males. There were no statistically significant differences for these groups when compared to the control group at the time of peak effect on study day 0, and on study days 7 and 14.

Handling Observations (Table 81):

Test substance-related effects on handling parameters were noted for the 150 and 250 mg/kg group females. Observations for the 2 females in the 250 mg/kg group that were found dead on study day 0 included moderate difficulty (animal rears, often following the investigator's hand) in removal from the cage, slight lacrimation, slight salivation, and/or chromodacryorrhea. For surviving females in this group, 1 female was moderately difficult to remove from the cage and 2 females had slight lacrimation at the 6-hour FOB evaluation on study day 0. Slight lacrimation was also noted for 1 surviving female in the 150 mg/kg group (also noted with slight tremors). There were no other test substance-related effects on handling parameters noted in the 150 or 250 mg/kg group females at the time of peak effect on study day 0, and no test substance-related effects were noted for these groups on study days 7 and 14. Handling parameters were unaffected by test substance administration for the 75 mg/kg group males and females and the 150 and 250 mg/kg group males. There were no statistically significant differences for these groups when compared to the control group at the time of peak effect on study day 0, and on study days 7 and 14.

Open Field Observations (Table 81):

Test substance-related effects on open field parameters were noted in the 150 and 250 mg/kg group males and females at the time of peak effect (6 hours post-dosing) on study day 0. Slight tremors were noted for 2 and 3 males and 5 and 2 females in the 150 and 250 mg/kg groups, respectively, compared to 0 animals in the control group; only the difference in the 150 mg/kg group females was significant ($p<0.05$). In addition, tonic convulsions were noted for one female (also noted with slight tremors) in the 250 mg/kg group at this same evaluation. Additional observations noted in the open field evaluation were observed for 2 females in the 250 mg/kg group that were found dead on study day 0 and included moderately impaired mobility, altered gait (hindlimbs splayed or dragging, unable to support weight), altered gait score (considerable or severe impairment), low or very low arousal, clonic tremors of the limbs, clonic convulsions, and/or higher mean time to first step. No other test substance-related effects on open field parameters were noted for the 150 and 250 mg/kg group males and females at the time of peak effect on study day 0, or on study days 7 or 14. Open field parameters in the 75 mg/kg group males and females were unaffected by test substance administration. There were no statistically significant differences for the test substance-treated males and females when compared to the control group at the time of peak effect on study day 0, and on study days 7 and 14.

Sensory Observations (Table 81):

Test substance-related effects on sensory parameters were noted for 1 of the 2 females that was found dead and 1 surviving male in the 250 mg/kg group at the peak time of effect (6 hours post-dosing) on study day 0. Both animals were noted with slightly uncoordinated air righting reflex and the female had no response to approach or touch and a lack of reaction to olfactory stimulus. One surviving female in the 250 mg/kg group was also observed with no response to approach and touch. Although the differences were not statistically significant when compared to the respective control groups, these effects occurred in the presence of other signs of toxicity and the aforementioned observations were therefore attributed to test substance administration. No other test substance-related effects on sensory parameters were noted at the time of peak effect on study day 0 and no effects were noted on study days 7 and 14. One female in the 250 mg/kg group exhibited no pupil and no eyeblink response on study day 7. However, these observations were also observed for a control group female during the pretest evaluation and therefore this difference was not considered to be test substance-related. Sensory parameters were unaffected by test substance administration for males and females in the 75 and 150 mg/kg groups. There were no statistically significant differences for these groups when compared to the control group at the time of peak effect on study day 0, and on study days 7 and 14.

Neuromuscular Observations (Table 81):

A test substance-related significantly ($p<0.01$) lower hindlimb footsplay was observed in the 250 mg/kg group females compared to the control group at the time of peak effect on study day 0. There were no other test substance-related effects on neuromuscular observations noted for the 250 mg/kg group females. Three females in the 250 mg/kg group were noted with reduced hindlimb resistance (indicative of weakness) at the time of peak effect on study day 0. The difference was not statistically significant compared to the control group and in the absence of a corresponding effect on hindlimb grip strength in the 250 mg/kg group females; this difference was not attributed to test substance administration. Neuromuscular parameters were unaffected by test substance administration for the 75, and 150 mg/kg group males and females and the 250 mg/kg group males. Significantly ($p<0.05$) lower hindlimb footsplays were also observed in the 250 mg/kg group males and 75 and 150 mg/kg group females at the time of peak effect on study day 0; however, these values were similar to the pretest values, were within the historical control data range of the conducting laboratory, and/or the differences did not occur in a dose responsive manner. Therefore, the lower hindlimb footsplays in

these groups were not attributed to test substance administration. In addition, a significantly ($p<0.05$) lower rotarod performance was noted for the 250 mg/kg group males on study day 7. In the absence of any other effects noted in this group at this time point, this difference was not attributed to test substance administration. There were no other statistically significant differences noted for these groups when compared to the control group at the time of peak effect on study day 0, and on study days 7 and 14. One male in the 250 mg/kg group and 2 females in each of the 75 and 150 mg/kg groups were noted with reduced hindlimb resistance (indicative of weakness). These differences were not statistically significant when compared to the control group and in the absence of a corresponding effect on hindlimb grip strength in these animals; this difference was not attributed to test substance administration.

Physiological Observations (Table 81):

Significantly ($p<0.05$ or $p<0.01$) lower mean body temperature was noted in the 250 mg/kg group males and females at the time of peak effect (6 hours following dose administration) on study day 0. Both values were below the respective minimum mean values for approximately age-matched animals in the historical control data of the conducting laboratory and were therefore attributed to test substance administration. A test substance-related increase (not statistically significant) in catalepsy time (the length of time spent motionless) was also observed in the 250 mg/kg group females compared to the control group at the time of peak effect on study day 0. All other physiological parameters in the 250 mg/kg group were similar to the concurrent control group values at the time of peak effect on study day 0, and on study days 7 and 14. Physiological parameters were unaffected by test substance administration for the 75 and 150 mg/kg group males and females. There were no statistically significant differences for these groups when compared to the control group at the time of peak effect o n study day 0, and on study days 7 and 14.

Table 81: Acute neurotoxicity study of metaldehyd in rats – Functional Observational Battery

	Male				**Female**			
Dose Level (mg/kg/day)	0	75	150	250	0	75	150	250
	study day -6 / 6h post dosing / study day 7 / study day 14							
Number of animals tested	**10/9/9/9**	**10/9/9/9**	**10/9/9/9**	**10/9/9/9**	**10/9/8/8**	**10/9/8/8**	**10/9/8/8**	**10/9/8/8**
Home Cage								
Posture								
Asleep, lying on side or curled up	4/6/5/7	4/4/3/3	2/1/3/7	5/1/2/5	5/5/0/1	3/1/1/2	3/**0***/1/0	8/2/2/2
Convulsions								
Slight	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
Clonic tremors of the limbs	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Whole body tremors	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**2**/0/0
Tremors								
Slight	0/0/0/0	0/0/0/0	0/0/0/0	0/**2**/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0	0/**3**/0/0
Markedly Coarse, Moderate/marked impairment of locomotion	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Feces consistency								
Pellets partially formed	0/0/0/0	0/0/0/0	0/0/0/0	0/1/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/2/0/0
Pellets absent	1/2/6/7	2/7/5/8	3/4/7/8	2/**8***/5/5	5/9/7/10	2/7/6/6	7/6/7/**5***	2/**3***/6/7
Handling								
Ease of removal from cage								
Moderately difficult: animal rears, often following investigator`s hand	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1**/0/0/0	0/0/0/0	0/**2**/0/0
Lacrimation								
Slight	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0	0/**3**/0/0
Salivation								
Present	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Chromodacryorrhea	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Red/crusty deposits								
Red deposits nose	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1**/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
Ease of handling animal in hand								
Slight Resistance to being handled, with/without vocalization	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1**/0/0/0	0/0/0/0	0/**1**/0/0	0/0/0/0
Open Field								
Convulsions								
Clonic tremors of the limbs	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Clonic Convulsions	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Tonic: Contraction of Extensors such that limbs are rigid and extended	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Tremors								

Slight	0/0/0/0	0/0/0/0	0/**2**/0/0	0/**3**/0/0	0/0/0/0	0/0/0/0	0/**5***/0/0	0/**2**/0/0
Mobility								
Moderately impaired	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**2**/0/0
Gait								
Hindlimbs splayed or dragging, unable to support weight	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**2**/0/0
Considerable impairment, without falling	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Severe impairment, cannot walk without falling	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Arousal								
Very low: stupor, coma, little or no responsiveness	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Low: somewhat stuporous	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Somewhat high: Slight excitement, tense, sudden darting or freezing	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1**/0/0/0	0/0/0/0	0/0/0/**1**
Sensory								
Approach response								
No reaction	0/0/0/0	**1**/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**2**/0/0
More energetic response, with/without vocalization	0/0/0/0	0/0/0/0	0/0/0/0	**1**/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1**/0/0/0
Startle response								
More energetic response, with/without vocalization	**1**/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
Pupil response								
No pupil response	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1**/0/0/0	0/0/0/0	0/0/0/0	0/0/**1**/0
Air righting reflex								
Slightly uncoordinated	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Touch response								
No reaction	0/0/0/0	**1**/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**2**/0/0
Tail pinch response								
More energetic response, with/without vocalization	**1**/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0	0/0/0/0	**1**/0/0/0	**1**/0/0/0	**1**/0/0/**1**
Eyeblink response								
No eyeblinking response	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1**/0/0/0	0/0/0/0	0/0/0/0	0/0/**1**/0
Olfactory orientation								
No reaction present	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0
Neuromuscular								
Hindlimb extensor strength								
Reduced hindlimb resistance, animal shows some weakness	0/0/0/0	0/0/0/0	0/0/0/0	0/**1**/0/0	0/0/0/0	0/**2**/0/0	0/**2**/0/0	0/**3**/0/0
Hindlimb foot splay (mm) – 6h post dosing	62,9	50,8	48,9	**46,3**$^{+}$	55,6	**37,5**$^{+}$	**38,6**$^{+}$	**26,9**$^{++}$
Rotarod performance (s) – study day 7	117,8	100,7	80,3	**69,0***	93,8	56,9	101,3	95,8
Physiological								
Body temperature (degrees C) – 6h post dosing	36,3	36,5	35,9	**35,3**$^{++}$	36,7	36,7	36,2	**35,7**$^{+}$
* = significantly different from the controls at the 0,05 level using fisher's test								

+ = significantly different from the controls at the 0,05 level using dunnett`s test ++ = significantly different from the controls at the 0,01 level using dunnett's test

Locomotor Activity: Within-session repeated measures analyses of variance were conducted across the subintervals of each test session for total and ambulatory counts and for the overall interval means (representing the entire 60-minute session activity) during each test session. Test substance-related effects on locomotor activity patterns were noted in the 250 mg/kg group males and females. Lower mean total and ambulatory counts were noted in the 250 mg/kg group males and females compared to the control group during the first 1 or 2 subintervals (0-10 minutes and 11-20 minutes) at the time of peak effect on day 0. The differences were generally significant ($p<0.002$) and corresponded to the altered gait, convulsions, altered mobility, and/or slight tremors that were observed in these animals during the FOB evaluations on study day 0. In addition, males and females in the 250 mg/kg group habituated slightly more slowly than the control group on study day 0, resulting in higher mean total and/or ambulatory counts generally throughout the remainder of the testing session (21-30 minutes, 31-40 minutes, 41-50 minutes, and/or 51-60 minutes). The differences were generally significant ($p<0.031$) in the 250 mg/kg group males and reached significance ($p=0.022$) during the 51-60 minute interval in the females. As a result, mean overall total counts in the 250 mg/kg group males and females were higher (not statistically significant) compared to the control group. The increases in total counts are likely due to the persistence of tremors/convulsions noted for this group during the FOB assessments as evidenced by increased non-ambulatory movements during these intervals. However, higher mean overall ambulatory counts were not observed in the 250 mg/kg group animals due to the decreased activity noted during the first 10 or 20 minutes of testing which resulted in significantly ($p=0.003$) lower mean overall ambulatory counts for the females while mean overall ambulatory counts in the males were slightly lower (not statistically significant) compared to the control group. There were no other test substance-related effects on locomotor activity observed at the time of peak effect on study day 0, or on study days 7 or 14. A significantly ($p<0.014$) higher mean ambulatory count was observed for the 250 mg/kg group females during the 0-10 minute interval on study day 7. No significant changes were noted for these females during the other subintervals and the value was similar to the maximum mean value in the historical control data of the conducting laboratory, therefore this difference was not attributed to test substance administration.
Test substance-related lower mean total and ambulatory counts were observed in the 150 mg/kg group females compared to the control group during the first 2 subintervals (0-10 minutes and 11-20 minutes) at the time of peak effect on day 0; the differences were generally significant ($p<0.043$). The lower activity noted in the 150 mg/kg group females corresponded to slight tremors observed in these animals during the FOB evaluation on study day 0. As a result of the deficits in mean ambulatory counts noted for the 150 mg/kg group females during the first 20 minutes of the testing session, significantly ($p<0.032$) lower mean overall ambulatory counts were noted in this group when compared to the control group on study day 0. There were no other test substance-related effects on locomotor activity observed in the 150 mg/kg group females at the time of peak effect on study day 0, or on study days 7 or 14.
Locomotor activity patterns (mean ambulatory and total motor activity counts) in the 75 mg/kg group males and females and 150 mg/kg group males were unaffected by test substance administration. The only significant ($p<0.017$) difference between the control and test substance-treated groups was an increase in mean ambulatory counts in the 75 mg/kg group females during the 0-10 minute interval on study day 7. This difference was not observed in a dose-responsive manner and was therefore not attributed to test substance administration. No remarkable shifts in the pattern of habituation occurred in the 75 and 150 mg/kg groups when the animals were evaluated on study days 0, 7, and 14.

Necropsy: There were no macroscopic changes observed for animals at the scheduled necropsy on study day 15. Brain weights and measurements were unaffected by administration of metaldehyde at 75, 150, and 250 mg/kg. There were no statistically significant differences between the control and test substance-treated groups.

Histopathology: No test substance-related microscopic lesions were observed in any of the central or peripheral nervous system tissues examined from 5 animals/sex in the 250 mg/kg group. All histologic changes were considered to be incidental findings or related to some aspect of experimental manipulation other than administration of the test substance. There was no test substance-related alteration in the prevalence, severity, or histologic character of those incidental tissue alterations. There were instances of axonal degeneration in the peripheral nerves and in the spinal nerve roots of animals from the control and 250 mg/kg groups. This axonal degeneration was of minimal severity, typically with only a single 'digestion chamber', and consistent with incidental alterations. Minimal axonal degeneration in the peripheral nerves and spinal nerve roots is a common background lesion[9].

[9]Eisenbrandt, D.L.; Mattsson, J.L.; Albee, R.R.; Spencer P.J.; Johnson, K.A. Spontaneous lesions in subchronic neurotoxicity testing of rats. *Toxicologic Pathology* **1990**, *18*, 154-164.

Conclusion:

The test substance, metaldehyde, was administered orally (gavage) to Crl:CD(SD) rats once at dosage levels of 75, 150, and 250 mg/kg. Morality was noted at 250 mg/kg. The predominant findings during the FOB evaluations in the 250 mg/kg group at the time of peak effect (6 hours after dosing) on study day 0 included tremors, clonic and tonic convulsions, impaired mobility, altered gait, low and very low arousal, shorter hindlimb footsplay, higher mean time to first step, increased mean catalepsy time, and/or lower mean body temperatures. FOB findings in the 150 mg/kg group included tremors and lacrimation. On study day 0, lower total and ambulatory locomotor activity was noted in the 150 mg/kg group females and 250 mg/kg group males and females during the first 10 or 20 minutes of testing and slower habituation was noted for the 250 mg/kg group males and females. There were no treatment-related neurological findings noted on study days 7 and 14, demonstrating that the acute effects of metaldehyde were reversible. There was no evidence of morphological or neuropathological changes at any dosage level. Based on the results of this study, the no-observed-adverse-effect-level (NOAEL) for neurotoxicity of a single dose of metaldehyde to rats was 75 mg/kg for males and females.

Reference:	**LZ1060, metaldehyde: ninety day repeated dose oral (dietary) neurotoxicity study in the rat**
Author(s), year:	Jones L., Finn J., Mullee D., 2003
Report/Doc. number:	Safepharm Laboratories Limited, Derbyshire, UK SPL Project Number: 102/420; Lonza Report No.3644; Doc. No. 533-004
Guideline(s):	OECD Guideline 424 (1997); JMAFF Guideline 12 Nohsan No.8147 (2000)
GLP:	Yes
Deviations:	No
Validity:	Yes

Material and Methods:

10 male and 10 female Sprague-Dawley Crl:CD rats (source: Charles River Ltd., Kent, UK) were fed diets containing concentrations of 0, 100, 500 and 2500 ppm metaldehyde (LZ1060, batch no. 31509, purity 98.3 %) over a period of ninety days. At the start of treatment the animals were six weeks old and weighed 179-243 g (males) and 143-186 g (females). The mean achieved dose levels were equivalent to 0, 8, 39 and 185 mg/kg bw/d. Dietary admixtures were prepared prior to treatment and then twice during the three month study period at approximately monthly intervals.

They were shown to be stable for 6 weeks. Homogeneity and concentration of the samples were confirmed by analysis.

Clinical observations: All animals were examined for overt signs of toxicity or behavioural changes once daily.

Functional observations: Prior to the start of treatment and during weeks 2, 4, 8 and 12, all animals were observed for signs of functional/ behavioural toxicity together with functional performance tests and an assessment of sensory reactivity to different stimuli. Behavioural assessment was performed for each animal using a purpose built arena, including the following parameters: gait, tremors, twitches, convulsions, bizarre/ abnormal/ stereotypic behaviour, salivation, piloerection, exophthalmia, lachrymation, hyperthermia, hypothermia, skin color, respiration, palpebral closure, urination, defecation, transfer arousal and tail elevation. Functional performance tests included motor activity and forelimb/ hindlimb grip strength. Motor activity was assessed with purpose built automated activity monitors. Animals of one sex were tested at each occasion and were randomly allocated to the activity monitors. The tests were performed at approximately the same time each day, under similar laboratory conditions. The percentage of time each animal was active and mobile was recorded for a one hour period. Forelimb/ hindlimb grip strength was tested using an automated grip strength meter. Each animal was allowed to grip the proximal metal bar of the meter with its forepaws. The animal was pulled by the base of the tail until its grip was broken. The animal was drawn along the trough of the meter by the tail until ist hind paws gripped the distal metal bar. The animal was pulled by the base of the tail until its grip was broken. A record of the force required to break the grip for each animal was made. Three consecutive trials were performed for each animal. Sensory reactivity: Each animal was individually assessed for sensory reactivity to auditory, visual and proprioceptive stimuli. The following parameters were observed: grap response, vocalisation, toe pinch, tail pinch, finger approach, touch escape, pupil reflex, startle reflex and blink reflex.

Assessment of motor activity:

Twenty purpose built 44 infra-red beam automated activity monitors were used to assess motor activity. Animals of one sex were tested at each occasion and were randomly allocated to the activity monitors. The tests were performed at approximately the same time each day, under similar laboratory conditions. The evaluation period was one hour for each animal. The percentage of time each animal was active and mobile was recorded for a one hour period and also during the final 20% of the period (considered to be the asymptotic period).

Bodyweight: Individual body weights were recorded on day 0 and at weekly intervals thereafter.

Food and water consumption: Food consumption was recorded for each cage group at weekly intervals throughout the study. Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.

Ophthalmoscopic examination: The eyes of all control and high dose animals were examined pre-treatment and during the final week of the study. Examinations included observation of the anterior structures of the eye, pupillary and corneal blink reflexes, and following pupil dilation with tropicamide solution, detailed examination of the internal structure of the eye.

Pathology: All surviving animals were killed by intravenous overdose of sodium pentobarbitone. Five males and five females from each dose group were then perfused with glutaraldehyde:paraformaldehyde via the heart, following initial perfusion with heparinised saline. The remaining animals were subjected to gross examination only, and any macroscopic abnormalities were recorded.

Organ weights: The brain from all perfused animals was weighed following immersion in buffered 10 % formalin.

Histopathology: Microscopic evaluation was performed on tissues from the perfused control and 2500 ppm dose groups. Samples of the following tissues from all animals were immersed in buffered 10 % formalin (spinal cord dissection was performed to reveal dorsal and ventral root ganglia/fibres following fixation): brain (olfactory bulb, forebrain centre of cerebrum including hippocampus, midbrain, cerebellum, pons and medulla oblongata), dorsal root ganglia (cervical and lumbar regions), dorsal and ventral root fibres (longitudinal cervical and lumbar sections), eyes (longitudinal sections), optic nerve (longitudinal sections), sciatic nerve (proximal-longitudinal and transverse sections), tibial nerve (proximal at knee and calf muscle branches – longitudinal and transverse sections), skeletal calf muscle (transverse sections), spinal cord (longitudinal and transverse cervical and lumbar sections).

Findings:

Clinical observations: A female receiving 2500 ppm showed loss of limb function on day 10 together with increased respiratory rate. The animal was housed individually and breathing recovered by day 13. Hind limb function, however, remained absent. The condition of the animal remained stable with full forelimb movement, but grooming appeared to regress over the following week. In the study report it is stated that loss of the hind limb function in this animal was considered to result from spinal cord injury, even though the lesion was not histopathologically examined. According to the study author in the absence of any similar effects in the other animals throughout the treatment period, this neurotoxic finding is considered to be consistent with an episode of acute toxicity associated with the large of metaldehyde administered at the beginning of the study. The doses received by the concerned animal can be estimated to be in excess of 240 mg/kg, which clearly lays in the acute toxic range of metaldehyde. The condition of the animal failed to improve and it was humanely killed on day 22. There was no evidence of neurotoxicity in any of the 2500 ppm animals. Incidents of hunched posture (and one incident of tiptoe gait on day 14) were noted in one male and three females from day 28 to day 31 but these were isolated and transient. The remaining clinical signs were confined to generalised fur loss and scab formation in treated and control animals. One control animal developed a limp on the left hind limb from day 77 onwards. All these findings were regarded as commonly seen, low incidence findings in laboratory rats.

Functional observations: Behavioural assessments: Detailed open-field observations during week 2, 4, 8 and 12 showed no evidence of neurotoxic effects. Functional performance tests: There were no treatment-related changes in the functional performance parameters measured. The statistically significant intergroup differences detected in motor activity parameters were considered to be isolated changes. A slight but statistically significant increase in hind limb grip strength was detected for 2500 ppm males during week 4. The increase was minimal and, in isolation was considered to be fortuitous. Sensory reactivity assessments: A statistically significant increase in startle reflex parameters was detected for 500 ppm females during week 12, but in the absence of a dose-response relationship, the differences were considered to be incidental and of no toxicological importance.

Some statistically significant intergroup differences were detected in motor activity parameters. As there were no clear dose-response-relationships and also large standard deviations occurred, these findings were considered to be incidental.

All group mean motor activity values for males and females are presented in the following table:

Table 82: Group mean motor activity values and standard deviations

Dietary concentration (ppm)	Number of animals	MALES				FEMALES			
		Motor activity				Motor activity			
		Overall		Final 20% of trial		Overall		Final 20% of trial	
		% activity	% mobile activity	% activity	% mobile activity	% activity	% mobile activity	% activity	% mobile activity
PRETEST									
0 (control)	10	19.6 Sd 4.3	5.0 Sd 1.2	9.2 Sd 7.6	0.8 Sd 1.5	8.2 Sd 5.7	0.1 Sd 0.2	26.9 Sd 10.2	7.6 Sd 3.7
100	10	29.9 Sd 19.0	4.6 Sd 1.5	25.0 Sd 28.6	0.9 Sd 1.2	11.5 Sd 9.6	0.6 Sd 1.0	30.3 Sd 6.7	9.7 Sd 2.0
500	10	30.2 Sd 15.6	6.3 Sd 1.3	19.8 Sd 29.5	0.4 Sd 0.4	13.4 Sd 15.8	4.3 Sd 8.2	38.6 Sd 10.8	13.9 Sd 4.6
2500	10	23.4 Sd 6.4	6.6 Sd 1.7	9.5 Sd 3.9	*1.1 Sd 1.1	8.2 Sd 4.6	0.8 Sd 1.3	27.5 Sd 13.6	8.3 Sd 3.6
WEEK 2									
0 (control)	10	8.4 Sd 9.8	0.4 Sd 0.5	18.8 Sd 6.2	5.1 Sd 2.2	29.7 Sd 26.7	4.7 Sd 5.6	41.4 Sd 20.0	8.9 Sd 3.3
100	10	5.8 Sd 7.0	0.9 Sd 1.0	18.3 Sd 6.7	4.6 Sd 1.8	15.0 Sd 19.9	3.8 Sd 6.4	44.3 Sd 11.0	13.8 Sd 3.9
500	10	*18.2 Sd 32.5	1.6 Sd 3.4	35.9 Sd 24.5	7.6 Sd 3.4	41.2 Sd 34.6	5.2 Sd 4.9	56.5 Sd 12.9	15.5 Sd 4.1
2500	10	**12.2 Sd 21.9	3.4 Sd 7.8	29.6 Sd 10.6	8.2 Sd 3.7	35.6 Sd 21.6	8.3 Sd 6.7	40.6 Sd 6.7	11.2 Sd 3.0
WEEK 4									
0 (control)	10	10.8 Sd 13,2	0.4 Sd 0.5	20.4 Sd 9.2	4.2 Sd 1.3	14.2 Sd 27.9	1.2 Sd 3.5	31.1 Sd 9.9	7.4 Sd 1.2
100	10	14.4 Sd 26.0	0.6 Sd 0.9	27.8 Sd 19.5	5.6 Sd 2.6	27.7 Sd 38.3	6.7 Sd 9.2	37.1 Sd 18.6	8.7 Sd 1.8
500	10	15.5 Sd 26.6	1.2 Sd 1.5	27.8 Sd 22.9	5.9 Sd 1.9	40.2 Sd 25.7	7.5 Sd 7.6	46.9 Sd 14.3	*11.7 Sd 3.9
2500	10	4.9 Sd 7.8	1.1 Sd 2.7	23.5 Sd 15.1	6.6 Sd 3.3	40.5 Sd 22.1	10.1 Sd 8.3	43.4 Sd 14.1	**11.7 Sd 6.6
WEEK 8									
0 (control)	10	21.2 Sd 25.3	2.7 Sd 3.7	29.5 Sd 11.5	8.2 Sd 4.1	17.3 Sd 29.5	1.6 Sd 3.7	36.0 Sd 15.3	7.0 Sd 2.4
100	10	20.7 Sd 17.3	2.1 Sd 1.7	35.1 Sd 14.5	8.6 Sd 4.2	17.0 Sd 24.1	3.9 Sd 7.8	40.0 Sd 12.3	10.8 Sd 3.6
500	10	15.8 Sd 19.8	3.0 Sd 4.5	31.0 Sd 13.6	8.4 Sd 3.8	20.7 Sd 20.6	3.3 Sd 4.3	47.7 Sd 14.0	12.6 Sd 5.3
2500	10	30.4 Sd 21.8	3.7 Sd 4.8	44.6 Sd 21.6	9.4 Sd 4.2	16.5 Sd 18.1	4.2 Sd 5.0	37.9 Sd 16.1	10.1 Sd 6.3
WEEK 12									
0 (control)	10	5.4 Sd 3.9	0.4 Sd 0.4	24.4 Sd 16.6	4.3 Sd 2.4	12.7 Sd 14.4	1.3 Sd 2.1	29.6 Sd 13.3	6.2 Sd 2.9
100	10	12.9 Sd 15.7	1.6 Sd 3.8	27.7 Sd 15.1	5.5 Sd 3.1	18.8 Sd 24.9	*4.1 Sd 8.9	44.4 Sd 13.1	12.0 4.3
500	10	6.5 Sd 9.4	0.9 Sd 2.2	22.3 Sd 10.6	5.2 Sd 2.8	**32.9 Sd 24.6	**5.6 Sd 5.4	51.8 Sd 15.5	12.7 Sd 3.2
2500	10	6.8 Sd 2.7	0.5 Sd 0.5	24.1 Sd 6.9	5.4 Sd 1.6	*36.7 Sd 16.2	*7.5 Sd 6.0	*45.9 Sd 15.2	12.1 Sd 6.3

Sd = Standard deviation

* significantly different from control group $p<0.05$

** significantly different from control group $p<0.01$

Body weight: Females receiving 2500 ppm showed a slight but statistically significant reduction in body weight gain during the first week of treatment when compared to controls. Thereafter body weight development was normal. No such effect was observed for 2500 ppm males or for animals of either sex treated with 500 or 100 ppm.

Food consumption: A slight reduction in dietary intake was evident for 2500 ppm females during weeks 3 and 4 but this was considered attributable to the physical condition of the solitary housed female which had suffered from spinal injury, and was unrelated to test material toxicity. Food efficiency was unaffected throughout the study period.

Table 83: 90 day neurotoxicity study in Sprague-Dawley Crl:CD rats Body weight / body weight gain

	Dose group level (ppm)							
	Males				**Females**			
	0	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Body weight (g)								
- day 0	219	220	221	222	164	164	162	164
- day 90	501	501	516	508	288	294	271	272
Body weight gain (g)								
- week 1	42	42	45	40	20	21	16	14*
- week 5	24	20	20	20	9	14	10	9
- week 9	14	9	8	13	5	6	5	6
- week 13	11	14	9	10	0	4	5	4

* ($p < 0.05$); significantly different from controls

Ophthalmoscopic examination: There were no treatment-related effects observed.

Brain weights: There were no treatment-related changes in brain weights, absolute and relative to body weight.

Necropsy: No macroscopic abnormalities were detected in test or control animals at terminal kill. The 2500 ppm female which developed loss of hind limb function and which was sacrificed prematurely showed no macroscopic abnormalities.

Histopathology: There were no treatment-related changes in the tissues examined.

Conclusion:

No evidence of neurotoxicity was revealed from the subchronic administration of metaldehyde at any dose level tested (100, 500 and 2500 ppm). The NOAEL for neurotoxic effects is therefore considered to be 2500 ppm (equivalent to 185 mg/kg bw/d). At the high dose level, one female showed loss of hind limb function together with an increased respiratory rate. Loss of the hind limb function was considered to result from spinal cord injury, even though the lesion was not histopathologically examined. In the absence of any similar effects in the other animals throughout the treatment period, this finding was considered to be an acute toxic effect. In the females receiving 2500 ppm, a slight and transient reduction of bodyweight gain was detected during the first week of treatment. Based on the finding of reduced body weight gain and the loss of hind limb function in one female at the high dose, the NOAEL for systemic toxicity was considered to be 500 ppm (equivalent to 39 mg/kg bw/d).

In the acute neurotoxicity study, CD rats were treated with a single oral dose of 75, 150 or 250 mg/kg metaldehyde by gavage. 250 mg/kg led to mortality in 5 of 10 female rats within 24

hours after administration. Treatment with 150 mg/kg and 250 mg/kg caused transient clinical signs and findings in the neurological screening with were recorded only on the administration day with females being more affected than males. The neurological screening revealed several changes like pilo-erection, tremor, diarrhea, increased body temperature, convulsions, impaired ability for wire maneuver, impaired gait, decreased resistance during limb rotation, increased hindleg splay, a reduced righting reflex and/or a reduced toe/tail pinch response. The histomorphological examination of the nervous system did not reveal any pathological evidence. The NOAEL of this study for both systemic toxicity and neurotoxicity is therefore set at 75 mg/kg metaldehyde, based on the findings in the neurological screening at the dose level of 150 mg/kg.

A 90 day neurotoxicity study was conducted in Sprague-Dawley Crl:CD rats. No evidence of neurotoxicity was observed following subchronic administration of metaldehyde at any dose level tested (100, 500 and 2500 ppm). At the high dose level of 2500 ppm (185 mg/kg bw/d), one female showed loss of hind limb function together with an increased respiratory rate. Loss of the hind limb function was considered to result from spinal cord injury, even though the lesion was not histopathologically examined. In the females receiving 2500 ppm, a slight and transient reduction of bodyweight gain was detected during the first week of treatment. The NOAEL for both systemic toxicity and neurotoxicity is considered to be 500 ppm (equivalent to 39 mg/kg bw/d) based on the finding of reduced body weight gain and the loss of hind limb function in one female at 2500 ppm.

Reference:	**Metaldehyd: A 90-day dietary combined toxicity and neurotoxicity study in Sprague-Dawley rats**
Author(s), year:	Gauvin, G. V., 2010
Report/Doc. number:	Report No. 1714-002 Conducting laboratory: MPI Research, Inc., Mattawan, Michigan
Guideline(s):	OECD Guideline 408 (1998); OECD Guideline 424 (1997) US EPA, OPPTS 870.6200 (1998)
GLP:	Yes
Deviations:	No
Validity:	Yes

Material and Methods:

Test material	Metaldehyde
Lot/Batch	080702
Purity	100.0%
Vehicle	LabDiet 5002 Certified Rodent Diet Meal
Species	Rat
Strain	CD, Crl:CD(SD)
Age	Approximately 6 weeks at receipt + 28 days acclimation period
Weight at dosing	Males: 244-285g; Females: 175-217g
Source	Charles River Laboratories, Portage, Michigan
Number of animals	60 males, 60 females

The animals were housed individually. The test material Metaldehyde was administered orally for 13 weeks as a diet additive. Diet and tap water were available ad libitum. Dose levels of 250, 750 and 2500 ppm were tested by using dose groups of 10 male and 10 female animals each for the

FOB (Functional Observational Battery) evaluation and 5 male and 5 female each for the locomotor activity evaluation (Table 84).

Table 84: 90-day dietary combined toxicity and neurotoxicity study in rats - Study Design

		Number of Animals	
Group Number[a]	**Dose Level (ppm)**	**Male**	**Female**
1	0	10	10
2	250	10	10
3	750	10	10
4	2500	10	10
5	0	5	5
6	250	5	5
7	750	5	5
8	2500	5	5
[a] Groups 1 through 4 were designated for FOB evaluations and Groups 5 through 8 were designated for locomotor activity evaluations.			

General Observations: All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily. Body weights for all animals were measured and recorded the day of receipt (not reported), prior to randomization (not reported) and weekly beginning day-1 during the study. Food consumption was measured and recorded daily during the study and reported weekly. Food efficiency was calculated for each week that food consumption was measured.

Detailed clinical examinations (DCE) of each animal were performed one week predose and during weeks 2, 6 and 10 of the study. Following each FOB detailed clinical observations (DCO) of each animal were performed one week predose and during weeks 1, 5, 9 and 13 of the study. Both DCE and DCO included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and bizarre behavior.

FOB and Locomotor activity evaluations were performed on all designate animals one week predose and during weeks 1, 5, 9 and 13 of the study. FOB evaluations were conducted without knowledge of the treatment groups and included those conducted in the home-cage, during handling, in the open field and others. During open-field evaluations, each animal was observed for a minimum of 3 minutes in a black plexiglass™ observation box measuring 20 x 20 x 8 inches. The parameters evaluated in the FOB were based on those outlined in Moser et al., 1988[1] and Moser et al., 1996[2]. The observations included, but were not limited to, evaluation of activity and arousal, posture, rearing, bizarre behavior, clonic and tonic movements, gait, mobility, stereotypy, righting reflex, response to stimulus (approach, click, tail pinch, and touch), palpebral closure, pupil response, piloerection, exophthalmus, lacrimation, salivation, and respiration. The amount, qualitative and/or quantitative measures, of defecation and urination were also recorded. Forelimb and hindlimb grip strength was measured using the procedure described by Meyer et al., 1979[3] and

hindlimb splay was quantitatively measured as described by Edwards and Parker, 1977[4]. Pain perception was assessed by measuring the latency of response to a nociceptive (thermal) stimulus when each animal was placed on a hot plate apparatus set to 52 ± 1°C as described by Ankier, 1974[5]. Body weight and temperature were also measured. For the Locomotor activity evaluations each animal was placed into the correct Hamilton-Kinder enclosure for monitoring. The duration of monitoring was 60 minutes with the data summarized into 10 minute segments. A range of different activities were assessed in a three dimensional array and were recorded. Only basic movement, fine movement, rearing, and distance (cm) were used in comparisons between treated and control animals as the most representative activity parameters.

[1] Moser VC, McCormick JP, Creason JP, MacPhail RC. Comparison of chlordimeform and carbaryl using a functional observational battery. Fund Appl Toxicol 1988;11:189-206.
[2] Moser VC, Ross JF. US EPA/AIHC Training video and reference manual for a functional observational battery. US Environmental Protection Agency 1996; Washington, D.C.
[3] Meyer OA, Tilson H A, Byrd WC, Riley MT. A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. Neurobehav Toxicol 1979;1:233-236.
[4] Edwards PM, Parker, VH. A simple, sensitive and objective method for early assessment of acrylamide neuropathy in rats. Toxicol Appl Pharmacol 1977;40:589-591.
[5] Ankier SI. New hot plate tests to quantify anti-nociceptive and narcotic antagonist activities. Eur J Pharmacol 1974;27:1-4.
[6] Latendresse JR, Warbrittion AR, Jonassen H, Creasy DM. Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. Toxicol Pathol. 2002 Jul-Aug;30(4):524-33.

Ophthalmoscopic examinations were conducted on all animals pretest and prior to terminal necropsy.

Clinical pathology evaluations were conducted on ten animals/sex/dose level selected from the FOB and locomotor activity animals prior to terminal necropsy. The animals had access to drinking water but were fasted overnight prior to sample collection. Blood samples were collected via the vena cava after carbon dioxide inhalation.

For Neuropathology evaluations five rats/sex/dose level were randomly selected for neuropathology evaluations from the 10 rats/sex designated for FOB examinations. These rats were euthanized. The brain, spinal cord at the cervical (C3-C7) and lumbar (T13-L4) swelling, proximal sciatic nerve, mid thigh, sciatic notch, sural, tibial, and fibular nerves, trigeminal, and dorsal root ganglia, and dorsal and ventral root fibers were excised and fixed in 3% paraformaldehyde and 3% glutaraldehyde in 0.1 M phosphate buffer.
Tissues were either embedded in plastic or paraffin. Central nervous system (CNS) and peripheral nervous system tissues and skeletal muscle were stained with hematoxylin and eosin and a sequential section from the same block was stained with Luxol fast blue/cresyl violet. For the initial qualitative analysis, microscopic examinations of paraffin and plastic sections were performed on all designated animals at 0 and 2500 ppm.

Necropsy examinations were performed on all surviving animals not designated for neuropathology evaluations at terminal necropsy. The animals were euthanized and examined carefully for external abnormalities including masses. The skin was reflected from a ventral midline incision taking care not to open the thoracic cavity, and any abnormalities were identified and correlated with antemortem findings. The trachea was exposed and clamped, the thoracic cavity opened, and the lungs removed and examined while inflated. The lungs were deflated and weighed with the trachea.

The abdominal, thoracic, and cranial cavities were examined for abnormalities and the organs removed, examined, and, where required, placed in fixative. The pituitary was fixed in situ. All designated tissues were fixed in neutral buffered formalin, except for the eye (including the optic nerve with contiguous Harderian gland) which was fixed using Davidson's fixative. Formalin was infused into the lung via the trachea and into the urinary bladder. A full complement of tissues and organs was collected from designated animals.

Organ weights were recorded for all surviving animals not designated for neuropathology evaluations at the scheduled necropsy and appropriate organ weight ratios were calculated (relative to body and brain weights). Paired organs were weighed together. The thyroid/parathyroid gland and pituitary gland were weighed following fixation. A combined weight of the thyroid gland with bilateral parathyroid glands was obtained.

Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on tissues from the control (0 ppm) and high dose groups (2500 ppm). A four-step grading system was utilized to define gradable lesions for comparison between dose groups. Slides from all treatment groups were examined and scored for both renal and hepatic tissues.

Results:

Mortatlity: One male rat from the high dose group died on Day 7 of the study. It was not necropsied at the time of death since the animal was in one of the "locomotor activity" designated groups which were exempt from this requirement in the protocol. In the opinion of the Study Director this single death was spontaneous and of idiopathic origin and not atypical in these types of toxicity studies.

Body Weight: There were slight between differences in the group mean bodyweights at the initiation of the study feeding schedule. The rank differences between the groups appeared to remain stable across the 13 weeks of exposure to metaldehyde feed admixtures. The rate of growth appeared unaffected by metaldehyde treatments. Male rats demonstrated a normal growth rate regardless of the dose of metaldehyde consumed. In contrast, the female rats exposed to the 2500 ppm metaldehyde food admixture did not gain weight during the first week of treatment compared with the gain in weight of controls and other dosed groups. This differential weight was not re-gained across the remaining 12 weeks of the study in the female high dose treatment group (2500 ppm), while all female rats showed normal growth rates. The reduced bodyweights noted for females in the high dose (2500 ppm) group were attributed to an initial palatability effect and a slightly reduced food intake during the study. There was no effect on food efficiency and the animals remained healthy and active. Therefore, although the reduced bodyweight is related to dietary exposure to metaldehyde it is considered unlikely to reflect a toxicologically significant effect.

Food Consumption: There was a decrease in voluntary food admixture consumption during the first week of exposure to metaldehyde in both male and female rats. The decrease was limited to an approximate 2-3 grams of daily ration in male and female rats, respectively. This decline was attributed to a common feature of rodent behavior, "bait shyness", attributed to an initial palatability effect, which was short lived. By Week 2, all rats demonstrated equivalent week-to-week variations in the amount of food consumed. Male rats did not appear to demonstrate any dose-related changes in group mean food consumption over the course of the 13 weeks of exposure. Whereas, female rats in the 2500 ppm group consistently ate slightly less than females in the control and the other

treatment groups. Food efficiencies in male and female rats demonstrated a differential pattern over the 13 weeks of data collection and there was more intergroup variation among females. However, no dose-dependent differences were noted for either male or female rats on this study and the variability between groups was not considered to be biologically relevant.

Clinical Examinations: No clear dose-related or notable changes in clinical examinations were found on this study. There were some incidences of "sparse hair" in both male and female metaldehyde treated rats that were the cause of animals not being categorized as "normal"; however, these signs were not clearly dose-dependent and not out of the normal expectations in a 13 week rodent study of this type.

Clinical Observations: Similar clinical signs were found during these observations as was reported for the interim examinations conducted as described above. While the total number of animals categorized as "normal" declined within and between weeks on study, these were not clearly dose-dependent. Overall, it is considered that there were no toxicologically significant detailed clinical observations among metaldehyde treated groups.

Functional Observational Battery (FOB): When reviewing the FOB, neurotoxicity is not defined by a single element or finding[7,8]. Generally, clusters of behavioral markers will most reliably support a claim on neurotoxicity [9,10]. Based on the behavioral and functional domains of the FOBs, no clear or definable neurotoxicity was demonstrated in any rat receiving up to 2500 ppm of metaldehyde over the 13 weeks of daily exposure in food admixtures.

[7] Chang LW (editor). Principles of Neurotoxicology. New York: Marcel Decker. 1994.
[8] Annau Z (editor). Neurobehavioral Toxicology. Baltimore: The Johns Hopkins University Press. 1986.
[9] Russell RW, Flattau PE, Pope AM (editors). Behavioral Measures of Neurotoxicity: Report of a Symposium. Washington DC:National Academy Press. 1990.
[10] Weiss B, O'Donoghue JL (editors). Neurobehavioral Toxicity: Analysis and Interpretation. New York:Raven Press. 1994.

Locomotor Activity: There were some group differences noted during the first 10 minutes of the 60 minute monitoring periods on individual parametric measurements of activity. Week-to-week variations in the temporal changes in rearing, fine movements, basic movements, and total distance traveled were similar across all treatment groups. All rats in all four treatment groups showed normal "habituation" to the environments with the normal and expected decline in activity across the 10 minute monitoring segments of the one-hour sessions. Metaldehyde did not have any significant effects on motor function, learning, and memory as assessed in the locomotor activity monitoring on this study.

Opthalmoscopic Examinations: There were no test article-related changes in the results of the opthalmoscopic examinations conducted in this study. Normal age and gender related findings were noted that were not attributed to metaldehyde exposures.

Clinical Pathology: There were no definitive test article-related effects among hematology parameters in either sex at any dose. In males there were minimal decreases in lymphocytes at 250 ppm (21%), 750 ppm (24%) and 2500 ppm (20%). The values remained within expected historic control ranges. There was an increase in neutrophils at 2500 ppm (70%) relative to controls, which was attributed to 2 individual animals with high values. Similar changes were not seen in females

and these changes lacked a dose response hence they are considered unlikely to be related to the test article. No other toxicologically relevant alterations among hematology parameters were observed.

There were no test article-related effects on coagulation parameters in either sex at any dose.

There were no definitive test article-related effects among chemistry parameters in either sex at any dose. Two individual male animals had mild increases in aspartate aminotransferase (AST) and creatine kinase relative to controls and other animals within the group. These changes indicate mild muscle injury which may be test article related or incidental; however, they did not reach statistical significant relative to controls.

Necropsy: There were no metaldehyde-related macroscopic effects at any dose level in either sex. All macroscopic findings were considered to be incidental.

Organ weights: Metaldehyde-related organ weight effects were limited to the liver. Statistically significant increased liver/body weight % was observed in males given ≥ 750 ppm (Table 85) and correlated with microscopic hepatocellular centrilobular hypertrophy. Although liver weight increases were not observed in females (with the exception of a slightly increased liver/body weight % in 2500 ppm females), hepatocellular centrilobular hypertrophy was also observed in females given ≥ 750 ppm. Statistically significant organ weight increases were present in the pituitary glands of females and adrenal glands of males. However, microscopic correlates were not observed in either the pituitary or the adrenal glands, the increases were of similar magnitude at higher dosages (i.e. lacked a strong dose dependent response) and similar trends were not observed in the opposite sex. Absolute pituitary weights were increased by 13 (not statistically significant), 19 and 21%, pituitary/body weight % were increased by 18, 24 and 29% at 250, 750 and 2500 ppm, respectively and pituitary/brain weight ratios were increased by 10, 15 and 17% at 250, 750 and 2500 ppm, respectively in females (statistically significant at 2500 ppm only). Statistically significant increases in absolute and relative/body weight % were observed in the adrenal gland of males given 2500 ppm by 22 and 28%, respectively. It should be noted that the mean control values in this study were slightly lower than those observed in control data of the conducting laboratory which may have accounted for the apparent organ weight increases. The mean historical control weight for female pituitary glands was 0.02021 compared to the study control mean of 0.0175. The mean historical control weight for male adrenal glands was 0.0641 compared to the study control mean of 0.060. Based on the lack of microscopic correlates and lower than average control mean weights, the increased pituitary and adrenal gland weights were not considered related to metaldehyde administration. All other organ weight changes were considered reflective of expected biological variation and not directly related to metaldehyde based on lack of statistical significance, microscopic findings and/or lack of dose dependency.

Table 85: Metaldehyde-related liver weight changes

Male and Female (Percent change relative to control)						
Dose level: ppm	250		750		2500	
Sex	M	F	M	F	M	F
Liver	↑2%	↓7%	↑9%	↓4%	↑12%	↓1%
Liver/BWt%	↑8%	↓3%	↑13%[b]	↓<1%	↑18%[b]	↑4%
Liver/BrWt ratio	↑5%	↓10%[a]	↑8%	↓7%	↑8%	↓5%

[a]Significantly different from control; ($p<0.05$)	↑ - Increased
[b]Significantly different from control; ($p<0.01$)	↓ - Decreased
BWt - Body Weight	BrWt - Brain Weight

Microscopic examinations: Metaldehyde-related microscopic effects were observed in the livers of males and females given ≥750 ppm. Hepatocellular centrilobular hypertrophy was characterized by enlargement of the hepatocytes surrounding the central vein with abundant cytoplasm. There were minimal increases in the incidence and severity of hepatocellular changes in the 750 ppm treatment group. The changes that were documented in this group were considered normative adaptive changes and not adverse. A notable increased incidence and severity of tubular mineralization was observed in the kidneys of females given 2500 ppm. Tubular mineralization is frequently observed in the kidneys of rats (i.e. background lesion). There were no reported abnormalities in renal clinical chemistry parameters and tubular mineralization was not observed in the males. Although the tubular mineralization was not likely directly related to metaldehyde administration, the possibility of metaldehyde involvement at 2500 ppm could not be fully excluded. All other microscopic findings were considered incidental and of the type infrequently observed in rats of this strain and age.

Neuropathology: There were no metaldehyde-related neuropathologic macroscopic or microscopic findings in either the central or peripheral nervous systems. Focal axonal/myelin degeneration was observed sporadically in the various peripheral nerve segments in both control and treated animals. Focal axonal/myelin degeneration can be a common background finding in the peripheral nerves of rats and often increases in severity and frequency with age. All observed axonal/myelin degeneration in this study was considered to be incidental.

Conclusion: Metaldehyde exposure of up to 2500 ppm in food admixtures did not produce any significant neurotoxicity in male or female rats on this study. A NOEL for neurotoxicity of 2500 ppm metaldehyde has been established by this study.

Structurally, a daily dose exposure of 2500 ppm metaldehyde for 13 weeks was found to produce significant changes in liver weights and incidences of hepatocellular centrilobular hypertrophy. Changes in the 750 ppm treatment group were minimal and not considered adverse. For these reasons the NOAEL for metaldehyde-induced systemic toxicity in the present study is set at 750 ppm (39.3 and 46.85 mg/kg/day for male and female rats, respectively).

In order to clarify the neurotoxic potential of metaldehyde, a position paper was requested from the notifier, as convulsions (or other signs of neurotoxicity) were observed in most of the studies and species (acute oral, 28-d rats, 1-year dogs, chronic toxicity rats, 2 generation rats, developmental rat and rabbit). The **notifier** considers the central nervous system not a specific target organ of short term toxicity of metaldehyde. The following argumentation was provided by the notifier:

- Metaldehyde is rapidly absorbed by rats after oral exposure with no evidence of accumulation as metaldehyde is rapidly and effectively metabolised to acetaldehyde, then further oxidised through the citric acid cycle and the major part expired as carbon dioxide (Selim, 1990, Doc. No. 512-001, see DAR).

- This kinetic profile determines the toxic profile which is characterized by acute and transient effects occurring immediately after dosing in the period of the most elevated plasma concentrations. Studies with repeated daily administration caused repeatedly daily clinical symptoms of acute intoxication which were transient.
- Both single and repeated treatments induced acute toxic effects including partly pronounced neurological symptoms.
- Metaldehyde induced neurofunctional effects like ataxia, twitching, tremors and/or convulsions following repeated exposure. As these effects did not persist, they were not in line with sustained dysfunctions normally induced by classical neurotoxins.
- Because of the distinct differences between Metaldehyde and classical neurotoxins, the term "neurotoxic effect" was not considered adequate to characterize the toxicity profile of metaldehyde.
- In part of the metaldehyde studies, there were individual cases of mortality. These cases were not seen frequently and did not follow a dose and time related incidence pattern that would point towards the presence of cumulative effects or other typical modes of actions related to subchronic dosing. With respect to the above kinetic and toxic profile these cases are interpreted as the combination of a rapid increase of significant metaldehyde plasma concentration with individual cardiovascular susceptibility of the animal in question at the day of administration.
- Hind limb paralysis/paresis due to mechanic vertebral lesions was not very frequently observed. They were considered to occur secondary to pronounced acute neurological symptoms causing mechanical impact on the lumbar region of the vertebral column. A genetic predisposition of certain rat in- and outbred strains for mechanical liability of the lumbar vertebrae could be a contributing factor for the observed lesions. This finding is clearly not caused by a neurotoxic mechanism leading to degeneration or other toxic damage to the central or peripheral nerve tissue. These single cases are relevant for the acute toxicity profile of metaldehyde, but not relevant for classification and labelling as neurotoxicant.

The **RMS (i.e. dossier submitter)** can follow the notifier´s arguments that the neurofunctional effects (e.g. tremor, convulsions, ataxia, paresis) following exposure to metaldehyde only occur at doses which are clearly acute toxic and are therefore covered by acute toxicity classification.

4.12.1.2 Immunotoxicity

No data available.

4.12.1.3 Specific investigations: other studies

Not relevant for classification and labelling of metaldehyde.

4.12.1.4 Human information

See also section 4.2 Acute Toxicity

Signs and symptoms of acute poisoning: Some information is presented in the Toxicology Update published in the Journal of Applied Toxicology (*Von Burg R., 1991; Doc.No. 592-028*), which is a brief review on toxicology data found in literature data bases.

However, more detailed information is found in the dissertation of *Borbely A. (1970, Doc.No. 592-001).* The neurological symptoms of metaldehyde are described as follows:

Confusion, restlessness, haziness, drowsiness, coma, spasms, tremor, muscle twitching, chorea, abnormal reflexes, ataxia, elevated muscle tone, hypersensitivity, Chvostek´s sign, Trousseau´s sign, disturbed vision, amnesia, respiratory arrest, risus sardonicus.

4.12.2 Summary and discussion

Neurofunctional effects (e.g. tremor, convulsions, ataxia, paresis) following exposure to metaldehyde are considered to occur only at doses which are clearly acute toxic. These effects do not persist (with exception of hind limb paresis following spinal cord injury) and are not in line with sustained dysfunctions normally induced by classical neurotoxins. Because of the distinct differences between metaldehyde and classical neurotoxins, the term “neurotoxic effect” was not considered adequate to characterize the toxicity profile of metaldehyde. Therefore in the EFSA peer review it has been agreed that metaldehyde does not require classification as neurotoxicant. The accurate conclusion drawn by EFSA is “Acute toxic effects following metaldehyde administration include partly pronounced neurological symptoms, without specific neurotoxic mechanism leading to degeneration or other toxic damage to the central or peripheral nerve tissue. Therefore these reversible effects at high doses are not relevant for classification and labelling as neurotoxicant.“ (see peer review report, evaluation table, 2.2. point of clarification, http://registerofquestions.efsa.europa.eu/roqFrontend/outputLoader?output=ON-1856)

4.12.3 Comparison with criteria

Not relevant, not required.

4.12.4 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: no classification proposed

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 86: Summary of relevant information on degradation

Method		Results			Remarks	Reference
Guideline	**Type of study**	**Matrix**	**Temp.**	**Result/Half-life**		
EPA Subdiv. N, Section 161-1	Hydrolysis	pH 5, 7 and 9, sterile buffer solutions	25 °C	hydrolytically stable		Carpenter M. (1989a)
EPA Subdiv. N, Section 161-2	Photolysis	pH 7, sterile buffer solution	25 °C	photolytically stable		Carpenter M. (1989b)
OECD 301E	Biological degradation (ready)	test medium inoculated with active sewage sludge	24 – 26.5 °C	not readily biodegradable		Wüthrich V. (1990a)
OECD 301F	Biological degradation (ready)	test medium inoculated with active sewage sludge	22 °C	not readily biodegradable		Lebertz, H. (2008)
OECD 302B	Biological degradation (inherent)	test medium inoculated with active sewage sludge	19 – 23.5 °C	not inherently biodegradable		Wüthrich V. (1990b)
BBA part IV-5-1	Water/Sediment study	water: pH 8.4 - 9 sediment: pH 7.8 – 7.9	20 ± 2 °C	**Water** **DT_{50}:** 11.35 d (S1), 10.25 d (S2) **DT_{90}:** 37.71 d (S1), 34.07 d (S2) **Whole system:** Metaldehyde **DT_{50}:** 4.10 d (S1), 4.42 d (S2) **DT_{90}:** 13.61 d (S1), 14.97 d (S2) Acetaldehyde **DT_{50}:** 30.98 d (S1), 19.01 d (S2) **DT_{90}:** 102.90 d (S1), 63.14 d (S2)		Möllerfeld J., Römbke J. & Heller M. (1993) – Calculations reviewed June 2009
OECD 308	Water/Sediment study	water: pH 5.1 – 7.8 sediment: pH 7.4 – 8.0	20 ± 2 °C	**Water** **DT_{50}:** > 1000 d (Silt loam), 473 d (Sand) **DT_{90}:** > 1000 d (Silt loam), > 1000 d (Sand) **Whole system:** Metaldehyde **DT_{50}:** >1000 d (Silt loam), >1000 d (Sand) **DT_{90}:** > 1000 d (Silt loam), > 1000 d (Sand)		Kane, T. (2009)
OECD 307	Aerobic degradation	pH: 6.1 – 7.3 OC 1 % - 4.2 %	20 °C	**DT_{50} Hockey-stick** 6.6 d – 19.4 d		Juozenaite, A. (2009)

	in soil					

5.1.1 Stability

Reference: Carpenter M. (1989a): Hydrolysis of metaldehyde as a function of pH at 25 °C. ; LONZA Report No. 1410; Document No. 711-001

Guideline: EPA, Pesticide Assessment Guidelines, Subdivision N, Section 161-1, Hydrolysis studies, October 1982

GLP: Yes

Test item: [U-^{14}C] metaldehyde, radiochemical purity >99 %, batch no. 8232

Material and methods:

The hydrolytic stability of [U-^{14}C] metaldehyde in an aqueous solution of a nominal concentration of 25 µg/L was studied at pH 5, 7 and 9. Two different pH 7 buffers were applied to evaluate buffer catalysis of the degradation process: TRIS buffer (tris(hydroxymethyl)aminomethane/HCL) and HEPES buffer (N-2-Hydroxyethylpiperazine-N`-2-ethane-sulfonic acid). The pH 5 and pH 9 buffer solutions consisted of acetic acid / sodium acetate and boric acid / borax. The test vessels were incubated in the dark under sterile conditions at 25°C up to 32 days. Duplicate samples were taken at days 0, 1, 3, 6, 9, 14, 22 and 32 and analysed by LSC, TLC and reverse phase HPLC. The DT_{50} values were calculated with linear regression assuming first order kinetics.

Findings:

The pH values measured on each sampling day showed deviations of only 0.01 units for the pH values showing sufficient stability of the buffer solutions. The concentration of metaldehyde remained constant until the end of the study. The measured amounts of applied radioactivity in all samples were within the range of 98.6 – 100 %. No hydrolytic degradation products were observed except for one sample at pH 5 at day 22 where small amounts of paraldehyde and acetaldehyde were detected. Since this was the only sample at which degradation was observed the sample was considered to be an outlier (rejected based on the "Q" test) and was not further taken into account.

Conclusion:

Metaldehyde was hydrolytically stable at a temperature of 25 °C and pH values of 5 – 9.

Comment (RMS):

The study was considered to be acceptable.

Photochemical Degradation

Reference: Carpenter M. (1989b): Photodegradation of metaldehyde in pH 7 buffered solution. LONZA Report No. 1412; Document No. 712-001

Guideline: EPA Pesticide Assessment Guidelines, Subdivision N, Section 161-2, Photodegradation studies in water, October 1982

GLP: Yes

Test item: [^{14}C]-metaldehyde, radiochemical purity 97.7 %, batch no. 8232

Material and methods:

^{14}C-metaldehyde was applied to a pH 7 buffer solution at an initial concentration of 30 µg/L. Additionally photosensitized samples were prepared containing 1 % by volume acetone. The test vessels were incubated at 25 ± 1 °C under sterile conditions and continuous irradiation with a xenon arc lamp (wavelength 300 – 750 nm, 0.02937 – 0.8649 W/m^2) up to 30 days. Duplicate samples were taken on days 0, 1, 3, 7, 14 and 30 and analysed by LSC and HPLC.

Findings:

No degradation of metaldehyde was observed in the dark controls, in irradiated sensitized

samples and in irradiated non-sensitized samples. The measured concentration of metaldehyde remained stable during the test within a range of 95.9 – 98.1 % of applied radioactivity. Since no photolytical degradation was observed (regardless of the presence of acetone as a photosensitizer) no estimation of the photolytical half-life was conducted.
Conclusion:
Metaldehyde is photolytically stable at pH 7 and a temperature of 25 °C.
Comment (RMS):
The study was considered to be acceptable.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

Reference: Wüthrich V. (1990a): Ready biodegradability: Modified OECD Screening Test for P0071. LONZA Report No. 1490; Document No. 713-002
Guideline: OECD 301 E: Ready Biodegradability: Modified OECD Screening Test (1981)
GLP: Yes
Test item: Metaldehyde, purity 99.3 %, batch no. 5448
Material and methods:
Metaldehyde was dissolved at a nominal concentration of 100 mg/L in a test medium containing 0.5 mL active sewage sludge per 1000 mL. The flasks were loosely covered with an aluminium foil. Duplicate test vessels with metaldehyde, aniline and blank controls were incubated for 28 days in the dark at 24 – 26.5°C. Samples were taken for DOC analysis on days 0, 7, 21 and 28.
Findings:
Aniline reached a level of biodegradation of 97 % within 7 days showing sufficient biological activity of the sewage sludge. 18 % degradation of metaldehyde was observed within the 28-day incubation period. Metaldehyde was slightly degraded but it failed clearly the trigger value of 70 % removal of DOC. Therefore it has to be classified as not readily biodegradable.
Conclusion:
Metaldehyde is not readily biodegradable.
Comment (RMS):
The pH value was not measured, but since the test medium was prepared according to OECD guideline 301, the study was considered to be acceptable.

Reference: Wüthrich V. (1990b): Inherent biodegradability: “Modified Zahn-Wellens Test”. LONZA Report No. 1488; Document No. 713-001
Guideline: OECD 302 B: Inherent Biodegradability: Modified Zahn-Wellens Test (1981)
GLP: Yes
Test item: Metaldehyde, purity 99.3 %, batch no. 5448
Material and methods:
Metaldehyde was dissolved in a test medium at a nominal concentration of 100 mg/L. The test medium was prepared according to OECD guideline 302. An amount of sludge corresponding to 0.2 g dry material was added per litre test medium. Two replicates of the sample treated with metaldehyde one sample treated with aniline and two blank controls were incubated in the dark for 28 days at 19 – 23.5 °C. The flasks were aerated with a flow rate of 0.5 – 0.7 L/minute

resulting in an oxygen concentration of 8.4 – 9.7 mg O_2 per litre. The pH values were in the range of 7.5 – 7.8. Samples were taken for DOC analysis immediately after application and at 3 hours, 7, 14, 21 and 28 days after treatment.
Findings: Aniline degraded by 98 % within 14 days showing sufficient biological activity of the sewage sludge. After 28 days only 6 % degradation of metaldehyde was observed. The level of 20 % reduction in DOC within 28 days was not reached and therefore metaldehyde has to be classified as not inherently biodegradable.
Conclusion:
Metaldehyde is not inherently biodegradable.
Comment (RMS):
The study was considered to be acceptable.

Reference:	**Study on ready biodegradability of Metaldehyde**
Author(s), year:	Lebertz, H., 2008
Report/Doc. number:	Lonza Report No. 4317, Doc. No. 713-003
Guideline(s):	OECD 301 F
GLP:	Yes
Deviations:	None
Validity:	Yes

Material and methods:
A test medium was prepared according to the OECD Guideline 301 F and inoculated with active sewage sludge. Metaldehyde was dissolved in the test medium at a nominal concentration of 100 mg/L. The incubation flasks were closed and incubated for 28 days at 22°C. The oxygen uptake was continuously measured by using a manometer. Sodium benzoate was used as a positive control.

Findings:

Metaldehyde showed a degradation of 2.8 % within the 28-day incubation period and is classified as not readily biodegradable in terms of this test (Table 87). The positive controle sodium benzoate reached a level of 91 % biodegradation within 28 days showing an adequate activity of the used inoculum.

Table 87: Ready biodegradability test with Metaldehyde and the positive control Sodium benzoate (results expressed as % degradation)

Time after application [days]	Degradation of Metaldehyde		Degradation of Sodium Benzoate
	Set 1	Set 2	
0	0.0	0.0	0
7	1.0	0.3	79
14	2.1	0.6	88
21	2.8	1.6	90
27	3.3	2.1	91
28	3.3	2.3	91

Conclusion:
Due to the fact that only 2.8 % of the Metaldehyde was degraded during the 28 day incubation period it has to be classified as not readily biodegradable in terms of this test.

Comments (RMS):
The study is considered acceptable

5.1.2.3 Simulation tests

Reference:	Determination of the degradation and persistence of ^{14}C-Metaldehyde in the water/sediment-system
Author(s), year:	Möllerfeld, J., Römbke, J., Heller, M., 1993
Report/Doc. number:	Lonza Report No. 2120, Doc. No. 714-001
Guideline(s):	BBA Guideline
GLP:	Yes
Deviations:	None
Validity:	Yes

For details on material and methods, please refer to DAR.

The study described under PD June 2009, IIA, 7.1/12 showed that Acetaldehyde is trapped by soda lime but not liberated with HCl. The rate of degradation of Metaldehyde in water/sediment systems was determined using non-linear regression assuming first-order reaction kinetics. On request of the member states and EFSA, the DT_{50} and DT_{90} values of Metaldehyde were re-calculated during dossier preparation according to the recommendations of the FOCUS Kinetic Working group.

Findings:
The formation of CO_2 was very high, accounting for a maximum of 61.57 % and 68.80 % of the applied radioactivity on day 100 for the sandy and loamy system, respectively. The study described under PD June 2009, IIA, 7.1/12 confirmed that only CO_2 is liberated by adding of HCl. Therefore, the radioactivity presented for the soda lime traps is only related to the volatile product CO_2. Other volatile compounds did not exceed 0.12 % of the applied radioactivity.

The amount of ^{14}C-Metaldehyde in the water phases decreased in the sandy and loamy system continuously to a minimum of 0.33 and 0.38 % of applied radioactivity at the end of the study, respectively (Table 89). In the sediment extracts of the sandy system, the ^{14}C-Metaldehyde concentration increased to 10.92 % of applied radioactivity on day 7 and subsequently decreased to 0.16 % of applied radioactivity on day 100. In the sediment of the loamy system, the Metaldehyde concentration increased to a maximum value of 19.49 % of applied radioactivity on day 7 and thereafter continuously decreased to 0.28 % of applied radioactivity on day 100.

The major degradation product both in the extract of the sediment and in the water phase was Acetaldehyde. The amount of Acetaldehyde in the water phases increased continuously to a maximum of 22.32 % and 21.73 % of applied radioactivity at day 30 in the sandy and loamy system, respectively. Thereafter, the amount of Acetaldehyde in the water phase decreased to 5.39 and 1.58 % of applied radioactivity on day 100, respectively. In the sediment,

Acetaldehyde occurred with a maximum of 4.70 % and 3.76 % at day 14 in the sandy and loamy system, respectively

The degradation of Metaldehyde in water/sediment systems was described by first order degradation kinetics. The DT_{50} values in the water phase were calculated to be 11.8 and 11.2 days for the sandy and loamy system, respectively, and the DT_{90} values were determined to be 39.1 days and 37.1 days, respectively. In the total water sediment systems Metaldehyde was degraded with half-lives of 12.4 days (sandy system) and 11.9 days (loamy system). The DT_{90} values for the total systems were calculated to be 41.0 and 39.4 days, respectively (Table 90). On request of the member states and EFSA, the DT_{50} and DT_{90} values of Metaldehyde were re-calculated during dossier preparation according to the recommendations of the FOCUS Kinetic Working group to separate between degradation and dissipation half-lives with the model KINGUI Version 1.1 (Peter, S. 2009, Doc. No. 782-015, PD June 2009, IIA, 7.2/10). The degradation DT_{50} values in the entire system were calculated to be 4.10 and 4.42 days for the sandy and loamy system, respectively, and the DT_{90} values were determined to be 13.61 days and 14.97 days, respectively (Table 91). In the water and sediment phase Metaldehyde dissipated with half-lives of 11.35 and 10.78 days (sandy system) and 10.25 and 9.78 days (loamy system). The DT_{90} values for the water and sediment phases ranged from 31.50 to 37.71 days in both systems.

The DT_{50} and DT_{90} values of the main degradation product Acetaldehyde in the total water/sediment systems were estimated during the dossier preparation according to the recommendations of the FOCUS Kinetic Working group. The DT_{50} values were calculated to be 30.98 and 19.01 days in the sandy and loamy system, respectively. The DT_{90} values were calculated to be 102.90 and 63.14 days, respectively (Table 92).

Table 88: Percent distribution of applied radioactivity and mass balance in water/sediment systems treated with 14C-Metaldehyde (results expressed as % of applied radioactivity)

Day	Organic Volatiles	CO_2	Water phase	Sediment Extractables		Sediment Un-extractables	Total Recovered
				DCM[1] Extract	Methane Extract		
Sandy system							
0	<0.1	0.06	98.97	1.45	0.51	<0.01	101.0
0.25	<0.1	0.13	96.18	3.31	0.95	0.15	100.7
1	<0.1	0.27	91.75	7.72	1.92	0.65	101.7
2	<0.1	0.27	86.48	7.74	2.17	0.57	102.3
7	<0.1	0.57	85.02	10.92	3.65	0.62	100.8
14	<0.1	6.39	59.47	9.28	4.70	8.58	88.42
30	0.11	27.48	23.10	1.09	3.17	20.54	75.49
62	<0.1	50.57	13.41	0.43	1.82	17.03	83.26
100	<0.1	61.57	5.72	0.16	0.87	19.05	87.37
Loamy system							
0	0	-	99.46	0.95	0.09	<0.10	100.5
0.25	0	0.12	96.75	4.66	0.62	0.11	102.3
1	0.12	0.22	88.53	9.85	1.42	0.29	100.2
2	0	0.35	83.61	12.82	1.80	0.48	99.1
7	0	0.68	76.44	19.49	2.73	0.84	100.2
14	0	5.08	57.23	15.01	3.76	6.13	87.2
30	0	36.82	22.99	1.26	2.84	13.33	77.2

Day	Organic Volatiles	CO_2	Water phase	Sediment Extractables		Sediment Un-extractables	Total Recovered
				DCM[1] Extract	Methane Extract		
Sandy system							
0	<0.1	0.06	98.97	1.45	0.51	<0.01	101.0
0.25	<0.1	0.13	96.18	3.31	0.95	0.15	100.7
1	<0.1	0.27	91.75	7.72	1.92	0.65	101.7
2	<0.1	0.27	86.48	7.74	2.17	0.57	102.3
7	<0.1	0.57	85.02	10.92	3.65	0.62	100.8
14	<0.1	6.39	59.47	9.28	4.70	8.58	88.42
30	0.11	27.48	23.10	1.09	3.17	20.54	75.49
62	<0.1	50.57	13.41	0.43	1.82	17.03	83.26
100	<0.1	61.57	5.72	0.16	0.87	19.05	87.37
Loamy system							
62	0.01	64.77	5.56	0.24	1.90	18.88	91.4
100	0.01	68.80	2.02	0.28	1.22	10.63	78.7

[1]Dichloromethan

Table 89: Percent distribution of applied radioactivity and mass balance in water and sediment phase of an aerobic aquatic systems treated with 14C-Metaldehyde

Day	Metaldehyde		Acetaldehyde	
	Water phase	Sediment	Water phase	Sediment
	[% of applied radioactivity]			
Sandy system				
0	95.87	1.45	3.11	0.51
0.25	93.28	3.31	2.90	0.95
1	89.10	7.72	2.66	1.92
2	87.14	7.74	2.34	2.17
7	82.61	10.92	2.41	3.65
14	40.05	9.28	19.42	4.70
30	0.78	1.09	22.32	3.17
62	3.20	0.43	10.21	1.82
100	0.33	0.16	5.39	0.87
Loamy system				
0	96.38	0.95	3.08	<0.10
0.25	93.68	4.66	3.07	0.62
1	85.87	9.85	2.66	1.42
2	81.00	12.82	2.61	1.78
7	74.10	19.49	2.35	2.73
14	36.01	15.01	21.22	3.76
30	1.27	1.26	21.73	2.84
62	0.38	0.24	5.18	1.90
100	0.44	0.28	1.58	1.22

Table 90: DT50 and DT90 values for Metaldehyde in water/sediment systems

	DT_{50} (days)	DT_{90} (days)	R^2	Method of Calculation
Sandy System				
Water Phase	11.8	39.1	0.749	First order

	DT_{50} (days)	DT_{90} (days)	R^2	Method of Calculation
Total System	12.4	41.0	0.698	
Loamy System				
Water Phase	11.2	37.1	0.643	First order
Total System	11.9	39.4	0.939	

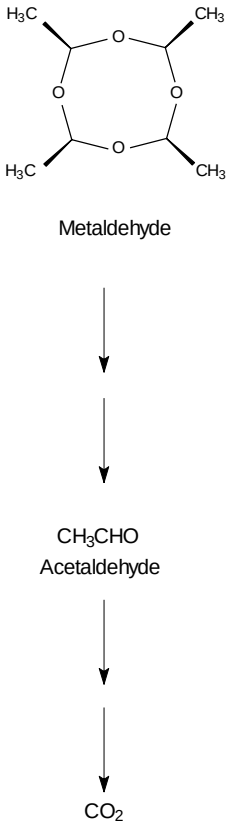
Table 91: DT50 and DT90 values for Metaldehyde in water/sediment systems

System	Phase	DT_{50} [d]	DT_{90} [d]	Model
Sandy System	System	4.10	13.61	Single first order[1)]
	Water	11.35	37.71	Single first order
	Sediment	10.78	35.82	Single first order
Loamy System	System	4.42	14.97	Single first order[1)]
	Water	10.25	34.07	Single first order
	Sediment	9.48	31.50	Single first order

[1)]Without taking into account the data of the lag phase (first 14 days of incubation)

Table 92: DT50 and DT90 values for Acetaldehyde in total water/sediment systems (re-calculated during dossier preparation with KINGUI Version 1.1)

System	DT_{50} (days)	DT_{90} (days)	Chi^2	Model
Sandy System	30.98	102.90	17.0	Single first order
Loamy System	19.01	63.14	16.7	Single first order



Proposed degradation pathways of Metaldehyde in aquatic systems

Conclusions:

Regarding the sequence of the used traps in the water/sediment study, it is very likely that the CO_2 trap was arranged at the last trapping vessel (i.e. any traps collecting metabolites are arranged between the incubation vessel and the CO_2 trap).

During the evaluation process, questions related to the trapping of Metaldehyde and its possible degradation products in laboratory studies have been raised in the reporting tables by the Member States and by EFSA. Therefore, several tests were conducted to identify appropriate media to trap Metaldehyde and any volatile degradation products which could be formed during the degradation of Metaldehyde in the laboratory studies (for details please see Juozenaite, A., 2009, Doc. No. 741-002, PD June 2009, IIA 7.1/12). Based on these results a trapping system was proposed and used in the new conducted water/sediment study (Kane, T. 2009).

Comment RMS: The review is considered acceptable.

Reference:	^{14}C-Metaldehyde Aerobic Transformation in Aquatic Sediment Systems
Author(s), year:	Kane, T., 2009
Report/Doc. number:	Lonza Report No. 4392, Doc. No. 714-003
Guideline(s):	OECD 308
GLP:	Yes
Deviations:	None
Validity:	Yes

MATERIAL AND METHODS:

Test item:	^{14}C-labelled Metaldehyde: Batch 2860DCR021-3 Radiochemical purity >97% (checked before use in this study) Specific radioactivity: 4.03 MBq/mg Radiochemical purity >97% (checked before use in this study) Non-radiolabelled test substance: Lot number 37801 Purity 99.6% (expiry date 26 February 2011)
Incubation temperature:	20 ± 2 °C
Application rate:	0.07 mg/L corresponding to 700 g ai/ha assuming a 30 cm water column

Table 93: Water/Sediment-systems used to investigate the route and rate of degradation of 14C-Metaldehyde

Parameters	**System I (Calwich Abbey Lake, Derbyshire, UK)**		**System II (Swiss Lake, Derbyshire, UK)**	
	Sediment			
Soil texture (UK classification)	Silt loam		Sand	
% sand (2000 µm-63 µm)	10		90	
% silt (63 µm-2 µm)	72		4.0	
% clay (<2 µm)	18		6.0	
Organic carbon (%)	4.1		0.6	
pH value (H_2O)	7.8		5.1	
Redox-Potential [mV]				
(beginning of study)	25		75	
(end of study)	22		26	
N-total (%)	0.39		0.23	
P-total (mg/kg sediment)	946		161	
CEC (mVal N/100 g dry soil)	15.5		2.2	
Microbial characterisation (colony forming untis/g)	Day 0	Day 97	Day 0	Day 97
Aerobic bacteria	747500	312250	590000	630000
Aerobic bacterial spores	146500	23050	60000	28000
Anaerobic bacteria	151500	61250	11950	11350
Anaerobic bacterial spores	54500	8850	9675	9225
Actinomycetes	3160	14575	1755	103
Fungi	1010	2150	1030	548
	Water			
N-total (mg/L)	2.3		1.2	
P-total (mg/L)	<0.1		<0.1	
Organic carbon (mg/L)	6.7		4.7	
Total hardness as $CaCO_3$(mg/L)	171		26	
pH value				
(beginning of study)	8.0		7.4	
(end of study)	7.2		6.7	
Oxygen content [mg/L]				
(beginning of study)	71.1		70.5	
(end of study)	71.0		63.9	
Redox-Potential [mV]				
(beginning of study)	368		364	
(end of study)	382		461	
Microbial characterisation (colony forming untis/g)	Day 0	Day 97	Day 0	Day 97
Aerobic bacteria	46200	11550	2270	4200
Aerobic bacterial spores	155	133	60	<18
Actinomycetes	<13	<223	<10	<10
Fungi	<13	120	<10	<13

The degradation of ^{14}C-Metaldehyde under aerobic aquatic conditions was studied in a silt loam and in a sand water/sediment system which were collected from two different lakes in Derbyshire, UK. The sediment and water characteristics of both systems are summarised in Table B.8.10.3-6. Incubation flasks for the test system were filled with wet sediment and water in such a way to obtain a sediment layer of 2.5 to 3 cm and supernatant water layer of 7.5 to 9 cm. Afterwards, the water/sediment systems were allowed to acclimatise in the dark at

20 ± 2°C for 19 days. The equilibrium period resulted in a complete phase separation and stabilisation of the oxygen concentration, pH and redox potential; these parameters were also measured at each sampling point. ^{14}C-Metaldehyde was dissolved in acetonitrile and applied separately onto the water surface of each test system at a rate of 0.07 mg/L corresponding to a field application rate of approximately 700 g ai/ha assuming an even distribution in a 30 cm water column. Acetonitrile was applied to control flasks at a corresponding rate to determine the microbial activity at the end of the study. Provisions were made for the quantitative trapping of any volatiles by the installation of a polyurethane foam bung (trapping of Metaldehyde), two silica gel cartridges coated with 4-dinitrophenylhydrazine (DNPH, trapping of Acetaldehyde), a vessel containing ethyl digol (any additional organic volatile) and two vessels containing 1 M KOH trapping solutions (trapping of CO_2). Several trapping media were tested before study start to have an appropriate trapping system for this study in order to trap and to quantify volatile Metaldehyde, any possible volatile organic degradation products and CO_2 separately. For details see point B.8.1.1. Samples were taken immediately after application and after 1, 2, 7, 14, 29, 59 and 97 days of incubation. The microbial activity of the water and sediment phase was measured separately for different groups of organisms (i.e aerobic and anaerobic bacteria, aerobic and anaerobic bacterial spores, actinomycetes and fungi) at study initiation and at 97 days after treatment. The results demonstrate that the microbial viability of the study soil at the time of dosing and during the incubation period was representative of microbially active systems.

The water and sediment phase was separated and the radioactivity in the water phase was determined by LSC. Sediment samples were extracted two times with approximately 400 mL and 200 mL methanol. Day 97 samples were additionally extracted with approximately 200 mL acetonitrile. The extracts were combined and the radioactivity in the extracts was quantified by LSC and characterised by HPLC. The results of selected samples were confirmed by TLC analysis. Extracted sediment samples were combusted to determine levels of un-extractable residues. Polyurethane bungs were extracted with acetonitrile. Each DNPH cartridge was eluted with acetonitrile and the eluates from the two cartridges associated with each sample were combined. Radioactivity in the trapping solutions, extracts and eluates was quantified by LSC. The presence of CO_2 in the KOH trapping solutions was confirmed by precipitation with barium carbonate.

The kinetic analysis of Metaldehyde in the water, sediment phase and entire system was assessed using different kinetic models according to the recommendations of the FOCUS Kinetic Working group.

Findings:
Total mean recoveries obtained during the study ranged from 96.8 % to 101.7 % and from 97.5 % to 105.8 % of the applied radioactivity for the silt loam and sand system, respectively (Table B.8.10.3-7).

Radioactivity in the water phases of both test systems decreased slowly throughout the incubation period reaching 68.2 % (silt loam system) and 73.6 % of applied radioactivity (sand system) after 97 days.

Non-extractable residues in the sediment did not exceed 4 % in the silt loam system and 1.0 % of applied radioactivity in the sand system during the entire incubation period.

Volatilised Metaldehyde, volatilised Acetaldehyde and other volatile organics did not exceed 2.0 % of applied radioactivity. The formation of CO_2 was low, accounting for a maximum of 4.8 % and 8.1 % of the applied radioactivity on day 97 for the silt loam and sand system, respectively.

The amount of ^{14}C-Metaldehyde in the water phases decreased in the silt loam and sand system to 72.7 % and 78.5 % of applied radioactivity at day 7 (Table 94). Afterwards, the level of Metaldehyde in the water phase remained stable in both systems ranging from 64.6 % to 67.4 % of applied radioactivity in silt loam system and from 71.1 % to 78.1 % in sand system. In the sediment extracts of the silt loam system, the ^{14}C-Metaldehyde concentration increased to 21.2 % of applied radioactivity on day 14 and remained at similar levels on day 97 (Table 95). In the sediment of the sand system, the Metaldehyde concentration increased to a maximum value of 13.5 % of applied radioactivity on day 29 and thereafter the residue level did not change obviously.

In both systems, up to three unknown compounds were detected during the entire incubation period none of them exceeded 2.0 % of applied radioactivity in the water phase as well in the sediment phase. The reaming radioactivity distributed throughout the regions of the chromatogram other than those specified was below 3.0 % of applied radioactivity in both systems and in both phases. The proposed degradation pathway of Metaldehyde in aquatic sediment systems under aerobic conditions is presented under Figure 1.

The degradation kinetics of Metaldehyde in water/sediment systems was evaluated by different kinetic models according to the recommendations of the FOCUS Group. The best fit model based on statistical values and visual assessment was chosen. The DT_{50} values in the water phase and total system were calculated to be > 1000 days for the silt loam system using DFOP and FOMC model (Table 97). For the sand system, the DT_{50} values in water and total system were determined to be 473 days and 714 days using HS and DFOP model, respectively. The corresponding DT_{90} values were determined to be > 1000 days in both phases of both systems. Since no decline of Metaldehyde occurred in the sediment phase of both systems from the maximum level onwards, no kinetic evaluation could be conducted for this phase.

Table 94: Percent distribution of applied radioactivity and mass balance in water/sediment systems treated with 14C-Metaldehyde (results expressed as % of applied radioactivity)

Time after application [d]	Volatiles				Water phase	Sediment		Total Recovered
	Met-aldehyde	Acet-aldehyde	Other organics	CO_2		Extract-ables	Un-extract-ables	
Silt loam system								
0	-	-	-	-	100.3	1.2	0.3	101.7
1	<LOQ	0.2	0.2	0.3	92.3	5.6	1.5	100.0
2	<LOQ	0.1	0.4	0.6	90.2	7.5	1.8	100.5
7	0.1	0.1	0.9	1.6	75.7	18.5	2.6	99.4
14	0.2	0.1	1.3	2.3	69.6	21.7	3.9	99.0
29	0.3	0.3	2.0	3.6	69.2	20.9	2.7	98.8
59	0.4	0.6	1.5	4.6	67.1	20.1	2.8	96.8
97	0.5	0.2	2.3	4.8	68.2	21.3	3.3	100.6

Sand system								
0	-	-	-	-	99.8	2.5	0.2	102.5
1	<LOQ	0.7	0.4	0.8	92.8	6.5	0.3	101.3
2	<LOQ	0.2	0.1	0.5	96.3	8.7	0.2	105.8
7	<LOQ	1.0	0.1	1.2	82.2	12.9	0.5	97.9
14	<LOQ	0.8	0.2	2.0	80.7	13.3	0.6	97.5
29	0.1	1.2	0.9	3.5	77.3	14.1	0.7	97.7
59	0.3	1.2	1.4	5.0	76.9	13.2	0.6	98.4
97	0.5	1.2	1.3	8.1	73.6	13.3	0.6	98.5

Table 95: Characterisation of radioactivity in the water phase of aerobic aquatic systems treated with 14C-Metaldehyde (expressed in % of applied radioactivity)

Time after application [d]	Metaldehyde	Unknown 1	Unknown 2	Unknown 3	Others[1)]
Silt loam system					
0	97.6	1.2	nd	nd	1.5
1	89.9	0.2	0.3	0.1	1.9
2	87.9	nd	0.1	nd	2.3
7	72.7	0.5	0.2	nd	2.4
14	67.4	0.3	0.2	nd	1.7
29	67.0	nd	nd	nd	2.2
59	64.6	0.1	nd	0.3	2.1
97	66.4	nd	nd	0.3	1.6
Sand system					
0	97.0	0.7	0.3	nd	1.9
1	90.0	1.3	nd	nd	1.6
2	92.3	1.6	0.4	nd	2.0
7	78.5	0.6	0.1	nd	3.0
14	78.1	0.3	0.1	nd	2.3
29	74.8	nd	0.1	nd	2.4
59	75.0	nd	nd	nd	1.9
97	71.1	nd	nd	nd	2.5

[1)] radioactivity distributed throughout regions of the chromatogram other than those specified and which did not contain any discrete radioactive components;
nd not detected

Table 96 Characterisation of radioactivity in the sediment phase of aerobic aquatic systems treated with 14C-Metaldehyde (expressed in % of applied radioactivity)

Time after application [d]	Metaldehyde	Unknown 1	Unknown 2	Unknown 3	Others[1)]
Silt loam system					
0	1.1	nd	nd	nd	0.1
1	5.1	nd	nd	0.1	0.4
2	7.0	nd	nd	< LOQ	0.2
7	17.4	0.1	nd	0.5	0.5
14	21.2	0.1	nd	nd	0.5
29	20.6	nd	nd	nd	0.3
59	19.5	nd	nd	0.1	0.9

Time after application [d]	Metaldehyde	Unknown 1	Unknown 2	Unknown 3	Others[1)]
97	20.5	nd	nd	0.1	0.8
Sand system					
0	2.5	nd	nd	nd	0.1
1	6.1	0.1	nd	nd	0.3
2	8.3	0.1	nd	nd	0.4
7	12.3	0.2	nd	nd	0.5
14	12.6	0.1	0.1	0.1	0.6
29	13.5	nd	0.1	0.1	0.4
59	12.8	nd	nd	0.1	0.3
97	12.5	nd	nd	0.1	0.7

[1)] radioactivity distributed throughout regions of the chromatogram other than those specified and which did not contain any discrete radioactive components;
nd not detected

Table 97: DT50 and DT90 values for Metaldehyde in water/sediment systems

Compartment	Kinetic model	DT_{50} (days)	DT_{90} (days)	Chi^2
Silt loam system				
Water Phase	SFO	184	610	5.5
	FOMC	>1000	>1000	1.8
	DFOP*	>1000	>1000	1.3
	HS	>1000	>1000	0.8
Total System	SFO	579	>1000	2.8
	FOMC*	>1000	>1000	1.1
	DFOP	>1000	>1000	1.1
	HS	nc	nc	nc
Sand system				
Water Phase	SFO	287	953	5.5
	FOMC	>1000	>1000	1.8
	DFOP	494	>1000	1.3
	HS*	473	>1000	0.8
Total System	SFO	486	>1000	2.1
	FOMC	>1000	>1000	0.8
	DFOP*	714	>1000	0.6
	HS	703	>1000	0.6

nc not calculated
* indicates the best fit with respect to Chi^2value and visual assessment of the fit to the measured data

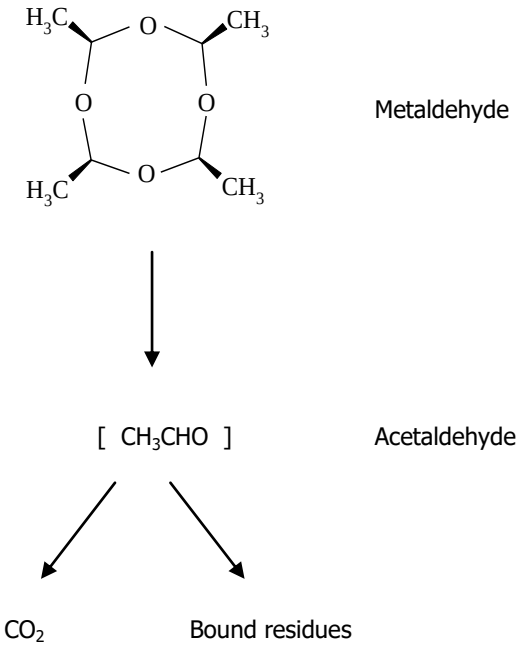
Conclusion:
Metaldehyde dissipated from the water phase with a DT_{50} value of >1000 days in silt loam system and of 473 days in sand systems. DT_{50} values for the dissipation from the total system were >1000 days in both systems. Metaldehyde was degraded within both water and sediment phases and up to four degradation products (including $^{14}CO_2$) were formed. Significant quantities of intermediate products were not produced during Metaldehyde degradation in both systems.

Comments (RMS):
The study is considered acceptable.

5.1.2.4 Degradation in soil under aerobic conditions

The *aerobic metabolism* of metaldehyde is suggested to occur via microbial and/or chemical degradation building mainly CO_2 (80.4% AR after 21 days). No volatilised Metaldehyde or other organic volatiles are observed at relevant concentrations. The aerobic metabolism studies showed that Metaldehyde degraded rapidly after a lag-phase between 6 and 19 days following hockey-stick kinetic. No metabolites are formed over 5 % AR. The non-extractable residues increased during the incubation period from a mean of 0.5% AR to a mean of about 36% AR.

The proposed metabolic pathway in soil is shown in the figure below:



DEGRADATION IN SOIL UNDER STANDARD CONDITIONS

The degradation rate of Metaldehyde under *aerobic* conditions was investigated in four soils, and the degradation occurred rapidly under aerobic conditions after a lag phase of 5.8 to 19 days, which was essentially no degradation of Metaldehyde during this period. The compound degraded almost completely over a very short period of time with extensive mineralisation to carbon dioxide and incorporation into bound residues. CO_2 accounted for up to 80 % of applied radioactivity and the maximum level of bound residues was 26 %. A decline of the non-extractable residues was observed during the entire incubation period. No intermediate degradation products were produced in relevant amounts (i.e. < 4 % of applied radioactivity). Using a modified hockey-stick kinetic model, the DT_{50} and DT_{90} values ranged from 6.6 to 19.5 days and from 8.5 to 22.1 days, respectively. The half-life of Metaldehyde during the rapid decline phase ranged from 0.5 to 4.1 days. The half-lives proposed for further evaluation were determined by back-calculation of the DT_{90} values (i.e. DT_{90}/3.32) resulting in pseudo-DT_{50} values ranging from 2.6 to 6.7 days.

The degradation rates are summarised in the tables below:

Table 98: Laboratory studies

Metaldehyde	*Aerobic conditions*						
Soil type (study Ref.)	OC	pH	Temperature/ Moisture content	DT_{50}/DT_{90} Hockey-stick (d)	Duration of lag-phase (d)	DT_{50} Pseudo single-first-order	Chi^2 (%)
Silt Loam, 02-A Juozenaite, A. (2009)	1.2	6.5	20 °C/pF 2	19.5/20.6	19.0	6.2	2.0
Sandy clay loam, Elmton (294) Juozenaite, A. (2009)	4.2	7.0	20 °C/pF 2	11.6/21.0	7.5	6.3	9.6
Sandy clay loam, Fladbury Juozenaite, A. (2009)	3.1	6.1	20 °C/pF 2	15.9/22.1	13.2	6.7	9.3
Sandy loam, Lanoe Juozenaite, A. (2009)	1.0	7.3	20 °C/pF 2	6.6/8.5	5.8	2.6	7.8
Geometric mean						5.1	

PHOTOLYTIC DEGRADATION ON SOIL SURFACE

It can be concluded that metaldehyde was found to be photolytically stable on soil surface under both tested conditions. Besides the parent compound no further radioactive fractions were observed in the extracts. Since no significant degradation of metaldehyde was detected during the study in either irradiated or non-irradiated systems it was not possible to estimate accurate DT_{50} and DT_{90} values.

5.1.3 Summary and discussion of degradation

Summary: Biotic degradation	Test guideline / design	GLP (y/n)	Reliability
Ready biodegradability Due to the fact that only 2.8 % of the Metaldehyde was degraded during the 28 day incubation period it has to be classified as not readily biodegradable in terms of this test.		--	--

Summary: Biotic degradation	Test guideline / design	GLP (y/n)	Reliability
Water/sediment system (simulation test) In laboratory incubations in dark aerobic natural sediment water systems (four systems investigated), metaldehyde exhibited low to very high persistence. In the 2 systems where metaldehyde exhibited low persistence (where conditions were more oxidising, as indicated by the negative sediment redox potentials measured for the pertinent systems), microbial degradation of Metaldehyde was rapid with DT50 values of 4.1 - 4.4 days for the whole systems and the major metabolite acetaldehyde was formed (max. ca. 22 % AR in water and 5% in sediment). In these systems acetaldehyde exhibited moderate persistence. In the two less oxidising systems metaldehyde exhibited very high persistence with DT50 (SFO) values in the range of 579 - 486 days and no major metabolites were formed. The unextractable sediment fraction (not extracted using methanol or dichloromethane) was a sink for the carbon radiolabels (all carbons uniformly labelled), accounting for 0.6 – 19 % AR at study end (97-100 days). Mineralisation of these radiolabels accounted for 5-8 % AR in the lower oxidation state systems and 62-69% AR under the more oxidising systems, at the end of the studies.		y	y
Degradation in soil: The degradation rate of Metaldehyde under aerobic conditions was investigated in four soils, and the degradation occurred rapidly under aerobic conditions after a lag phase of 5.8 to 19 days, which was essentially no degradation of Metaldehyde during this period. The compound degraded almost completely over a very short period of time with extensive mineralisation to carbon dioxide and incorporation into bound residues. Mineralisation of the carbon radiolabels (all carbons uniformly labelled) to carbon dioxide accounted for 50 - 78 % AR after 22-60 days (termination times of the incubations). The formation of unextractable residues (not extracted using methanol) for these radiolabels accounted for 13 – 20 % AR after 60 days.		y	n

Summary: Abiotic degradation	Test guideline / design	GLP (y/n)	Reliability
Hydrolysis: Metaldehyde was hydrolytically stable at a temperature of 25 °C and pH values of 5 – 9.		y	n
Photolysis Metaldehyde was photolytically stable at a temperature of 25 °C and a pH value of 7.		y	n
Soil Photolysis It can be concluded that metaldehyde was found to be photolytically stable on soil surface under both tested conditions. Besides the parent compound no further radioactive fractions were observed in the extracts. Since no significant degradation of metaldehyde was detected during the study in either irradiated or non-irradiated systems it was not possible to estimate accurate DT50 and DT90 values.		y	n

Conclusion: The criteria for rapid degradation are not fulfilled because

Metaldehyde is hydrolytically and photolytically stable at a temperature of 25 °C and environmentally relevant pH values.

Metaldehyde is not readily biodegradable under test conditions within 28 days.

In UK simulation study (two less oxidising systems) DT50 whole system is >> 16 d, therefore Metaldehyde is considered not to be ready biodegradable/rapid degradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The adsorption behaviour of metaldehyde was studied in 8 soils using the batch equilibrium method. The K_F values were in the range of 0.432 to 0.977 L/kg. The K_{FOC} values were calculated to be in the range of 38 to 149 L/kg with a median value of 60.4 L/kg, indicating very high to high mobility. The Freundlich coefficient 1/n was in the range of 0.675 to 1.023 with a median value of 0.96. A dependency between organic content and K_{FOC} values is given. The K_{FOC} values increased with decreasing organic content values.

The results are summarised in the table below:

Table 99: Soil adsorption/desorption

Metaldehyde					
Soil Type (Ref. Study)	**OC %**	**Soil pH**	**K_F**	**K_{FOC}**	**1/n**
Sand (Heim & Daly,1999)	0.29	7.4	0.432	173	0.9099
Sandy loam (Heim & Daly,1999)	0.46	6.5	0.644	161	0.9651
Silt loam (Heim & Daly,1999)	1.39	7.1	0.685	57	0.9918
Clay loam (Heim & Daly,1999)	1.51	7.5	0.962	75	0.9953
Humic sand (de Vette & Aalderink, 2002)	1.9	5.3	0.735	38	0.974
Sandy loam (de Vette & Aalderink, 2002)	1.56	7.7	0.633	40	1.023
Loam (de Vette & Aalderink, 2002)	1.45	7.5	0.807	56	0.961
Low humic content sand (de Vette & Aalderink, 2002)	0.76	7.8	0.675	78	0.675
Median				**60.4**	**0.96**

Metaldehyde					
Soil Type (Ref. Study)	**OC %**	**Soil pH**	**K_F**	**K_{FOC}**	**1/n**
pH dependence, Yes or No	No				

5.2.2 Volatilisation

With a Henry´s constant of 3.5 Pa m^3/mol (20°C) and a vapour pressure of 4.4 Pa (20°C) metaldehyde is expected to volatilise.
In addition, the volatility of metaldehyde from a liquid formulation applied to a soil surface was experimentally investigated. The experiment showed that metaldehyde was only slowly released from the formulated product. However once released, most of the metaldehyde was found in the trapping solutions for volatile products whereas only small amounts were found as extractable or un-extractable residues in soil. Therefore, it can be concluded that the potential for volatilization of metaldehyde in formulated form seems to be significantly reduced.
The photochemical oxidative half-life was estimated by a model calculation according to Atkinson to be 1.7 hours (12 hours day - 1.5 x 10^6 OH-radicals/cm^3) indicating a quick degradation of metaldehyde in the troposphere.

Summary: Evironmental Distribution (not relevant for classification and labelling)	
Adsorption/Desorption	$K_{F,OC}$ values were calculated to be in the range of 5.7 to 83.3 L/kg with an arithmetic mean of 36.4 L/kg, indicating high mobility.
Volatilisation	Henry´s constant of 1.6 x 10^{-6} Pa m^3/mol (20° C) Vapour pressure of 1.3 x 10^{-5} Pa (20° C)

5.2.3 Distribution modelling

No data/information available

5.3 Aquatic Bioaccumulation

Table 100: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water OECD 107 (Shake flask method)	Purified product purity: 99.3 % (w/w) at 19.9-20.1 °C log P_{ow} = 0.12 P_{ow} = 1.33 ± 0.04 at pH 6.7 Effect of pH (4 to 10) is not required, because Metaldehyde is neither an acid nor a base. Because metaldehyde is not an ionisable compound the water phase is not buffered	Acceptable The method is comparable to the EEC/A8 shake flask method	Cardinaals, J.M. (1988b) (Doc. No. 114-002)
Bioconcentration in fish OECD 305 E (1981), EPA Pesticide Assessment Guidelines, Subdivision N Series 165-4 (1989)	Active substance: purity 99% Test dose: 0.1 mg/L BCF (steady-state, whole fish, based on total radioactive residues) : 11 Lipid content: Not measured	*	Sved, D. W., Holmes, C. M., Smith, G. J. (1992), Document No. 872-001
* Due to the low and variable residues in fish tissues, negligible depuration, and the lack of detailed information about the kinetic models used for the estimation of uptake rate and depuration rate constants in the study report (k1 and k2) the estimates for these parameters and the kinetic BCF estimates are not considered to be reliable. However, the study is of sufficient quality to demonstrate that metaldehyde does not bioconcentrate in fish and hence the study is acceptable.			

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

5.3.1.2 Measured bioaccumulation data

Reference: Sved, D. W., Holmes, C. M., Smith, G. J. (1992): A bioconcentration study with metaldehyde in the bluegill (*Lepomis macrochirus*). Document No. 872-001
Test guideline: OECD 305 E (1981), EPA Pesticide Assessment Guidelines, Subdivision N Series 165-4 (1989)
GLP: Yes
Material and methods:
Test substance:
Metaldehyde, purity: 99 %, batch: 5448; [U-^{14}C]metaldehyde, radiochemical purity: ≥ 98.4 %
Test organism:
Bluegill sunfish (*Lepomis macrochirus*), mean length of fish collected during the test: 55 mm (s.d. 10.4), mean weight of fish collected during the test: 2.4 g (s.d. 1.57). Detailed information on lengths and weights of fish at the start of exposure and the length and weight development during the test are not given in the study protocol. No information on age of fish at test initiation is provided.
Treatments: 0.1 mg/L, methanol was used as a solvent (0.1 mL/L), solvent control: 0.1 mL methanol/L

Number of animals: 2 replicates (A and B) per treatment and control, 70 fish per replicate
Duration:
Replicates A: 28 days uptake phase, 21 days depuration phase, replicates B: 28 days uptake phase plus 1 additional day of exposure at an increased rate of specific radioactivity to increase the concentration of radioactivity in fish tissues for possible metabolite identification
Test medium:
Dilution water: Fresh water obtained from a well 45 m deep, pumped through a sand filter, aerated, filtered to remove microorganisms and particles, analytical results of the used well water indicate acceptable quality for the purpose of this study, hardness: 132 - 148 mg/L as $CaCO_3$, dissolved oxygen: 5.4 - 8.3 mg/L (> 60 % saturation throughout the study), pH: 7.7 - 8.2
Test conditions and test design:
Flow-through system, 6.4 volume additions every 24 hours, test chambers: Teflon®-lined, loading: 0.61 g fish/L test medium that passed through test chambers in 24 hours, at any given time the biomass of fish/ L of test water did not exceed 3.9 g fish/L, feeding: flaked fish food, excess food was siphoned from the test chambers daily after feeding, photoperiod: 16 hours light, 8 hours dark, temperature: 20.4 - 22.6 °C.
Water and fish data from replicates A were used to determine the BCF, the rate and degree of uptake, and the rate of depuration. Water and fish samples from replicates B were used to characterise the radioactive residues with regard to their polarity, extractability, and possible identification.
Biological observations:
Daily fish were observed for mortality and sublethal signs of toxicity.
Analytical measurements:
Concentration verifications: On days 0, 3, 7, 14, 21 and 28 of the uptake phase and on days 1, 3, 7, 10, 14 and 21 of the depuration phase water samples were collected from the solvent control and treated test chambers and analysed for total radioactivity by LSC. The concentrations of radioactivity in the water samples were converted to mg metaldehyde equivalents/L. Radioactivity in the samples was corrected for background radioactivity and counting efficiency by an external standard method. LOQ: 0.0078 mg/L.
Determination of total radioactivity in fish tissues: On days 0, 3, 7, 14, 21 and 28 of the uptake phase and on days 1, 3, 7, 10, 14, and 21 of the depuration phase four fish were sampled from replicates A of the treated and solvent control groups. Non edible (fins, heads, and viscera) and edible tissue fractions from the four fish were pooled. Analysis of total radioactivity was performed by homogenisation of tissue samples followed by combustion and LSC analysis of the trapped CO_2. The concentrations of radioactivity in the tissue samples were converted to mg metaldehyde equivalents/kg of tissue. Radioactivity in the samples was corrected for background radioactivity, counting efficiency, and combustion efficiency. LOQ: 0.29 mg/kg.
Determination of polar, non polar and non extractable residues in fish tissues: On day 28 of the uptake phase 10 fish were collected from replicates B of the treated and solvent control groups. The edible parts were pooled for each group. Proportions of polar, non polar, and non extractable residues were determined by sequential extraction with methanol and hexane and combustion (trapping of CO_2) followed by LSC.
Collection of water and fish tissues for possible metabolite identification: Water samples were collected from chambers A and B of the treated and control groups on days 14 and 28 of the uptake phase and from chamber B of the treated group on day 29 after delivering [^{14}C]-metaldehyde at an increased specific activity for approximately 21 hours. Fish samples (20 individuals) were taken from chambers B of the treated and control groups on days 14 and 28 of

the uptake phase. The 18 fish remaining in chamber B of the treated group were sampled on day 29 after exposure to [^{14}C]-metaldehyde at an increased specific activity. The edible parts of fish were pooled for each fish sample and frozen for possible metabolite identification. However, this identification was not conducted, because there was evidence that metaldehyde is metabolised to acetaldehyde which is used in anabolic pathways (see findings described below).

Statistical evaluation:

The BIOFAC computer program for characterising the rates of uptake and clearance of chemicals in aquatic organisms (The Dow Chemical Company, Midland, MI) was used to estimate uptake rate constants (k2), depuration rate constants (k1) and kinetic BCF values.

Findings:

Verification of test concentrations:

Mean concentrations of metaldehyde in replicates A and B of the treated group were 0.10 mg/L (s.d. 0.022) and 0.10 mg/L (s.d. 0.017), respectively. Total radioactivity in test chambers ranged from 0.078 – 0.14 mg metaldehyde equivalents/L. The concentration of metaldehyde in water of the control replicates and in all replicates during the depuration phase was less than the LOQ.

Table 101: 14C-residues in water samples and tissue samples of Lepomis macrochirus during the uptake and depuration phase of a bioconcentraiton test with metaldehyde

	Sample (chamber A)	Residues [metaldehyde equivalents/L or kg]					
		day 1	day 3	day 7	day 14	day 21	day 28
Uptake phase	Water	0.088	0.10	0.078	0.14	0.09	0.11
	Fish edible	< LOQ	< LOQ	< LOQ	0.75	0.30	0.87
	Fish non edible	< LOQ	< LOQ	0.62	2.0	1.0	2.0
	Whole fish	< LOQ	< LOQ	0.38	1.4	0.66	1.4
Depuration phase	Water	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Fish edible	0.87	1.1	0.48	0.64	0.75	1.2
	Fish non edible	1.4	2.8	1.2	0.97	0.96	1.7
	Whole fish	1.1	1.9	0.83	0.79	0.85	1.4

The amount of radioactivity in edible and non edible tissues did not decrease during the 28 days of depuration. In the study report this finding is explained as follows: In mammals metaldehyde is quantitatively metabolised to acetaldehyde which is readily converted to acetyl-CoA. Acetyl-CoA is utilised in various anabolic pathways. These anabolic reactions should account for the slow depuration and persistence of ^{14}C-residues in tissues if the metabolic pathways for metaldehyde in fish are similar to those in mammals.

Fractionation of metaldehyde residues in edible tissues after 28 days of uptake into polar, non polar and non extractable residues yielded 28 % polar, 12 % non polar and 59 % non extractable residues expressed as % of total residues. The high portion of non extractable residues supports the view that metaldehyde is metabolised to acetyl-CoA which is transferred via anabolic pathways to body carbon pools.

Table 102: Fish tissue bioconcentration factors for [U-14C]-metaldehyde in Lepomis macrochirus

Days of uptake	Residues in water and fish tissues [mg ai equiv./L or kg]				Bioconcentration factors		
	Water *	Edible	Non edible	Whole fish	Edible	Non edible	Whole fish
0	0.088	< LOQ	< LOQ	< LOQ	-	-	-
3	0.094	< LOQ	< LOQ	< LOQ	-	-	-
7	0.089	< LOQ	0.62	0.39	-	-	4.4
14	0.10	0.75	2.0	1.4	7.5	20	14
21	0.099	0.30	1.0	0.61	3	10	6.2
28	0.10	0.87	2.0	1.4	8.5	20	14
mean BCF at steady state (days 14 - 28)					6.3	17	11

* Average concentration over time.

Table 103: Estimated uptake and depuration parameters of 14C-residues in Lepomis macrochirus estimated with the BIOFAC computer program

Parameters	Edible tissues [a]	Non edible tissues	Whole fish
Uptake rate constant k1 [mg/g/d]	-	0.94	0,62
Depuration rate constant k2 [1/d]	-	0.024	0.017
Time to reach 90 % steady state [d]	-	96	135
Time to reach 50 % clearance [d]	-	29	41
Estimated BCF		39	36

[a] The BIOFAC model could not produce reasonable parameter estimates due to the low and variable tissue residues and the apparent lack of depuration

Lipid content: Not measured
Mortalities and clinical signs:
Four fish in the control group and two fish in the treated group died during the uptake phase. No fish died during the depuration phase. Since post-mortem examinations revealed no signs of toxicity, these deaths are not considered to be treatment related.
Conclusions:
The uptake of metaldehyde in fish was slow and low. Depuration of total radioactivity was negligible during 28 days. The low depuration can be explained by the metabolisation of metaldehyde to acetaldehyde which enters anabolic pathways via acetyl-CoA and hence ^{14}C-residues are incorporated into body carbon pools. This interpretation is supported by the high portion of non extractable residues found in edible fish tissues after 28 days of exposure.
The data of total radioactive residues in fish tissues suggest that steady-state is reached after 14 days, however, residues thereafter were variable.
BCF (steady-state, whole fish, based on total radioactive residues) : 11
Comments (RMS):
Due to the low and variable residues in fish tissues, negligible depuration, and the lack of detailed information about the kinetic models used for the estimation of uptake rate and depuration rate constants in the study report (k1 and k2) the estimates for these parameters and the kinetic BCF estimates are not considered to be reliable. However, the study is of sufficient quality to demonstrate that metaldehyde does not bioconcentrate in fish and hence the study is acceptable.

5.3.2 Summary and discussion of aquatic bioaccumulation

The determined **BCF (steady-state, whole fish, based on total radioactive residues) 11** and the log P_{ow} (0.12at pH 6.7) demonstrate that metaldehyde does not bioconcentrate in fish. This was

Summary Bioconcentration	
	Active substance Metaldehyde
log$P_{O/W}$	0.12 (19.9 - 20.1 °C, pH: 6.7)
Bioconcentration factor (BCF)	11 (whole fish at steady state)
Conclusion	Metaldehyde is considered to have **a low bioaccumulation potential**

5.4 Aquatic toxicity

Table 104: Summary of relevant information on aquatic toxicity

Method	Test material	Results							Remarks	Reference / acceptability
		Test organism	Test condition	Duration	Endpoint	Test conc	NOEC [mg ai/L]	EC_{50}/LC_{50} [mg ai/L]		
EPA Pesticide Assessment Guidelines, Subdivision E, 72-1 (1985), OECD 203	Metaldehyde, purity 99.3%	*Oncorhynchus mykiss*	semi-static	96 h	mortality	n	56	75		Bogers (1990a) / yes
OECD 203	Metaldehyde, purity not stated	*Cyprinus carpio*	semi-static	96 h	mortality	n	100	> 100		Wetton et al. (2001) / yes
OECD 202 (1984), US EPA 540/9-85-005	Metaldehyde, purity 99.3%	*Daphnia magna*	static	48 h	immobility	mm	90	> 90		Wuethrich (1990a) / yes
No guideline available, OECD 202 (2004) was taken into account	Metaldehyde, purity 99.5%	*Planorbarius corneus*	static	48 h	immobility	n	200	> 200		Egeler et al. (2007) / yes
OECD 201	Metaldehyde, purity not stated	*Scenedesmus subspicatus*	static	96 h	biomass growth rate	n		---	no reliable toxicity values could be derived	Wuethrich (1990b) / no
OECD 201 (2006)	Metaldehyde, purity 99.5%	*Desmodesmus subspicatus*	static	72 h	yield growth rate	n	25 25	> 200 > 200		Egeler et al. (2007) / yes
OECD 204	Metaldehyde, purity 99.3%	*Oncorhynchus mykiss*	semi-static	21 d	mortality body weight	n	37.5	-		Bogers (1990b) / yes
OECD 202, Part II	Metaldehyde, purity 99.3%	*Daphnia magna*	semi-static	21 d	immobility reproduc.	n	90	-		Wuethrich (1990c) / yes

n = nominal; mm = mean measured;

5.4.1 Fish

Short-term toxicity to fish

Reference: Bogers, M. (1990a): 96-hour acute toxicity study in the rainbow trout with P0071 in a semi-static system. Document no. 821-001
Test guideline: EPA Pesticide Assessment Guidelines, Subdivision E, 72-1 (1985), OECD 203 (1984)
GLP: Yes
Material and methods:
Range finding test
In a preliminary 96-hour fish toxicity study (semi-static system) rainbow trouts were exposed to a range of 0.1 to 1000 mg metaldehyde/L forming a geometric progression with a factor of 10 (1000 mg/L was a supersaturated solution with undissolved substance particles)
Main test:
Test substance: P0071 (metaldehyde), purity: 99.3 %, batch: 5448
Test organism:
Rainbow trout (*Oncorhynchus mykiss*), mean length: 5.3 cm (s.d. 0.64), mean weight: 1.64 g (s.d. 0.389)
Treatments: 0, 32, 56, 100 and 180 mg/L
Number of animals: 2 replicates with 5 fish per concentration and control
Duration: 96 hours
Test conditions:
Semi-static conditions, renewal of the test media after 48 hours, test vessel were of glass and had a volume of 12 L, no feeding from 24 hours prior to the test start and during the total test period, 16 hours photoperiod daily, temperature between 11 and 13.5 °C (on one occasion 9.5 – 9.7 °C), loading: 0.68 g fish/L
Test medium:
Analytical results of the used tap-water indicate in general adequate quality for the purpose of this study, however the pH was relatively high, hardness: 2.2 mmol/L (220 mg $CaCO_3$/L), pH between 8.4 and 8.8, dissolved oxygen concentration between 10.5 and 12.9 mg/L
Biological observations:
Mortality and sublethal effects were recorded at 2, 24, 48, 72 and 96 hours
Analytical measurements:
Stability test: Additionally to the test vessels with fish a vessel without fish and 100 mg metaldehyde/L was set up to confirm the stability of the test substance. From this vessel duplicate samples were taken at 0 and 48 hours.
Concentration verifications: From each replicate of all treatment groups samples were taken at test start and from replicates of the 32 and 0.56 mg/L treatment groups samples of new test media were taken at 48 hours.
Method of analysis: Derivatisation with 2,4-dinitrophenylhydrazin followed by HPLC
Statistical evaluation:
Since no test concentration resulted in partial mortalities, no statistical evaluation of the data was performed. The LC_{50} was estimated as the geometric mean between the lowest concentration with 100 % mortality and the highest concentration with 0 % mortality (this is in accordance with OECD 203).
Findings:
Range finding study:
1000 mg/L (supersaturated solution): 100 % mortality, 100 mg/L: one out of the four fish exposed was found dead
Main study:
Analytical results:
Stability test: Measured concentrations were 92 % (0 hours) and 87 % (48 hours) of nominal.

Concentration verification: Measured concentrations were between 82 and 100 % of nominal.
Mortality:
Up to and including 56 mg/L no fish died, at 100 mg/L and 180 mg/L all fish died within 24 hours of exposure.
Sublethal effects:
At 56 mg/L one fish was found discoloured 48 hours after exposure. Since this was the only incidence that a sublethal effect was observed it is not considered to be treatment related.
Conclusion:
LC_{50}: 75 mg/L (nominal), NOEC: 56 mg/L (nominal)
Comment (RMS):
Deviations from test guidelines: On one occasion temperatures of both replicates from the 100 mg/L and of one replicate from the 180 mg/L treatment group had decreased to values ranging from 9.5 to 9.7 °C, respectively. This deviation from the guideline is not considered to have affected the test results.
Due to the relatively high pH of the used tap water the pH of test media (8.4 – 8.8) exceeded slightly the limit of the optimal range given in the guidelines. However, no effects were observed in the control and measured test concentrations were acceptable. Therefore this deviation from guidelines is also considered to have not affected the results of the study.
The study is considered acceptable.

Reference: Wetton, P. M., Mullee, D. M. (2001): Meta®-Metaldehyde (Code LZ1060): Acute Toxicity to common carp (*Cyprinus carpio*). Document no. 821-002
Test guideline: OECD 203 (1992)
GLP: Yes
Material and methods:
Range finding study
3 fish per treatment were exposed to nominal concentrations of 0, 1, 10 and 100 mg/L
Main study:
Test substance: META^R metaldehyde (tech. metaldehyde), purity: not stated, batch: 29103
Test organism:
Common carp (*Cyprinus carpio*), mean length: 4.2 cm (s.d. 0.1), mean weight: 1.82 g (s.d. 0.21) at the end of the study, loading rate: 0.91 g/L, food: commercial crushed carp pellets. Feeding was discontinued approximately 24 hours prior to the start of exposure.
Treatments: Based on the results of the range-finding study a limit test at 100 mg/L was conducted.
Number of animals: 10 fish for the control and 2 replicates with 10 fish each per treatment group
Duration: 96 hours
Test conditions:
20 L glass vessels, semi-static test regime, daily renewal of test media, photoperiod: 16 hours light and 8 hours dark with 20 minutes transition periods, temperature was held constant at 21 °C.
Test medium:
Laboratory tap water of sufficient quality, dechlorinated by passage through an activated carbon filter and partly softened, total hardness of approximately 100 mg $CaCO_3$/L, pH: 7.6 – 7.9, dissolved oxygen: 7.4 – 8.7 mg/L
Biological observations:
Mortalities and sublethal effects were recorded at 3, 6, 24, 48, 72 and 96 hours.
Analytical measurements:
Method of analysis: Gas chromatography using an external standard

Test substance verification: Samples from the control and each replicate test vessel were taken at 0, 24 and 96 hours.
Stability test: Pre-study test samples were prepared and analysed initially and after storage for approximately 24 hours (period between media renewal) in sealed vessels at ambient temperature in light and dark conditions.
Findings:
Range finding study
No mortalities or sublethal effects could be observed up to the highest tested concentration of 100 mg/L.
Main study
Analytical results:
Analysis of the test preparations at 0, 24 and 96 hours gave measured values ranging from 84 % to 108 % of nominal concentrations. The test material was stable in the test media for the period between renewals (24 hours).
Mortalities and sublethal effects:
After 48 hours one fish was observed to be moribund and killed in extremis. Since no sublethal effects were observed in the remaining fish after 96 hours, this mortality was considered not to be treatment related. No further mortalities occurred.
Conclusion:
LC_{50}: > 100 mg/L, NOEC: 100 mg/L, based on a nominal limit concentration
Comment (RMS):
The study is considered acceptable.

Long-term toxicity to fish

Reference: Bogers, M. (1990b): Prolonged toxicity study in the rainbow trout with P0071. Document No. 826-001
Test guideline: OECD 204 (1984)
GLP: Yes
Material and methods:
Test substance: P0071 (metaldehyde), purity: 99.3 %, batch: 5448
Test organism:
Rainbow trout (*Oncorhynchus mykiss*), mean length: 4.8 cm (s.d. 0.12, n=10), mean weight: 1.18 g (s.d. 0.022, n=10)
Treatments: Control, 4.5, 9.5, 19, 37.5 and 75 mg/L
Number of animals: One replicate with ten fish per concentration and control
Duration: 21 days
Test medium:
Analytical results of the used tap water indicate acceptable qualitiy for the purpose of this study. Oxygen concentrations were generally in the range of 6.5 and 10.9 mg/L which corresponds to saturation levels above 60 %. However, on days 8 and 16 saturation levels had dropped to about 35 % and 50 % in old test media of the 75 mg/L and 19 mg/L treatments (% saturation levels were estimated by the RMS from measured temperatures and measured oxygen concentration levels). pH: 7.6 to 8.7, values above 8.5 were only found at the test concentration of 75 mg/L; hardness: 220 mg $CaCO_3$/L
Test conditions:
Semi-static test system with renewal of test media every 48 hours, test vessels: 15 L all-glass, photoperiod: 16 hours light and 8 hours dark, temperature: range of 12.0 to 15.0 °C except on day 1 and day 9 where temperature had dropped to 9.5 °C (75 mg/L) and 10.5 °C (at all test concentrations), loading: 0.91 g fish/L at the start of the test, feeding: daily 0.6 g (first week),

0.9 g (second week) and 1.2 g (third week) Trouvit per vessel
Analytical measurements:
Method of analysis: Derivatisation with 2,4-dinitrophenylhydrazin followed by HPLC
Sampling: At the start of the test, after 48 hours (old media) and just before the last renewal duplicate samples from the 4.5, 19, and 75 mg/L test vessels were taken. Extra samples were taken from all test concentrations and stored at -20 °C for additional analyses if necessary.
Biological observations:
Fish were daily observed for mortality. Effects on behaviour and appearance were recorded every 48 hours at test media renewals.
Statistical evaluation:
Fish weights and lengths recorded for treated groups at the end of the test were compared to those of the control with the Steel-test.
Findings:
Analytical measurements:
Only samples taken from concentrations that were considered relevant in terms of effects (19, 37.5 and 75 mg/L) were analysed because the analytical method used was of relatively low sensitivity. Measured concentrations were in the range of 86 to 105 % of nominal concentrations.
Mortalities:
Only at 75 mg/L mortalities were observed, one fish was found dead on day 1 and one on day 10 of exposure (total mortality: 20 %).
Sublethal effects: Up to and including the test concentration of 37.5 mg/L no effects on fish weights and body lengths could be observed. At 75 mg/L the mean weight of fish was statistically significantly reduced compared to the control.

Table 105: Mean weights and lengths of rainbow trouts after 21 days of exposure to metaldehyde

Test concentration [mg/L]	Mean weight (s.d.) [g]	Mean length (s.d.) [cm]
0 (control)	2.79 (0.59)	6.0 (0.49)
4.5	2.79 (0.56)	6.2 (0.58)
9.5	2.69 (0.62)	6.1 (0.51)
19	2.66 (0.90)	6.1 (0.64)
37.5	2.46 (0.90)	5.9 (0.68)
75	1.63 (0.43) *	5.3 (0.50)

* statistically significantly different from control ($p<0.05$)

Conclusion:
NOEC = 37.5 mg/L (nominal) based on mortalities and reduced weights of fish at 75 mg/L
Comment (RMS):
At test concentrations of 19 and 75 mg/L on one occasion dissolved oxygen concentrations were found to be below 60 % and at the highest test concentration pH values were repeatedly slightly above 8.5 (max 8.7), the upper limit given in OECD 204. The RMS is of the opinion that the singular cases of lowered oxygen saturation and the slightly elevated pH at the highest test concentration as well as the short periods of reduced temperature did not have an influence on the integrity of the study. Hence the study is considered acceptable.

5.4.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

Reference: Wuethrich V. (1990a): 48-hour acute toxicity of P0071 to *Daphnia magna* (OECD-immobilization test). Document no. 822-001

Test guideline: OECD 202 (1984), US EPA 540/9-85-005 Acute Toxicity Test for Freshwater Invertebrates (1985)

GLP: Yes

Material and methods:

Test substance: P0071 (metaldehyde), purity: 99.3 %, batch: 5448

Test organism: *Daphnia magna*, less than 24 hours of age

Treatments: Control, solvent control (0.01 % methanol per volume), 1.125, 2.25, 4.5, 9 and 90 mg/L. The concentration of the solvent methanol was 0.01 % per volume at the highest.

Number of animals: 2 replicates with 10 animals each per control and treatment

Duration: 48 hours

Test conditions:

Test vessels: 50 mL glass beakers, loading: 10 daphnids per 20 mL of test medium, static system, temperature: 21 °C (no values for individual measurements provided in the study protocol), photoperiod: 16 hours light and 8 hours dark, no information about feeding is provided

Test medium:

Reconstituted water prepared according to Directive 84/449 EEC, Part C.2: "Acute Toxicity for Daphnia" was used. Total hardness: 250 mg $CaCO_3$/L, dissolved oxygen concentration: 8.0 – 8.8 mg/L, pH: 8.1 – 8.3. Oxygen and pH were measured at the start and end of the test.

Biological observations:

Mobility of daphnids was recorded after 24 and 48 hours of exposure. Observations for sublethal effects were not conducted.

Analytical measurements:

Method of analysis: Derivatisation with 2,4-dinitrophenylhydrazin followed by HPLC.

Concentration verification: Samples from the solvent control and 1.125, 4.5 and 90 mg/L vessels were taken at the start and end of the test.

Stability test: From an additional test vessel containing 90 mg/L samples were taken at the beginning and after 48 hours of exposure.

Statistical evaluation:

Due to the lack of treatment related effects, no statistical evaluation of the data was performed.

Findings:

Analytical results:

The test substance was shown to be stable over the test duration. Measured concentrations (mean of two replicates) were in the range of 75.4 - 81.7 % of nominal concentrations at the beginning of the test and 71.6 - 95.7 % of nominal concentrations at the end of the test. Since measured concentrations were repeatedly below 80 % of nominal, the EC50 was derived from mean measured concentrations.

Immobility:

Table 106: Effect of P0071 (metaldehyde) on immobility of Daphnia magna

Treatment (nominal concentrations)	Replicate	Cumulative immobilisation [%]	
		24 h	48 h
Control without methanol	A	0	0
	B	0	0
Control with methanol (0.01 % per volume)	A	0	0
	B	0	0
1.125 mg/L	A	0	0
	B	0	0
2.25 mg/L	A	0	0
	B	0	0
4.5 mg/L	A	0	0
	B	10	10
9 mg/L	A	100	100
	B	0	0
90 mg/L	A	0	0
	B	0	0

The observed 100 % immobilisation in one replicate at a test concentration of 9 mg/L is not regarded to be treatment related because at the second replicate at this concentration and at a treatment rate of 90 mg/L no immobilisation was recorded.
Conclusion:
EC_{50} > 78.4 mg/L based on mean measured concentrations
Comments (RMS):
The study is acceptable.

Long-term toxicity to aquatic invertebrates

Reference: Wuethrich, V. (1990c): Influence of P0071 on the reproduction of *Daphnia magna.* Document No. 827-001
Test guideline: OECD 202, Part II
GLP: Yes
Material and methods:
Test substance: P0071 (metaldehyde), purity: 99.3 %, batch: 5448
Test organism: *Daphnia magna*, age: < 24 hours
Treatments:
Control, solvent control, 5.63, 11.25, 22.5, 45 and 90 mg/L, the concentration of the solvent methanol was in all test solutions and in the solvent control 0.01 % v/v
Test duration: 21 days
Test medium:
A mixture of 2/3 of reconstituted water (Directive 84/449 EEC, Part C.2: "Acute Toxicity for Daphnia") and 1/3 of pond water (Anwiler Weiher, CH-4461 Anwil) was used as dilution water. Hardness of the mixture: 256-278 mg $CaCO_3$/L (measured once per week), pH: 8.0 – 8.6 in fresh test media and 8.0 – 9.0 in old test media (measured in the control, solvent control, the

highest and lowest test concentrations), dissolved oxygen: 5.9 – 11.3 mg/L (≥ 60 % saturation) and on one occasion 5.2 mg/L (slightly below 60 % saturation) at the test concentration of 5.63 mg/L.

Test design:

Daphnids were kept in groups of 2 x 10 individuals per treatment in glass beakers of 200 mL until eggs could be observed in the brood pouches (day 5). Then for each treatment ten daphnids with eggs in their brood pouches were separated and kept individually in beakers with 50 mL test medium. The reproduction rate of these individually held daphnids was recorded until the end of the study. The daphnids not selected for individual holding were further on kept in groups (at least 5 daphnids per beaker) and were observed for mortality until the end of the test. In the study protocol no information is provided on the criteria which were applied to select 10 daphnids with eggs in their brood pouches for the reproduction part of the test out of the original 20 individuals per concentration.

Test conditions:

Semi-static test system, test medium renewal: every Monday, Wednesday and Friday, temperature: 20 – 21 °C (checked at renewal days), photoperiod: 16 hours light and 8 hours dark, food: a mixture of yeast and algae (*Scenedesmus subspicatus*) was fed, feeding was performed with increasing amounts of food, the information provided in the study report is not conclusive on the amounts of food used per test chamber

Analytical measurements:

Method of analysis: Derivatisation with 2,4-dinitrophenylhydrazin followed by HPLC
Concentration verification: From the solvent control, the lowest, medium and highest test concentration samples were taken at days 0, 2 and 19 (fresh media) and at day 21 (old media).
Stability test: From an additional test vessel containing 90 mg/L test article (without feed and daphnids), samples were taken on days 0, 2 and 21.

Biological observations:

The mortality of adults and the number of young was recorded 3 times per week at test medium renewals.

Statistical evaluation: Steel-test

Findings:

Analytical measurements:

Table 107: Measured concentrations of metaldehyde in test media

Sampling day	Test medium	Test concentrations [% of nominal]			
		5.63 mg/L	22.5 mg/L	90 mg/L	90 mg/L (stability test)
0	fresh	83.1	80.0	73.0	90.1
2	fresh	94.7	90.1	86.2	95.9
19	fresh	82.2	82.3	67.7	not sampled
21	old	89.5	83.9	101.4	99.4

The measured concentrations from day 19 (fresh test media) and day 21 (respective old test media) indicate that the test article was not completely dissolved in fresh test media and that the dissolution process continued throughout the period between medium renewals. This finding was more pronounced at the highest test concentration of nominal 90 mg/L.

Biological observations:

At the second water renewal on day 5 eggs were observed in the brood pouches of daphnids and

then 10 daphnids per test concentrations were selected and held individually.

Table 108: Effects of metaldehyde on survival and reproduction of Daphnia magna after 21 days of exposure

Treatment [mg ai/L]	Cumulative no. of young / 10 adults	Mean no. of live young / adult ± s.d.	Immobile out of 20 individuals [%]
Control	1209	121 ± 9	10
Solvent control	1337	134 ± 13	5
5.63	1389	139 ± 7	5
11.25	1390	139 ± 5	5
22.5	1213	135 ± 8	5
45	1263	126 ± 33	5
90	1421	142 ± 18	0

Conclusion:
NOEC = 90 mg/L based on nominal concentrations or 68 mg/L based on mean measured concentrations of fresh test media of the 90 mg/L treatment group, LOEC > 90 / 68 mg/L (nominal / mean measured)
Comment (RMS):
The study was conducted according to a test design which deviates from accepted test guidelines. Daphnids were first held in groups of 2 x 10 individuals per concentration. On day 5 of exposure, when eggs were observed in the brood pouches of daphnids, 10 animals per concentration with eggs in their brood pouches were selected and then held individually. Such a change from group exposure to individual exposure of a subsample of the original group is not foreseen in any accepted test guideline. In the study protocol no information is provided on the criteria which were applied for the selection of the 10 daphnia for individual exposure per treatment level. Also the number of adults with eggs in their brood pouches per concentration on the day of change of exposure regime (from group to individual exposure) is not given.
Further deviations from the applied test guideline (OECD 202, part II) are: Daphnids were fed three times a week, they should have been fed daily, the loading was 20 mL of test medium per animal, it should have been 40 mL per animal, the dilution water was a mixture of reconstituted water and pond water, the processing of pond water (e.g. filtration) is not stated, no water quality parameters such as TOC of the pond water are provided in the study protocol.
Despite these deviations from accepted test guidelines and shortcomings of the study design, the RMS thinks that the results can be accepted for risk assessment for the following reasons: Up to the highest tested concentration of 90 mg/L no reproductive effects were observed on the 10 selected and individually held daphnids, additionally no treatment related mortalities were observed. The validity criteria of the guidelines OECD 211 and EPA OPPTS 850.1300 are met: mortality of parent animals ≤ 20 % (met for all daphnids of the test), mean number of offspring per surviving control parent at the end of the test is ≥ 60 and no ephippia are produced by control animals (met for the individually held daphnids for which offspring was recorded). Since no effects were observed, it can be assumed, that the quality of the used pond water was acceptable for this test. The mean Koc of metaldehyde is 85 (s.d. 53, n = 8) and hence the adsorption to organic carbon will be rather low. Therefore it can be assumed that the TOC added with the pond water (1/3 of the dilution water) will not have had a major effect on the bioavailability of the test substance.

The study is considered acceptable.

5.4.3 Algae and aquatic plants

Reference: Wuethrich, V. (1990b): Acute toxicity of P0071 to *Scenedesmus subspicatus* (OECD- Algae growth inhibition test). Document no.: 823-001
Test guideline: OECD 201
GLP: Yes
The study is not considered valid for the following reasons:
Concentrations of 0, 1.56, 3.13, 6.25, 12.5, 25, 50 mg metaldehyde/L were tested. Regarding the appearance of the test substance in the test medium the following is stated in the study protocol: "not completely soluble, slightly turbid, particles similar to glass splinters rise on glass – was above liquid level". It was not mentioned at which test concentrations this was observed. Therefore it is not clear whether the test substance was sufficiently solved and hence bioavailable in the test media at the highest test concentrations (25 and 50 mg/L).
Regarding the composition of the nutrient solution for algal cultivation only a reference is provided in the study protocol. From this reference it cannot be judged whether the limits for P, N, chelators and hardness as given in OECD 201 are met.
The pH was adjusted to 7.7 at the beginning of the test, but it was not stated which buffer was used.
After 24 and 48 hours the number of algae was estimated by means of a microscope. After 72 and 96 hours the number of algae was determined spectrophotometrically using a standard curve established with known (counted) cell densities. No information on the validity of the spectrophotometrical biomass estimation (e.g. calibration data) was provided and hence the reliability of the used method could not be evaluated.
No values for growth rates were provided in the study report. Therefore the RMS calculated growth rates from the provided cell density data and found that growth rates were unexpectedly high for the used species *Scenedesmus subspicatus* within the first 24 hours of the study. From 24 to 48 hours growth rates dropped drastically and recovered from 48 to 72 hours (see table B.9.2.1-2).
The ErC_{50} values (72 h and 96 h) as given in the study report are not considered valid by the RMS. The 72 h ErC_{50} was stated to be 21.7 mg/L although even at 50 mg/L (the highest test concentration) the inhibition of the mean growth rate was below 20 %. Also the derivation of the ErC_{50} value for 96 hours could not be comprehended.
In the OECD guideline it is stated that the highest test concentration should inhibit growth at least by 50 % and preferably stop growth completely. As demonstrated in other toxicity tests on fish and *Daphnia magna*, higher test concentrations than 50 mg/L can be achieved and therefore should be tested.

Table 109: Changes in growth rates of Scenedesmus subspicatus over time at different treatments with metaldehyde

Time period	Treatments [mg ai/L]						
	Control	1.56	3.13	6.25	12.5	25	50
0-24 h	2.44	2.83	2.97	3.05	2.71	1.82	1.36
24-48 h	0.51	0.39	0.54	0.42	0.22	0.53	0.44
48-72 h	0.93	0.92	0.32	0.18	0.47	0.76	1.39

Time period	Treatments [mg ai/L]						
	Control	1.56	3.13	6.25	12.5	25	50
72-96 h	1.65	1.49	1.39	1.65	1.62	1.87	1.91
Mean 0-72 h	1.30	1.38	1.28	1.22	1.14	1.04	1.06
Mean 0-96 h	1.39	1.41	1.30	1.33	1.26	1.25	1.28

Reference:	**Metaldehyde - A study on the toxicity to algae (*Desmodesmus subspicatus*) over 72 hours**
Author(s). year:	Egeler, P., Junker, T. Knoch, E. (2007)
Report/Doc. number:	823-002
Guideline(s):	OECD 201 (2006)
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance: Metaldehyde, batch no.: 36605, purity: 99.5 % (w/w)

MATERIAL AND METHODS:

Test species:	*Desmodesmus subspicatus*
Test concentrations:	Nominal: 0 (medium control), 12.5, 25, 50, 100 and 200 mg/L
No. of replicates:	3 replicates per test concentration and 6 replicates for the control, 2 replicates for a test substance stability check without algae
Initial loading:	5×10^3 cells/mL
Test type / duration:	Static, 72 hours
Nutrient medium:	AAP-medium adjusted to a pH of 7.5
Test conditions:	Continuous illumination at 76.4 ± 4.1 µE m^{-2} s^{-1} (fluorescent tubes, universal white type) Continuous stirring Temperature: 22.8 ± 0.24 °C pH at test start: 7.3 – 7.6. pH at test end: 8.1 – 8.8
Observations:	Cell concentrations were evaluated by fluorescent measurements and conversion of fluorescence units into biomass concentration using a calibration curve. Cell measurements after 24, 48 and 72 Microscopic observation of algal cells at the beginning and at the end of the test
Analytical measurements:	For chemical analysis of the test substance samples of test solutions from all treatment levels were taken at test start and test end. The lowest test concentration sample (12.5 mg/L) was not analysed. Analytical method: Gas chromatography with mass selective detection (GC-MSD)
Statistical evaluation:	EC_{50}: no statistics, NOEC: ANOVA followed by Williams' Test

RESULTS

Validity criteria:	Biomass increase in the control cultures: 132 (required: ≥ 16 within 72 h) Mean coefficient of variation for section-by-section specific growth rates in control cultures: 11.6 % (required: ≤ 35 %)

	Coefficient of variation of average specific growth rates during the test period in replicate control cultures: 6.7 % (required: ≤ 7 %)
Analytical results:	Measured concentrations were found to be 98.2 – 102.8 % of nominal at test start and 91.3 – 102.2 % at test end
Morphological effects:	In the pre-culture cells appeared normal and healthy 0 up to 25 mg/L: Cells appeared healthy at the end of the test ≥ 50 mg/L: Cells were partially deformed at the end of the test

Table 110: Effects of metaldehyde on yield and growth rate of Desmodesmus subspicatus after 72 hours of exposure

Metaldehyde [mg/L]	Inhibition after 72 hours [%]	
	Yield	Growth rate
12.5	6.1	1.5
25	1.5	0.2
50	30	7.6
100	39	9.9
200	39	10.3

CONCLUSION:
E_yC_{50} (72 h): > 200 mg/L
E_rC_{50} (72 h): > 200 mg/L
NOEC (72 h): 25 mg/L (based on inhibition of yield and growth rate)
Based on mean nominal concentrations.

5.4.4 Other aquatic organisms (including sediment)

Reference:	**Metaldehyde- A study on the toxicity to the Great Ramshorn Snail (*Planorbarius corneus*) over 48 h**
Author(s). year:	Egeler, P., Goth, M., Knoch, E. (2007)
Report/Doc. number:	825-001
Guideline(s):	No guideline available, OECD 202 (2004) was taken into account
GLP:	Yes
Deviations:	Not applicable (no guideline available)
Validity:	Acceptable

Test substance:	Metaldehyde technical, batch no.: 36605, purity: 99.5 % (w/w)
Test species:	Great Ramshorn Snail (*Planorbarius corneus*), snails of similar size were used, body index (ratio of fresh weight to volume): 0.51 – 1.41
Treatments:	Nominal: 0 (control), 9, 19, 41, 91 and 200 mg/L
No. of replicates:	4 replicates with 5 snails each per treatment and control
Test type / duration:	Static, 48 h followed by a 24 h post exposure period
Test medium:	Elendt Medium M4
Test conditions:	Temperature: 20.1± 0.5 °C, pH: 7.8 Dissolved oxygen: 4.6 – 9.5 mg/L (51 – 103 % saturation) Hardness: 268 mg/L as $CaCO_3$ Photoperiod: 16 light / 8 h dark, No aeration, No feeding during the exposure period
Observations:	Behaviour: The number of moving snails was recorded before and 1 minute after gentle agitation of the vessels. Immobilisation: After 48 h exposure snails were transferred to vessels containing clean medium and a piece of cucumber as food source. The location of each animal in the vessel was marked by clearly visible circles on the outer bottom of the vessel. The location of each snail was also recorded by photography immediately after transfer. Observations were performed 3, 6 and 24 h after exposure. All animals having changed location were recorded as mobile.
Analytical measurements:	At the beginning of the test and at test termination (48 h) samples from all test levels were taken, however, only samples from the control, 91 and 200 mg/L groups were analysed for the test substance. Analytical method: Gas chromatography with mass selective detection (GC-MSD)
Statistical evaluation:	None (all snails were mobile after 24 hours)
RESULTS	
Analytical results:	Measured concentrations were in the range of 80 – 98 % of nominal.
Sublethal effects (behaviour):	After 24 h of exposure in the two highest test concentrations (91 and 200 mg/L) 95 and 90 % of snails did not move even after gentle agitation of the test vessels. After 48 h 85 and 100 % of snails did not move at the two highest test concentrations. In the highest test concentration snails were lying on the lateral side, foot retracted into the shell.
Immobilisation:	All animals up to and including the highest test concentration appeared to be mobile 24 h after termination of exposure.

CONCLUSION: Based on nominal concentrations.

EC_{50} (48 h): > 200 mg/L (immobility 24 h after termination of exposure)

NOEC (48 h): 200 mg/L (immobility 24 h after termination of exposure)

5.4.5 Summary and discussion: Acute (short-term) aquatic toxicity

Data element: Acute (short-term) aquatic toxicity Generally expressed in terms of LC_{50} or EC_{50} (mg/L)					
	L(E)C_{50} [mg/L]		Test guideline / design	GLP (y/n)	Reliability
Fish (96 hr LC_{50}):					
Oncorhynchus mykiss	75		EPA Pesticide Assessment Guidelines, Subdivision E, 72-1 (1985), OECD 203	y	y
Cyprinus carpio	> 100		OECD 203	y	
Crustacea (48 hr EC_{50}):					
Daphnia magna	90		OECD 202 (1984), US EPA 540/9-85-005	y	y
Algae (72 or 96 hr E_rC_{50}):					
Scenedesmus subspicatus	biomass growth rate	-----	OECD 201	y	n
Desmodesmus subspicatus	yield growth rate	> 200 > 200	OECD 201 (2006)	y	y
Gastropods					
Planorbarius corneus	> 200 (48 h)		No guideline available, OECD 202 (2004) was taken into account	n	y
Conclusion: For classification and labeling the most sensitive species *Oncorhynchus mykiss* with a **LC50 = 75 mg /L** was used.					

5.4.6 Summary and discussion: Chronic (long-term) aquatic toxicity

Data element: Chronic (long-term) aquatic toxicity Generally expressed in terms of NOEC (mg/L)					
	NOEC [mg/L]		Test guideline / design	GLP (y/n)	Reliability
Fish (21 d NOEC):					
Oncorhynchus mykiss	mortality body weight	37.5	OECD 204	y	y
Crustacea (21 d NOEC):					
Daphnia magna	immobility reproduction	90	OECD 202, Part II	y	y
Algae/aquatic plants (NOEC):					
Scenedesmus subspicatus	biomass growth rate	---	OECD 201	y	n
Desmodesmus subspicatus	yield growth rate	25 25	OECD 201 (2006)	y	y
Conclusion: For classification and labeling the most sensitive species *Desmodesmus subspicatus* with a **NOEC = 25 mg /L** was used.					

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Endpoint	Classification Criteria (criteria in bold)	Conclusion for Metaldehyde
	CLP (2nd ATP)	
Degradation		
	Metaldehyde is **photolytically stable and hydrolytically stable** at environmentally relevant pH values. Metaldehyde is **not readily biodegradable** under test conditions within 28 days. In **UK simulation study** (two less oxidising systems) **DT50 whole system is > 1000 d**, therefore based on these data, the substance is considered **not to be ready biodegradable/rapid degradable.**	The active substance is not considered as ready biodegradable/rapid degradable.
Bioaccumulation		
Criteria LogKow	**Log K_{ow} is < 4** Metaldehyde Log K_{ow} = 0.12	The measured BCF is 11 and is below the classification criteria of 500 (CLP). Therefore depending on classification criteria Metaldehyde is considered to have **a low bioaccumulation potential.**
Criteria BCF	**BCF < 500** Metaldehyde BCF is 11	
Acute aquatic toxicity		
Criteria	**E/LC50 < 1 mg/L** Metaldehyde: Acute toxicity values > 1 mg/L for fish, daphnids, algae, and aquatic snails	No acute classification is required. The E/LC50 values for fish, daphnids and algae are > 1 mg/L. Based on an acute toxicity study with the water snail *Planorbarius corneus* (LC50 > 200 mg/L) metaldehyde is considered not toxic to water snails.
Chronic aquatic toxicity		
Criteria	**NOEC < 1 mg/L** Metaldehyde: Chronic toxicity values > 1 mg/L for fish, daphnids and algae	No chronic classification is required. The NOEC values for fish, daphnids and algae are > 1 mg/L. Based on an acute toxicity study with the water snail *Planorbarius corneus* (LC50 > 200 mg/L, NOEC = 200 mg/L) metaldehyde is considered not toxic to water snails.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Conclusion of environmental classification according to Regulation EC 286/2011 (2nd ATP to EC 1272/2008)

Based on the CLP Regulation, no classification is required for metaldehyde.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Metaldehyde is a molluscicide for the control of slugs and snails, it was approved under Council Directive 91/414/EEC in 2008. Metaldehyde is already listed in Annex VI of the CLP Regulation with harmonised classifications as Flam. Sol. 2 and Acute Tox. 4*.

Degradation

The DS proposed to not consider metaldehyde as rapidly degradable for classification purposes. The basis for this proposal was that metaldehyde is hydrolytically stable at a temperature of 25 °C and pH values of 5 – 9 (Carpenter, 1989a), photolytically stable at pH 7 and a temperature of 25 °C (Carpenter, 1989b), not readily biodegradable following OECD TG 301 E (Wüthrich, 1990a) and OECD TG 301 F (Lebertz, 2008), and not inherently biodegradable following OECD TG 302 B (Wüthrich, 1990b). In addition, metaldehyde was shown to have long residence times under OECD TG 308 (Kane, 2009) and dissipated from the water phase with a DT_{50} value >1000 days in silt loam system and 473 days in sand systems. DT_{50} values for the dissipation from the total system were >1000 days in silt loam system and 714 days in sand system (these values were incorrectly reported in the CLH dossier). In a BBA (German, Federal Biological Research Centre for Agriculture and Forestry) Guideline water/sediment-study (Möllerfeld *et al*., 1993), the DT_{50} values were calculated to be 30.98 and 19.01 days with sandy and loamy system, respectively. However, these were based on acetaldehyde, the major degradation product of metaldehyde.

Aquatic Bioaccumulation

The DS proposed to not consider metaldehyde as being bioaccumulative in the aquatic environment for classification purposes. The basis for this proposal was an OECD TG 107 (Shake flask method) test with a measured partition coefficient n-octanol/water log K_{ow} of 0.12 (19.9 - 20.1 °C, pH: 6.7) (Cardinaals, 1988b) and an OECD TG 305 E test with a measured BCF value of 11 (whole fish at steady state) (Unpublished, 1992).

Acute Toxicity

The DS proposed to not classify metaldehyde as acutely hazardous to the aquatic environment. The basis for this proposal was that the available short-term (acute) aquatic toxicity test results are >1 mg/L, showing toxicity of 75 mg/L for fish (96 h LC_{50}), 90 mg/L for crustacea (48 h EC_{50}), >200 mg/L for algae (72 h ErC_{50}) and > 200 mg/L for *Planorbarius corneus* (Great Ramshorn Snail) (48 h EC_{50}; immobility 24 h after termination of exposure and after recovery in clean media). The results indicated that fish were the

most sensitive taxon and that the most sensitive acute endpoint is a 96 h LC_{50} = 75 mg/L (nominal concentration) for *Oncorhynchus mykiss* (Rainbow Trout).

Chronic Toxicity

The DS originally proposed to not classify metaldehyde as chronically hazardous to the aquatic environment. The basis for this original proposal was that the available long-term (chronic) aquatic toxicity test results are > 0.1 mg/L for a non-rapidly degradable substance, showing toxicity of 37.5 mg/L for fish (21 d NOEC), 90 mg/L for Crustacea (21 d NOEC) and 25 mg/L for algae (NOEC).

Comments received during public consultation

Four MSs commented on the proposal, with three agreeing with the proposal to not classify metaldehyde for aquatic environmental hazards. The fourth MS pointed out that the OECD TG 204 "Prolonged Toxicity Test: 14-Day Study" (Unpublished, 1990b) is not considered suitable for generating chronic (long-term) toxicity data (Information requirements Chapter R.7b: Endpoint specific guidance, Version 4.0, June 2017, page 30f) and that the test guideline has been withdrawn by the OECD. The MS argued that as such a data gap on chronic toxicity exists and acute fish data should be used in conjunction with degradation data under the surrogate approach to derive the potential chronic classification. In their response to this comment, the DS agreed to withdraw the NOEC value of the 21 d prolonged fish test and to base the chronic hazard assessment on the lowest LC_{50} for fish and environmental fate data via the surrogate approach, resulting in a classification as Aquatic Chronic 3; H412.

The same MS also commented on the acute toxicity study in snails (Egeler *et al*., 2007) and indicated that the EC_{50} should be based on immobility after 48 h (exposure termination) and not 24 h after exposure termination and recovery (when the snails had been transferred into vessels containing clean medium and a piece of cucumber as a food source). The DS agreed and estimated that, instead of the proposed endpoint of 48 h EC_{50} > 200 mg/L, the correct 48 h EC_{50} would be between 19 and 41 mg/L. However, the DS did not provide a new statistical evaluation of the study. Instead, the DS emphasised this would not change the proposal for acute aquatic classification of metaldehyde.

The same MS also pointed out that given the test species (*P. corneus*) is the only available ecotoxicity data for a mollusc species and metaldehyde is a molluscicide, this test result is important for classification. The MS proposed to include the EC_{50} value as a surrogate for chronic classification. Also, given ecotoxicity data is only available for one snail species and other gastropod molluscs may be more sensitive, the classification should be reconsidered in the future if further data becomes available. This might include data from efficacy studies if usable or snail studies following new OECD TGs 242 or 243.

Additional key elements

Letter (29 August 2017) from the director of the study by Egeler et al. (2007)

The RAC Secretariat received a letter from the director of the study Egeler *et al*. (2007). The letter is a direct response to comments made under public consultation and the request for recalculation of their results by one commenting MS. In the letter, the author of the

study states that "*This test system allows a verification of the status "alive" or "dead" at the end of the exposure phase only in retrospect, i.e. based on the test organism mobility during the post-exposure phase. Therefor it is justified to determine an EC_x (mortality/immobility; 48h) based on the number of surviving animals as determined after the exposure phase, i.e. during the post-exposure phase.*"

In the original study report by Egeler *et al*. (2007), it is explained that during the exposure phase "*the number of moving snails per vessel was recorded before and 1 minute after gentle agitation of the test vessel*".

All introduced test animals (n=20) in the control and test concentrations of 9 and 19 mg/L were unambiguously identified as "mobile" without gentle agitation, while in test concentrations of 19 and 41 mg/L mobile individuals were identified only after gentle agitation of the test vessel. Individuals in the highest test concentrations of 91 and 200 mg/L were identified as "immobile", and it was further recorded that test animals were "lying on the lateral side with the foot retracted into shell", indicating a clear concentration-response relationship. Consequently, RAC concludes that the test design allowed a proper measurement of "immobility", it being in line with the definition found in Annex I of OECD TG 202.

RAC concludes that, if a snail (e.g. *P. corneus*) is immobile as a reaction to chemical exposure, this is a significant and adverse effect because it could indicate an influence on the physiology of the animal such as adverse effects on metabolism, aerobiosis, and food intake. The animal also may be more easily predated. Although immobility might be a strategy of a snail to avoid exposure and to survive a period of hostile conditions (*e.g.* high temperature or low oxygen level) this must not diminish the effect of chemical exposure. RAC concludes that such an effect has to be taken into account for the purpose of classification. While immobility might already be assessed as abnormal behaviour itself, this is clearly the case if the snail is lying on the lateral side with the foot retracted into the shell, as reported in Egeler *et al*. (2007).

RAC further concludes that for the purpose of classification, a recovery period should not be considered in aquatic toxicity testing at all. RAC agrees with a MS comment received under PC and with the response of the DS that the derived endpoint from Egeler *et al*. (2007) is not justified and instead an EC_{50} at the end of exposure at 48 h must be used.

New chronic toxicity fish study following OECD TG 210 (Unpublished, 2016)

The RAC Secretariat was informed on 31 August 2017 about a new chronic fish toxicity study finalised on 23 December 2016. The full study report was received on 4 September 2017 and the study summary on 6 September 2017. RAC subsequently assessed the GLP compliant chronic fish toxicity study conducted following OECD TG 210 (Fish Early Life Stage (FELS)) (Unpublished, 2016).

The study used *Danio rerio* (zebra fish) in a flow-through set up and exposed individuals for 35 days. The nominal concentrations used were 0 (control), 0.250, 0.791, 2.5, 7.91, and 25.0 mg/L (using 99.5% technical metaldehyde), with analytical recoveries of >80% for mean measured concentrations over the exposure period. As such, findings are reported for nominal concentrations.

The test used 4 replicates of 20 fertilised eggs per concentration level, with hatching starting on day 3 and ending on day 7. The test was continued under 30 days after 96%

of the eggs had hatched. The endpoints to be observed during the 35 day study period were hatching success & start and end of hatching, post-hatch success (Survival), macroscopic morphological abnormalities, behavioural abnormalities, dry weight of the surviving fish, and length of the surviving fish.

For all endpoints measured, no dose-response relationship could be observed. Similarly, no statistically significant difference was found between any concentration level and the controls. For the endpoints 'behavioural and morphological abnormalities', no observations were made that could be ascribed to exposure to the test substance and they are not reported individually. Furthermore, as all surviving fish appeared healthy no separate statistical analysis was made for numbers of healthy fish. In conclusion, no toxicity was observed in *D. rerio* over the concentration range tested and as such the NOEC for all endpoints is ≥ 25 mg/L.

All validity criteria were met, for both the abiotic criteria regarding the conditions of the study and the biotic criteria regarding the hatching success and post hatch survival in the controls for *D. rerio*. In conclusion, the study appears to be acceptable for the purpose of classification.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the proposal and argumentation of the DS to not consider metaldehyde as rapidly degradable for classification purposes.

Aquatic bioaccumulation

RAC agrees with the proposal and argumentation of the DS that metaldehyde is not bioaccumulative and therefore does not meet the criteria for bioaccumulation.

Acute Toxicity

RAC agrees with a commenting MS and with the response of the DS that the derived endpoint from Egeler *et al*. (2007) is not valid and instead an EC_{50} at the end of exposure at 48 h must be used. In general, RAC assesses this study as relevant since metaldehyde is a molluscicide. It is also important to note that the study was considered relevant by the RMS (Rapporteur Member State) under Reg. (EC) No 1107/2009 and by the DS when preparing the dossier. RAC assesses the test methodology as acceptable (according to Section 1 of Annex XI to the REACH Regulation (EC) No 1907/2006) and suitable for classification (CLP Regulation, Annex I, section 4.1.2.7.1). RAC considers the study to be reliable and concludes that immobility of *P. corneus* is an adverse effect which shall be taken into account for the purpose of classification. Consequently, this study is given the same weight as standard tests, meaning it is appropriate to consider it in an overall weight of evidence approach (CLP Regulation, Annex I, section 4.1.2.1). RAC has reassessed the study by Egeler *et al*. (2007) and concludes that the measured values show a clear concentration-response relationship at the end of the exposure period (48 h). As a result, RAC concludes that a 48 h EC_{50} of 78.2 mg/L (nominal), as re-calculated by RAC, should be used for classification.

In addition, RAC has reassessed the OECD TG 204 study (Unpublished, 1990b) as a prolonged acute study. No lethality was observed and only a 21 d EC_{50} (body weight) of 89.1 mg/L (nominal) for *O. mykiss* was derived from this study. However, as this endpoint is not equivalent to the available standard acute toxicity test results on fish, RAC concludes that the study is not relevant for the purpose of acute classification.

The following data for acute (short-term) aquatic toxicity are relevant for the purpose of acute classification:

	fish	**Crustacea**	**algae**	**gastropod molluscs**
included in dossier	75 mg/L (96 h LC_{50})	90 mg/L (48 h EC_{50})	>200 mg/L (72 h ErC_{50})	-
new after RAC evaluation	-	-	-	78.2 mg/L (48 h EC_{50} immobility)

RAC concludes that, besides fish, gastropod molluscs are the most sensitive group for acute (short-term) aquatic toxicity of metaldehyde, with Crustacea as the third tropic level with results in the same range. Overall - and including the reassessment of the study by Egeler *et al*. (2007) – all acute toxicity results are > 1 mg/L and RAC agrees with the proposal of the DS that metaldehyde does not meet the criteria for aquatic acute hazard classification.

Chronic Toxicity

RAC agrees with a commenting MS and with the DS's response that adequate long-term fish toxicity data cannot be derived from OECD TG 204 and as such the data from the study (Unpublished, 1990b) must not be considered for chronic classification. This is in line with ECHA guidance (Information requirements Chapter R.7b: Endpoint specific guidance Version 4.0 June 2017). RAC concludes that this study is not relevant for chronic classification.

RAC assessed the new chronic toxicity fish study OECD 210 (Unpublished, 2016) and accepts the results presented in the study report.

The following data for chronic (long-term) aquatic toxicity are relevant for the purpose of classification:

	fish	**Crustacea**	**algae**	**gastropod molluscs**
included in dossier	-	90 mg/L (21 d NOEC)	25 mg/L (NOEC)	-
newly available	> 25 mg/L (NOEC)	-	-	-

RAC notes that data are available for the three standard trophic levels from chronic aquatic toxicity studies with metaldehyde. These NOECs for fish, Crustacea and algae are all > 0.1 mg/L and do not meet the criteria for aquatic chronic classification of a non-rapidly degradable substance. However, fish (along with gastropods) were the most sensitive group under acute testing and the fish species used under chronic testing is not the same as that from the acute data set, so it is unclear if the chronic fish data truly represents the long-term hazard for this trophic level.

Furthermore, as metaldehyde is a molluscicidal substance and the Egeler *et al*. study (2007) is considered suitable for consideration under acute aquatic classification, RAC notes that no chronic toxicity data for molluscs are available.

RAC concludes that in the case of metaldehyde a weight of evidence approach is appropriate and that all information besides the standard dataset for the three trophic levels should be taken into account for a full description of the hazard of metaldehyde. As a consequence, RAC concludes it is appropriate to use a combination of environmental fate and acute toxicity data to derive aquatic chronic toxicity by applying the surrogate approach. In doing so, taking into account that the substance is not rapidly degradable and the snail 48 h EC_{50} (immobility) of 78.2 mg/L, a classification of metaldehyde as Aquatic Chronic 3; H412 is derived. As the classification derived from the provided standard long-term toxicity data is 'no classification', the surrogate approach provides the basis for classification as it gives the most stringent outcome.

In conclusion, following a weight of evidence approach and using acute toxicity data from molluscs in combination with environmental fate data, metaldehyde meets the criteria for classification as **Aquatic Chronic 3; H412**.

RAC further notes that the classification may be reconsidered in the future if further data on chronic (long-term) aquatic toxicity for gastropod molluscs becomes available. This might include data from efficacy studies, if usable for classification purposes, or gastropod mollusc studies following the new OECD Test Guidelines 242 or 243.

Supplemental information - In depth analyses by RAC

RAC reassessment of the study by Egeler et al. (2007)

RAC has reassessed the original study report of "Metaldehyde- A study on the toxicity to the Great Ramshorn Snail (*Planobarius corneus*) over 48 h" by Egeler, P., Goth, M., Knoch, E. (2007) with Report/Doc. number: 825-001.

RAC notes that there are no raw data for each of the four replicates concerning the mobility of the snails, as made in all test vessels at 24 h and 48 h documented in the original study report. Only a summary of observations at 24 h and 48 h is available in the original study report. Consequently, the data presented in the following Tables had to be used for the statistical analysis using the software ToxRat Professional Version 3.2 (www.toxrat.com/). The Figure shows the concentration-effect curve. The results are presented in the Tables below.

Table: Total number of immobile individuals of *Planorbarius corneus* over time as dependent on concentration of metaldehyde

Treatm. [mg/L]	**Control**	**9**	**19**	**41**	**91**	**200**
0 h:	0	0	0	0	0	0
24 h:	0	0	0	0	19	18
48 h:	0	0	0	0	17	20

Table: Effects on immobility in *Planorbarius corneus* at 48 h (end of exposure)

Treat.[mg/L]	Total Introduced	Mobile	Immobile	% Immobility
Control	20	20	0	0,0
9,0	20	20	0	0,0
19,0	20	20	0	0,0
41,0	20	20	0	0,0
91,0	20	3	17	85,0
200,0	20	0	20	100,0

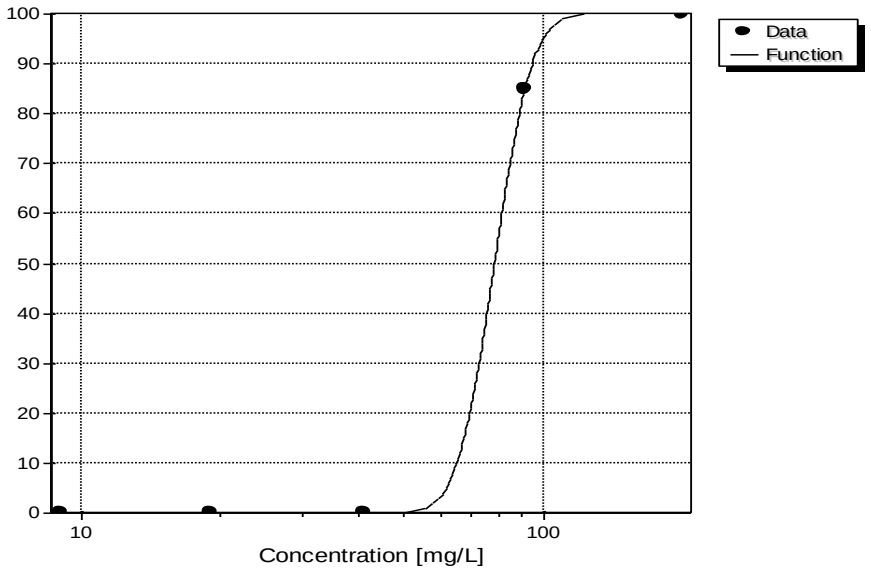


Figure: Concentration-effect curve showing the influence of metaldehyde on immobility of the introduced *Planorbarius corneus* as observed after 48 h (end of exposure).

Table: LOEC and NOEC determination with immobility: Arithmetic means and significance marks as computed for immobility for all inspection intervals (top); bottom part: obtained LOEC and NOEC with indication of statistical test used; *casd: Step-down Cochran-Armitage test procedure, significance level was 0,05, one-sided greater.

Treatm. [mg/L]	0-24 h	0-48 h
9	0,0 -	0,0 -
19	0,0 -	0,0 -
41	0,0 -	0,0 -
91	95,0+	85,0+
200	90,0+	100,0+
LOEC	91 *casd	91 *casd
NOEC	41 *casd	41 *casd

+: Significant difference to control (p <=0,050)

Table: Summary of Results for all Endpoints: Critical effect and threshold concentration as observed at end of experimental time; EC: Effective concentration for xx% reduction; 95%-CL: 95% Confidence limits

Critical Conc.s [mg/L]		0-24 h	0-48 h
Immobility			
	EC_{10}	40.2	64.8
95%-CL	lower	n.d.	n.d.

	upper	n.d.	n.d.
	EC_{20}	49.3	69.1
95%-CL	lower	n.d.	n.d.
	upper	n.d.	n.d.
	EC_{50}	72.7	78.2
95%-CL	lower	n.d.	n.d.
	upper	n.d.	n.d.

n.d.: not determined due to mathematical reasons or inappropriate data

RAC notes that the raw data for the four replicates should be used for the derivation of the 48 h EC_{50} value, however these data are not documented in the original study report.

RAC concludes that as this data is not available, a 48 h 78.2 mg/L (nominal) EC_{50} for *P. corneus* (Great Ramshorn Snail) should be used for the purpose of classification.

6 OTHER INFORMATION

Environmental fate properties and environmental hazard assessments of this CLH report are based on studies and summaries of the Draft Assessment Report and its addenda.

7 REFERENCES

7.1 Physico-chemical properties

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Bohle, J. F.	1989	Determination of the water solubility of P0071 RCC, 026021 LR 1390 N No	N	Lonza
Bohle, J. F.	1989	Determination of the solubility of P0071 in different solvents RCC, 016457 LR 1391 Yes No	No	Lonza
Cardinaals, J. M.	1988	Determination of the vapour pressure of P0071 RCC, 00854/C559; LR 1386 No No	N	Lonza
Cardinaals, J. M.	1988	Calculation of Henry's law constant of P0071 RCC, 00854/C588 LR 1389 No No	N	Lonza
Cardinaals, J. M.	1988	Determination of the solubility of P0071 in water RCC, 00854/C558 LR 1388 No No	N	Lonza
Cardinaals, J. M.	1988b	Determination of the partition coefficient of P0071 RCC, 00854/C560 LR 1387 No No	N	Lonza
Carpenter, M.	1989	Hydrolysis of Metaldehyde as a function of pH at 25°C ABC, 37146 LR 1410 No No	N	Lonza
Carpenter, M.	1989a	Photodegradation of Metaldehyde in ph 7 buffered solution ABL, 37766 LR 1412 No No	N	Lonza
Carpenter, M.	1989a	Photodegradation of Metaldehyde in ph 7 buffered solution ABL, 37766 LR 1412 No No	N	Lonza

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Comb, A.L.	2007	Metaldehyde - Melting temperature Huntingdon Life Science Report No.: LZA 0287/072574 LR 4077 GLP, unpublished Doc. No.: 112-002	Y	Lonza
Comb, A.L.	2009	!! Confidential !! - Acetaldehyde Spectra Huntingdon Life Science Lonza Rep. No. 4384 Report No.: LZA0315 GLP, unpublished Doc. No.: 157-001	Y	Lonza
Comb, A.L.	2007	Metaldehyde - Solvent solubility Huntingdon Life Science Report No.: LZA0288/072599 LR 4078 GLP, unpublished Doc. No.: 114-006	Y	Lonza
Hogg, A. S.	1998	Meta Metaldehyde: determination of general physico-chemical properties SPL, 102/278 LR 2993 Yes No	N	Lonza
O'Connor, B. J., Mullee, D. M.	2001	Meta Metaldehyde (CODE LZ1060): determination of general physico-chemical properties and spectra SPL, 102/353 LR 3340 Yes No	Y	Lonza
O'Connor, B. J., Mullee, D. M.	2001	Meta Metaldehyde (CODE LZ1060): determination of general physico-chemical properties and spectra SPL, 102/353 LR 3340 Yes No	Y	Lonza
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O'Connor, B. J., Mullee, D. M.	2001a	Meta Metaldehyde (CODE LZ1060): determination of solubility in ethyl aceto acetate SPL, 102/388 LR 3332 Yes No	Y	Lonza

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Tremain, S. P.	2001	Meta Metaldehyde (Code LZ1060): determination of hazardous physico-chemical properties SPL, 102/354 LR 3338 Yes No	Y	Lonza
Voget, M.	1994	Calculation of the photochemical oxidative half-life of Meta Metaldehyde ECO, 94-23-11 LR 2293 no No	N	Lonza
Weiss, A.	2009	Statement related to the oxidising properties of Metaldehyde Scientific Consulting Company, Wendelsheim, Germany Report No.: 262-013 Not GLP, unpublished Doc. No.: 143-001	Y	Lonza

7.2 Toxicological properties

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
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Jones J., Collier T.	1987	P0071: OECD 401 Acute oral toxicity test in the rat, Project Number 102/9A Safepharm Laboratories Ltd., Derby, UK Doc.No. 521-002, Lonza Report No.1354 GLP: yes unpublished	N	Lonza
Coles R.	1990a	P0071: Acute oral toxicity test in the mouse, Project Number 102/50 Safepharm Laboratories Ltd., Derby, UK Doc.No. 521-001, Lonza Report No. 1325 GLP: yes unpublished	N	Lonza
Durando, J.	2009	Acute Oral Toxicity Study with META Metaldehyde techn. CAS No. 108-62-3: Up-And-Down Procedure in Rats Eurofins/Product Safety Laboratories, USA Laboratory Identification No. 26776 Lonza Report No. 4377 / Doc. No.: 521-003 GLP Unpublished	Y	Lonza
Davies R., Collins C.	1974	Acute percutaneous toxicity to rats of metaldehyde Huntington Research Centre, UK Doc.No. 522-001, Lonza Report No. 1360 GLP: no unpublished	N	Lonza
Berczy Z., Cobb L., Cherry C.	1973	Acute inhalation toxicity to the rat of metaldehyde dust Huntington Research Centre, UK Doc.No. 523-001, Lonza Report No. 1362 GLP: no unpublished	N	Lonza
Griffiths, D.R.	2009	Outcome of technical pre-trials for an acute inhalation toxicity study with Metaldehyde Harlan Laboratories Ltd., Derbyshire, UK; Ref: L260109-01/vm Lonza Report No. 4336, Doc. No.: 581-004 GLP: not applicable Unpublished	Y	Lonza
Jones J.	1983	P0071: A primary skin irritation and corrosivity study in the rabbit Hazleton Laboratories Europe Ltd., UK Doc.No. 565-001, Lonza Report No. 1373 GLP: yes unpublished	N	Lonza

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Nitka S.	1984	Guinea pig sensitization (Buehler) Consumer Product Testing, NJ, USA Doc.No. 567-001, Lonza Report No. 1378 GLP: yes unpublished	N	Lonza
Bull, A.D.	2007	LZ1060 Metaldehyde. Assessment of skin sensitization potential using the Local Lymph Node Assay in the mouse Huntingdon Life Sciences Limited, Cambridgeshire UK; LZA 0292/064237/LN Lonza Report No. 4064, Doc. No.: 567-003 GLP Unpublished	Y	Lonza
Dreher, D.M.	2008	Metaldehyde: Local Lymph Node Assay in the mouse (Pooled Method) Covance Laboratories, North Yorkshire UK; 2904/001 GLP Unpublished	Y	Metaldehyde Task Force
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Thomas O., Bartlett A., Brooks P.	1998	P0071: Ninety day sub-chronic oral (dietary) toxicity study in the rat Safepharm Laboratories Ltd., Derby, UK Doc.No. 533-003, Lonza Report No. 2974 GLP: yes unpublished	Y	Lonza
Gill M., Wagner C.	1990	Ninety-Day Dietary Dose Range Finding Study with Metaldehyde in Mice Bushy Run Research Center, PA, USA Doc.No. 533-002, Lonza Report No. 1546 GLP: yes unpublished	N	Lonza
Leuschner, J.	2002	4-week dose-range-finding study for a 52-week chronic toxicity study of metaldehyde by oral administration via the diet to Beagle dogs LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany; LPT Report No. 14543/01 Lonza Report No. 3506, Doc. No.: 532-003 GLP Unpublished	Y	Lonza

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Leuschner J.	2003	52-week chronic toxicity study of metaldehyde by repeated oral administration via the diet to Beagle dogs LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany Doc.No. 537-003, Lonza Report No. 3657 GLP: yes unpublished	Y	Lonza
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Hermansky S., Wagner C.	1991	21-day repeated cutaneous dose toxicity study with metaldehyde in New Zealand White rabbits Bushy Run Research Center, PA, USA Doc.No. 532-002, Lonza Report No. 1800 GLP: yes unpublished	N	Lonza
Thompson P.	1998	P0071: Reverse mutation assay "Ames Test" using *Salmonella typhimurium* and *Escherichia coli* Safepharm Laboratories Ltd., Derby, UK Doc.No. 557-006, Lonza Report No. 2972 GLP: yes unpublished	Y	Lonza

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May K.	1992	P0071: Assessment of its ability to cause lethal DNA damage in strains of *Escherichia coli* Life Science Research Ltd., Suffolk, England Doc.No. 557-004, Lonza Report No. 1900 GLP: yes unpublished	N	Lonza
Jenkinson P.	1990	P0071: OECD 474 Micronucleus test in the mouse Safepharm Laboratories Ltd., Derby, UK Doc.No. 557-005, Lonza Report No. 1507 GLP: yes unpublished	N	Lonza
Gill M., Wagner C.	1992	Chronic dietary toxicity / oncogenicity study with metaldehyde in rats Bushy Run Research Center, PA, USA Doc.No. 537-002, Lonza Report No. 1550 GLP: yes unpublished	N	Lonza
Chun J., Wagner C.	1993	Chronic dietary oncogenicity study with metaldehyde in mice Bushy Run Research Center, PA, USA Doc.No. 537-001, Lonza Report No. 1549 GLP: yes unpublished	N	Lonza

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Beyrouty P.	1998	A chronic dietary oncogenicity study with metaldehyde in mice ClinTrials BioResearch Ltd., Senneville, Quebec H9X 3R3, Canada Laboratory Project I.D. 87013 Lonza Report No. 2976, Doc. No.: 555-002 GLP Unpublished	N	Lonza
Harder V., Roth T., Hofer M.	2010	Review of carcinogenicity studies with Metaldehyde SCC Scientific Consulting Company, Wendelsheim, Germany SCC Project No. 262-004; Report date 2010-04-22, Doc. No.: 581-009 GLP: not applicable Unpublished	Y	Lonza
Chun J., Neeper-Bradley T.	1993	Two generation reproduction study in CD rats with metaldehyde administered in the diet Bushy Run Research Center, PA, USA Doc.No. 543-001, Lonza Report No. 1544 GLP: yes unpublished	N	Lonza
Neeper-Bradley T., Chun J.	1990	Developmental toxicity evaluation of metaldehyde administered by gavage to CD (Sprague Dawley) rats Bushy Run Research Center, PA, USA Doc.No. 551-003, Lonza Report No. 1545 GLP: yes unpublished	N	Lonza
Neeper-Bradley T.	1990a	Developmental toxicity dose range-finding study of metaldehyde administered by gavage to New Zealand White rabbits Bushy Run Research Center, PA, USA Doc.No. 551-001, Lonza Report No. 1503 GLP: yes unpublished	N	Lonza
Neeper-Bradley T.	1990b	Developmental toxicity evaluation of metaldehyde administered by gavage to New Zealand White rabbits Bushy Run Research Center, PA, USA Doc.No. 551-002, Lonza Report No. 1504 GLP: yes unpublished	N	Lonza
Haferkorn J.	2009	Acute neurotoxicity study in rats by oral administration of metaldehyde LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany; LPT Report No. 23443, Doc. No.: 541-002 GLP Unpublished	Y	Lonza

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Jones L., Finn J., Mullee D.	2003	LZ1060, metaldehyde: ninety day repeated dose oral (dietary) neurotoxicity toxicity study in the rat Safepharm Laboratories Ltd., Derbyshire, UK Doc.No. 533-004, Lonza Report No. 3644 GLP: yes unpublished	Y	Lonza
Harder V., Roth T., Hofer M.	2010	Relevant aspects of neurological effects associated with exposure to Metaldehyde SCC Scientific Consulting Company, Wendelsheim, Germany SCC Project No. 262-004; Report date 2010-04-22, Doc. No.: 581-008 GLP: not applicable Unpublished	Y	Lonza
Flügge C.	2009	Validation of an analytical method for the determination of metaldehyde in test item formulations by GC-FID method LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany; LPT Report No. 23202, Doc. No.: 411-002 GLP Unpublished	Y	Lonza
Booze T., Oehme F.	1986	An investigation of metaldehyde and acetaldehyde toxicities in dogs Fundamental and Applied Toxicology 6, 440-446 (1986) Doc.No. 592-026 GLP: no Published	N	Published data
Sparks S.E. et al.	1996	Metaldehyde molluscicide action in mice: distribution, metabolism and possible relation to GABAergic system Pesticide Biochemistry and Physiology 55, 226-236, 1996 Doc. No. 592-005 GLP: no Published	N	Published data
Joos R., Matter S.	2003	“To whom it may concern” (Statement regarding medical information on plant personnel) Lonza AG Doc.No. 574-002 GLP: not applicable unpublished	N	Lonza
Borbely A.	1970	The problem of metaldehyde poisoning Inaugural dissertation for obtaining a doctorate at the Medical Faculty of the University of Zurich Doc.No. 592-001 GLP: no published	N	N.R.
Moody J., Inglis F.	1992	Persistence of metaldehyde during acute molluscicide poisoning Human & Experimental Toxicology 11, 361-362 (1992) Doc.No. 592-029 GLP: no published	N	N.R.

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Thompson J., Casey P., Vale J.	1995	Deaths from pesticide poisoning in England and Wales 1990-1991 Human & Experimental Toxicology 14, 437-445 (1995) Doc.No. 592-030 GLP: no published	N	N.R.
Longstreth W., Pierson D.	1982	Metaldehyde poisoning from slug bait ingestion The Western Journal of Medicine 137, 134-137 (1982) Doc.No. 592-033 GLP: no published	N	N.R.
Booze T. and Oehme F.	1985	Metaldehyde toxicity: a review Vet Hum Toxicol 27(1), 11-19 (1985) Doc.No. 592-025 GLP: no published	N	N.R.
Lisi P., Caraffini S., Assalve D.	1987	Irritation and sensitization potential of pesticides Contact Dermatitis 17, 212-218 (1987) Doc.No. 592-002 GLP: no published	N	N.R.
Booze T. and Oehme F.	1985	Metaldehyde toxicity: a review Vet Hum Toxicol 27(1), 11-19 (1985) Doc.No. 592-025 GLP: no published	N	N.R.
Von Burg R.	1991	Toxicology Update: Metaldehyde Journal of Applied Toxicology 11(5), 377-378 (1991) Doc.No. 592-028 GLP: no published	N	N.R.
Borbely A.	1970	The problem of metaldehyde poisoning Inaugural dissertation for obtaining a doctorate at the Medical Faculty of the University of Zurich Doc.No. 592-001 GLP: no published	N	N.R.
Mayer S.	1991	Poison metaldehyde In Practice, March 1991 Doc.No. 592-010 GLP: no published	N	N.R.
Booze T. and Oehme F.	1986	An investigation of metaldehyde and acetaldehyde toxicities in dogs Fundamental and Applied Toxicology 6, 440-446 (1986) Doc.No. 592-026 GLP: no published	N	N.R.

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Anonymous	1991	Safety Data Sheet Meta Metaldehyde techn. Lonza Group, Version 20.08.2001 Doc.No. 955-004 GLP: not applicable unpublished	N	Lonza
Mayer S.	1991	Poison metaldehyde In Practice, March 1991 Doc.No. 592-010 GLP: no published	N	N.R.
Mayer S.	1991	Poison metaldehyde In Practice, March 1991 Doc.No. 592-010 GLP: no published	N	N.R.

7.3 Environmental effects

7.3.1 Environmental fate and behaviour

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Cardinaals, J. M.	1990	Calculation of the volatility of P0071 Generated by: RCC Document No: 1510 GLP / GEP Yes unpublished	N	Lonza
Carpenter, M.	1989 a	Hydrolysis of Metaldehyde as a function of pH at 25°C Generated by: ABC Document No: 1410 GLP / GEP Yes unpublished	N	Lonza
Carpenter, M.	1989 b	Photodegradation of Metaldehyde in ph 7 buffered solution Generated by: ABL Document No: 1412 GLP / GEP Yes unpublished	N	Lonza
Cranor, W.	1990 a	Aerobic soil metabolism of ^{14}C-Metaldehyde Generated by: ABC Document No: 1472 GLP / GEP Yes unpublished	N	Lonza

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Cranor, W.	1990 b	Anaerobic soil metabolism of ^{14}C-Metaldehyde Generated by: ABC Document No: 1424 GLP / GEP Yes unpublished	N	Lonza
De Vette, H. Q. M. Aalderink, G. H.	2002	A study on the adsorption of [U-14-] Metaldehyde to soil particles in four soil types Generated by: TNO Document No: 3551 GLP / GEP Yes unpublished	Y	Lonza
Groß, R.	2000	Determination of the aerobic soil degradation of ^{14}C-Metaldehyde – Recalculation of DT_{50} and DT_{90} values Generated by: SCC Document No: 1634 GLP / GEP not applicable unpublished	N	Lonza
Heim, D. Daly, D.	1999	Soil/ sediment adsorption-desoprtion of ^{14}C-Metaldehyde Generated by: ABC Document No: 3123 GLP / GEP Yes unpublished	N	Lonza
Kabler, K.	1990	Determination of the photolysis rate of Metaldehyde on the surface of soil Generated by: ABC Document No: 1425 GLP / GEP Yes unpublished	N	Lonza
Möllerfeld, J. Römbke, J. eller, M.	1993	Determination of the degradeability and persistence of ^{14}C-Metaldehyde in the water / sediment-system Generated by: BAT Document No: 2120 GLP / GEP Yes unpublished	N	Lonza
Mônego, J.G.	2005	Inherent biodegradability of ^{14}C-Metaldehyde in Brazilian Soils. Generated by: Bioensaios Document No: 3949 GLP / GEP Yes unpublished	Y	Lonza
Römbke, J. Möllerfeld, J.	1991	Determination of the aerobic soil degradation of ^{14}C-Metaldehyde Generated by: BAT Document No: 1634 GLP / GEP Yes unpublished	N	Lonza
Juozenaite, A.	2009	^{14}C-Metaldehyde route and rate of degradation in aerobic soil ‘02-A’ Doc. No. 722-004 GLP Yes unpublished	Y	Lonza

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Juozenaite, A.	2009	^{14}C -Metaldehyde Route and Rate of Degradation in Aerobic Soil 'Elmton (294)' Doc. No. 722-005 GLP Yes unpublished	Y	Lonza
Juozenaite, A.	2009	^{14}C -Metaldehyde route and rate of degradation in aerobic soil 'Fladbury' Doc. No. 722-006 GLP Yes unpublished	Y	Lonza
Juozenaite, A.	2009	^{14}C -Metaldehyde route and rate of degradation in aerobic soil 'Fladbury' Doc. No. 722-007 GLP Yes unpublished	Y	Lonza
Juozenaite, A.	2009	Investigation of Media to Trap Volatile Products of Metaldehyde Degradation Doc. No. 741-002 GLP No unpublished	Y	Lonza
Selim, S.	1993	Laboratory volatility of Metaldehyde from soil Generated by: BTC Document No: 742-001 GLP / GEP Yes unpublished	N	Lonza
Voget, M.	1994	Calculation of the photochemical oxidative half-life of Meta Metaldehyde Generated by: ECO Document No: 2293 GLP / GEP Yes unpublished	N	Lonza
Wuethrich, V.	1990 a	Ready biodegradability: Modified OECD screening test for P0071 Generated by: RCC Document No: 1490 GLP / GEP Yes unpublished	N	Lonza
Wuethrich, V.	1990 b	P0071: Inherent biodegradability "modified Zahn-Wellens Test" Generated by: RCC Document No: 1488 GLP / GEP Yes unpublished	N	Lonza
Lebertz, H.	2008	Study on ready biodegradability of Metaldehyde Document No: 713-003 GLP / GEP Yes unpublished	Y	Lonza
Kane, T.	2009	^{14}C-Metaldehyde Aerobic Transformation in Aquatic Sediment Systems Document No: 714-003 GLP Yes unpublished	Y	Lonza

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Klein, C.	2009	91/414/EEC Review of Metaldehyde – Revised estimation of the degradation rates of Metaldehyde to be used in environmental fate modelling Doc. No. 782-011 Not GLP unpublished	Y	Lonza
Peter, S.	2009	91/414/EEC Review of Metaldehyde - Evaluation of the degradation kinetics of Metaldehyde and its degradation product Acetaldehyde in aquatic systems Document No: 782-015 GLP No unpublished	Y	Lonza
Römbke, J.	2009	Statement about the water/sediment study with ^{14}C-Metaldehyde - Arrangement of the traps Doc. No: 714-002 GLP No unpublished	Y	Lonza

7.3.2 Ecotoxicology

Author(s)	**Year**	**Title** **Source** (where different from company) **Company name,** **Report No.,** **GLP status** (where relevant) **published or not**	**Data protect. claimed**	**Owner**
Bogers, M.	1990a	96-hour acute toxicity study in the rainbow trout with P0071 in a semi-static system RCC Report No. 000348, LR 1489 Document No.: 821-001 GLP: Yes, not published	No	Lonza
Bogers, M.	1990b	Prolonged toxicity study in the rainbow trout with P0071 RCC Report No. 029295, LR 1517 Document No. 826-001 GLP: Yes, not published	No	Lonza
Brooke, L. T., Call, D. J., Geiger, D. L., Northcott, C. E. (eds.)	1984	Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*), Volume I, Center for Lake Superior Environmental Studies, University of Wisconsin-Superior, pp 1-13 and 29-30. Document No. 892-009 GLP: No, published	No	not re-ported
Egeler, P. Junker, T. Knoch, E.	2007	Metaldehyde - A study on the toxicity to Algae (Desmodesmus subspicatus) over 72 h SGS Institut Fresenius GmbH Report No.: AR1AO LR 4090 GLP, unpublished Doc. No.: 823-002	Yes	Lonza
Geiger, D. L., Brooke L. T., Call, D. J. (eds.)	1990	Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*), volume V, Center for Lake Superior Environmental Studies, University of Wisconsin-Superior, pp 33-34. Document No. 892-010 GLP: No, published	No	not re-ported
Sved, D. W. Holmes, C. M. Smith, G. J.	1992	A bioconcentration study with Metaldehyde in the bluegill (*Lepomis macrochirus*) WLD Report No. 289A-102, LR 1959 Document No. 872-001 GLP: Yes, not published	No	Lonza
Wetton, P. M., Mullee, D. M.	2001	Meta Metaldehyde (Code LZ1060): aute toxicity to common carp (*Cyprinus carpio*) SPL Report No.: 102/355, LR 3345, Document No. 821-002 GLP: Yes, not published	Yes	Lonza
Wuethrich, V.	1990a	48-hour acute toxicity of P0071 to *Daphnia magna* (OECD-immobilization test) RCC Report No. 267658, LR 1539 Document No. 822-001 GLP: Yes, not published	No	Lonza

Author(s)	**Year**	**Title** **Source** (where different from company) **Company name,** **Report No.,** **GLP status** (where relevant) **published or not**	**Data protect. claimed**	**Owner**
Wuetherich, V.	1990b	Acute toxicity of P0071 to *Scenedesmus subspicatus* (OECD - algae growth inhibition test) RCC Report No. 259953, LR 1540 Document No. 823-001 GLP: Yes, not published	No	Lonza
Wuetherich, V.	1990c	Influence of P0071 on the reproduction of *Daphnia magna* RCC Report No. 259942, LR 1538 Document No. 827-001 GLP: Yes, not published	No	Lonza
Scholtz, R	2004	Toxicity of Metaldehyde-containing slug pellets to Golden Snails in rice paddy fields. Summary and calculation of acute lethal effect dose using data from efficacy trials conducted by Dupoh Bio Research Centre during 1997 in the Philippines. Lonza Report No. 3873 GLP: No	Yes	Lonza
Egeler, P., Goth, M., Knoch, E.	2007	Metaldehyde- A study on the toxicity to the Great Ramshorn Snail (*Planorbarius corneus*) over 48 h Report/Doc. number: 825-001 Guideline(s): No guideline available, OECD 202 (2004) was taken into account GLP: Yes	Yes	Lonza

8 ANNEXES