CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Phosmet

EC Number: **211-987-4**

CAS Number: **732-11-6**

Index Number: **015-101-00-5**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Phosmet
EC number:	211-987-4
CAS number:	732-11-6
Annex VI Index number:	015-101-00-5
Degree of purity:	≥ 95%

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	Acute Tox. 4*; H312 Acute Tox. 4*; H302 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 M = 100
Current proposal for consideration by RAC	Acute Tox. 3, H301 Acute Tox. 4, H332 STOT RE 1, H372 (nervous system) Aquatic Acute 1, H400 M = 100 Aquatic Chronic 1, H410 M=10
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 3, H301 Acute Tox. 4, H332 STOT RE 1, H372 (nervous system) Aquatic Acute 1, H400 M = 100 Aquatic Chronic 1, H410 M=10

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	-	-	-	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	-	-	-	Conclusive, but not sufficient for classification
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	-	-	-	Conclusive, but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	-	-	Conclusive, but not sufficient for classification
2.13.	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	Acute Tox. 3; H301	-	Acute Tox. 4; H302	
	Acute toxicity - dermal	-	-	Acute Tox. 4; H312	Conclusive, but not sufficient for classification
	Acute toxicity - inhalation	Acute Tox. 4; H332	-	-	

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	-	-	-	Conclusive, but not sufficient for classification
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	STOT RE 1 H372	-	-	
3.10.	Aspiration hazard	-	-	-	Conclusive, but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic acute 1; H400 Aquatic chronic 1; H410	M = 100 M= 10	H400/H410 M = 100	
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:

GHS Pictograms:


GHS 06


GHS08


GHS 09

Signal word: Danger

Hazard statements:
H301: Toxic if swallowed
H332: Harmful if inhaled.
H372: Causes damage to nervous system through prolonged or repeated exposure.
H410: Very toxic to aquatic life with long lasting effects.

Precautionary statements: No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry: no notes proposed.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Phosmet is an insecticide and acaricide used as an active substance in plant protection products included in the Annex of Regulation (EU) No. 540/2011 which contains the list of active substances included in Annex I to Directive 91/414/EEC at the moment of the adoption of this Regulation and deemed approved under Regulation (EC) No. 1107/2009. Phosmet was notified as an existing active substance, with Spain as RMS and the applicant Gowan International (now Margarita Internacional). Data referred to assessment made under PPP regulation is attached to the IUCLID 5 dossier [Draft Assessment Report, May 2004 _ v.3 (07/05) and subsequent final addendum, compiled by EFSA in March 2006] [EFSA Scientific Report, 2011].

Currently, there is an application for the renewal of phosmet as an active substance for plant protection products in accordance to Commission Implementing Regulation (EU) No 844/2012 (September 2014). Industry has informed that studies conducted in the context of the US EPA Endocrine Disruptor Screening Program (EDSP) (US EPA, 2009) to assess the potential of phosmet to affect estrogen, androgen or thyroid hormone systems will be provided in this process.

Phosmet is currently listed in Annex VI of Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP). It was included in Annex I of Dangerous Substance Directive 67/548/EEC (DSD) in April 2004 (29th ATP; Commision Directive 2004/73/EC). The current classification listed in Annex VI of CLP is as follows: Acute Tox. 4*; H312 (minimum classification), Acute Tox. 4*(minimum classification); H302, Aquatic Acute 1; H400, Aquatic Chronic 1; H410 and Xn; N; R21/22 – R50/53.

Subsequently, phosmet was further discussed at the former Technical Committee on Classification and Labelling of the European Chemicals Bureau (ECB TC C&L). A discussion regarding a change of the classification for environmental hazards and the human health endpoints (acute toxicity and reproductive toxicity) took place at the TC C&L meetings [summary record ECBI/20/07, Rev.2 of the November 2006 meeting and Follow up V (Ispra, 29 May 2008) of the May 2007 meeting for health effects].

During the November 2006 meeting it was agreed by the TC C&L experts to delete Xn; R21 classification from the DSD Annex I entry, as this classification was considered to be based on a read across between the different routes of exposure only and not on the available experimental data for the dermal route. The environmental classification as N; R50 (concentration limit ≥0.25%) was agreed during the January 2007 meeting and Aquatic Acute 1, H400 proposed in accordance with the CLP regulation.

During the last May 2007 meeting it was agreed not to classify phosmet for effects on fertility. No consensus was reached on classification and labelling for the oral toxicity of phosmet as the TC C&L experts were more or less equally divided between the Xn; R22 and T; R25 classification due to a difference in opinion about the influence of impurities on the acute oral toxicity. Also, no agreement was found on the need to classify phosmet for acute inhalation toxicity (Xn; R20). As a possible solution a split entry was created by ECB for phosmet, above and below 70% and it was suggested to classify “Phosmet ≥ 70%” with Xn; R20. This split entry was, however, not accepted by MS experts.

At the last ECB TC C&L meeting, as it was not reached a final agreement, the Spanish Competent Authority was requested to send a C&L proposal to ECHA. In the document “Hand-over to the European Chemicals Agency” document (CA/29/2008 – Annex III of 29 May 2008), phosmet was listed with the acute oral and inhalation toxicity as issues for discussion together with the following relevant documents: ECBI/88/06, Add. 1, 3, 5, 6, 7 (MS only), 8 and 9.

Summary records of the meetings at which the human health and environmental classification of phosmet were discussed are attached in IULCID file.

Furthermore, EFSA recommends the following classification with regard to mammalian toxicological data, T, R25 and with regard to ecotoxicological data, N, R50 [EFSA Scientific Report, 2011].

Based on the review of the available data, an update in the classification is needed. A proposal for changing the current harmonised classification and labelling in accordance to Regulation 1272/2008 has been prepared in the present CLH dossier. This proposal focuses on the changes in the classification of phosmet as discussed by the TC C&L in November 2007. Besides, available data on phosmet supports a harmonised classification for repeated dose toxicity. However, information on all other hazard classes is included as additional information. No REACH registration dossiers are available for phosmet at time of submission of the present CLH dossier.

2.2 Short summary of the scientific justification for the CLH proposal

During the evaluation of phosmet by TC C&L no final conclusion was reached on classification regarding the acute oral and inhalation toxicity in the human hazard assessment. There was no discussion on the repeated dose exposure toxicity.

Justification for the proposal with respect to human health effects:

The available data on phosmet supports a harmonised classification for acute oral toxicity, acute inhalation toxicity and repeated dose toxicity.

Acute Tox 3, H301

This classification is based on the reported acute oral LD_{50} value of 113 (101-127) mg/kg bw for male rats and 113 (98-130) mg/kg bw for females rats obtained in an acute oral toxicity study (Mccabe, 1978) and also supported by data in mice.

Acute Tox. 4, H332

LC_{50} (vapour) in a rat study (Leong, 1977) was calculated to be greater than 0.152 mg/L (maximum attainable concentration under the conditions of the test) for male and female rats during 4 hours of exposition.

According to data submitted by notifier it is not possible to generate respirable atmosferes of the technical material without destructive grinding of the test substance and therefore classification for acute inhalation toxicity should not be required. However, in an acute inhalation toxicity test in male and female rats performed with a wettable powder formulation (Imidan 70 WP) containing 70 % (w/w) phosmet an LC_{50} (4 hours) of 1.6 mg/L was determined for the product (Mould, 1995). The only component of this formulation that can potentially induce toxicity by inhalation is phosmet. Classification as H332 is purposed taking into account data from an acute inhalation toxicity study (Mould, 1995) with formulation Imidan 70 WP since it is not clear that generation of respirable atmosferes with technical phosmet inducing toxicity by inhalation can be discarded.

STOT RE 1, H372

Adverse effects such as brain and erythrocite cholinesterase inhibition and some clinical signs associated with neurotoxicity were observed after oral and dermal repeated dose exposure with phosmet. These findings were seen below the cut-off values for classification as STOT RE 1 and at

lower dosages than for single exposure. Therefore, classification as STOT RE 1 is warranted for phosmet.

With respect to environmental hazards, the current entry in Annex VI Table 3.2 of Regulation (EC) 1272/2008 needs to be replaced by the classification agreed during the TC C&L meeting in January 2007, i.e. N; R50 (regarding Directive 67/548/EEC). And the current entry in Annex VI Table 3.1 as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) should not be changed; only a chronic M factor should be added. For this environmental hazard category corresponds an M factor for chronic toxicity of 10, justified due to the invertebrates' chronic NOEC value of 0.00078 mg/l and the fact that the substance is rapidly degradable in the aquatic environment.

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:

Acute Tox 4*; H312
Acute Tox 4*; H302
Aquatic Acute 1; H400
Aquatic Chronic 1; H410

Labelling:

GHS07, GHS09, Wng
H312, H302, H410

Specific concentration limits and M factor:

M = 100

2.4 Current self-classification and labelling

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

The self-classification according to the ECHA inventory of notified classification and labelling on 27 July 2015 was:

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)			
Acute Tox. 4	H302	H302		GHS07 GHS09 Wng			45
Acute Tox. 4	H312	H332					
Aquatic Acute 1	H400						
Aquatic Chronic 1	H410	H410					
Acute Tox. 4	H302	H302		GHS07 GHS09 Wng			23
Acute Tox. 4	H312	H332					
Aquatic Acute 1	H400						
Aquatic Chronic 1	H410	H410					
Acute Tox. 4	H302	H302		GHS07 GHS09 Wng	M = 100		20
Acute Tox. 4	H312	H332					
Aquatic Acute 1	H400						
Aquatic Chronic 1	H410	H410					
Acute Tox. 4	H302	H302		GHS07 GHS09 Wng	M = 100		3
Acute Tox. 4	H312	H332					
Aquatic Acute 1	H400	H400					
Aquatic Chronic 1	H410	H410					
Acute Tox. 4	H302	H302		GHS07 GHS09 Wng	M = 100		1
Acute Tox. 4	H312	H312					
Aquatic Acute 1	H400						
Aquatic Chronic 1	H410	H410					
Acute Tox. 4	H302	H302		GHS07 GHS09 Wng	M = 100		1
Acute Tox. 4	H312	H312					
Aquatic Acute 1	H400						
Aquatic Chronic 1	H410	H410					

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Phosmet is an active substance included in the list of active substances approved under Regulation (EC) no. 1107/2009 and therefore no justification is required.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

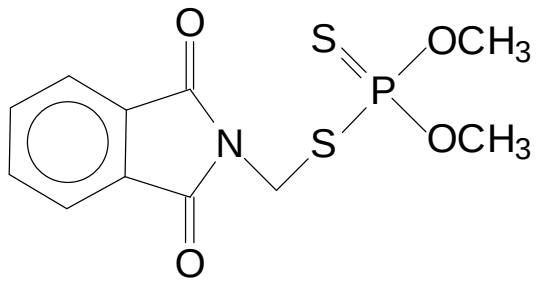
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	211-987-4
EC name:	Phosmet
CAS number (EC inventory):	732-11-6
CAS number:	732-11-6
CA index name:	Phosphorodithioic acid, S-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)methyl] O,O-dimethyl ester
IUPAC name:	S-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl] O,O-dimethyl phosphorodithioate
CLP Annex VI Index number:	015-101-00-5
Molecular formula:	$C_{11}H_{12}NO_4PS_2$
Molecular weight range:	317.33 g/mol

Structural formula:



1.2 Composition of the test substance

Table 5: Constituents

Constituent	Typical concentration	Concentration range	Remarks
Phosmet	≥ 950 g/kg		-

Current Annex VI entry: H302, H312, H400, H410.

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Phosmet oxon: IUPAC name: S-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl] O,O-dimethyl phosphorothioate CAS number: 3735-33-9	≤ 0.8 g/kg	-	-
Iso phosmet: IUPAC name: S-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl] O,S-dimethyl phosphorodithioate	≤ 0.4 g/kg	-	-

Current Annex VI entry: Not listed.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives	-	-	-	-

1.2.1 Composition of test material

Phosmet manufactured has a minimum purity of 95% with two identified relevant impurities, phosmet oxon(≤ 0.8 g/kg) and iso phosmet ≤ 0.4 g/kg).

1.3 Physico-chemical properties

Table 8: Summary of physico-chemical properties

Property	Value	Reference/Comment
State of the substance	Clear colourless, crystalline, solid at room temperature (purity 99.8%)	Myers,1987
Melting/freezing point	71.6-72 ºC (purity: 99.8%) 66.0-69 ºC (purity 94.3%)	Myers,1987 OECD 102
Boiling point	The decomposition of phosmet was observed before boiling occurred. Exothermic decomposition = 208.5 ºC (purity 100%).	Widmer, 2005a EEC A2
Relative density	1.439 g/cm^3 at 20 ºC (purity: 99.8%)	Myers, 1987 OECD 109
Vapour pressure	6.5 x 10^{-5} Pa (25 °C) (purity: 99.8%)	Myers, 1987 OECD 104
Surface tension	σ = 71.8 mN/m at 20°C (90% of saturated solution) (purity: 97%)	Ramsay, 2003 EEC A5
Water solubility	Water solubility = 15.2 mg/L (± 0.68 mg/l) (20 ºC. pH 4.4 and purity 99.8%).	Widmer, 2005b EEC A6
Partition coefficient n-octanol/water	log $P_{O/W}$: 2.96 at 25 °C (purity: 99.8%)	Myers, 1987 EECA8
Flash point	Not required because melting point is above 40 ºC.	
Flammability	Not flammable (purity 97.0%).	Jackson, 2003 ECCA10
Explosive properties	Not explosive (purity 97.0%).	Jackson, 2003 EEC A14
Self-ignition temperature	The substance does not ignite below the melting point (purity 97.0%).	Jackson, 2003 EEC A16
Oxidising properties	Phosmet contains only oxygen as dithiophosphoric ester, carbonyl and amide function that do not support oxidation.	
Granulometry	Particle size distribution: 0.053-0.106 mm: 0% 0.106-0.250 mm: 6.19% 0.250-0.500 mm: 66.19% 0.500-1.190 mm: 16.79% > 1.190 mm: 12.19%	Pessin, 1998 OECD guideline
Stability in organic solvents and identity of relevant degradation products	Solubility (purity of 97.0%): Xylene: 50-57 g/L Ethyl Acetate: 57-67 g/L Acetone: 143-167 g/L 1,2-Dichloroethane: 400-500 g/L Methanol: 29.2 g/L n-Heptane: 1.04 g/L	Ramsay, 2003 CIPAC MT 181 CIPAC MT 157
Dissociation constant	Not applicable	
Viscosity		

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for Classification and Labelling.

2.2 Identified uses

Phosmet is an insecticide and acaricide used as active substance in plant protection products.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

It was agreed not to classify phosmet for physico-chemical properties at ECB level.

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Flash point	Not required because melting point is above 40 ºC.	None	
Flammability EEC A10	Not flammable (purity 97.0%).	None	Jackson,2003
Explosive properties EEC A14	Not explosive (purity 97.0%).	None	Jackson, 2003
Self-ignition temperature EEC A16	The substance does not ignite below the melting point (purity 97.0%).	None	Jackson, 2003
Oxidising properties	Phosmet contains only oxygen as dithiophosphoric ester, carbonyl and amide function that do not support oxidation.	None	

4 HUMAN HEALTH HAZARD ASSESSMENT

Phosmet has been produced by the applicant at three different plants, one in the USA (Stauffer Chemical Company, Zéneca) one in Mexico (Teckhem) and one in Spain (General Química, S.A). There are some differences in the profile of impurities of the three plants since there are differences in the manufacturing processes.

The currently manufactured technical material in the EU is produced by General Quimica. Considering that most toxicological studies provided were carried out with the technical material from Stauffer Chemical Company, the old source no longer manufactured or used, the notifier was required to justify that the technical material from General Quimica and from Stauffer do not differ significantly.

The technical phosmet from General Quimica is a significantly improved product which contains substantionally lower levels of the relevant impurities (isophosmet and phosmet oxon) than the original Stauffer material. IUCLID dossier includes identity of impurities of the current technical material. Since most of toxicological studies were performed with the old technical material, it can be concluded that the levels of these two impurities in the new technical material are acceptable. However, three new impurities (<1%) were detected in the new technical material. Besides, another

impurity, only detected qualitatively in the old technical material, was quantified up to 2% in the new technical material.

For the assessment of the toxicity of the impurities the notifier submitted an acute oral study in rats and a study testing the repeated toxicity in dogs with the technical material from General Química.

Table 10: Comparison of oral toxicity studies from different sources

Species	Source	Purity	Reference	Results
Wistar Rat (acute toxicity)	General Química, S.A	95.4%	Navarro Aragay, C. 1998	LD_{50}= 230 mg/kg (151-347 mg/kg)
Sprague Dawley Rat (acute toxicity)	Stauffer	96.1%	McCabe, J., 1978	LD_{50}=113 mg/kg
Dog (28 days) (range finding oral)	General Química, S.A	97.0%		NOEL=1.5 mg/kg bw /day
Dog (90 days)	Stauffer	98%±0.5		NOEL=1.9 mg/kg bw /day

Peer review experts agreed that the two sources were comparable for acute and subacute toxicity and that the results of the studies from these two sources were in the same order of magnitude, which suggests that the two sources are reasonably comparable. However, the assessment of the toxicological equivalence of the two materials was only based on acute and subacute toxicological data and was not fully demonstrated according to the Guidance Document on the Assessment of the Equivalence of Technical materials of Substances Regulated under Council Directive 91/414/EEC (SANCO/1059/2003). Further assessment would be helpful in getting a full picture of the toxicological equivalence (available information, structure-activiy relationship assessment, etc). The toxicological risk assessment was finally based on the assumption that studies have been performed with a test material that covers the current technical specificaction (EFSA, 2011).

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

For non-human toxicokinetics information on phosmet, please refer to paragraph 4.1.3.

4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

Absorption: Rapid and almost complete within 24 h (84.4%) based on urinary excretion, cage wash and tissue radioactivity from rats dosed with 1 mg/kg bw.

The peak concentration of blood and plasma radioactivity in pharmacokinetic studies with single acute doses of 1 and 25 mg/kg bw was observed at 0.5 h after dosing. Phosmet was eliminated from the blood in a biphasic manner. First phase correspond to the distribution of the compound to the tissues, and showed a short half-life (0.2-6 h). The second phase corresponded to the elimination and presented longer life (41-1543 h).

Excretion: Mainly by urine (68.9%-82.6%) and faeces (4.5%-9.9%) at 24 h in rats dosed with single acute doses of 1 and 25 mg/kg bw and 14-day repeated dose of 1 mg/kg bw/day. More than 50% of the radioactivity was eliminated within first 12 h. There appeared to be slight differences related to

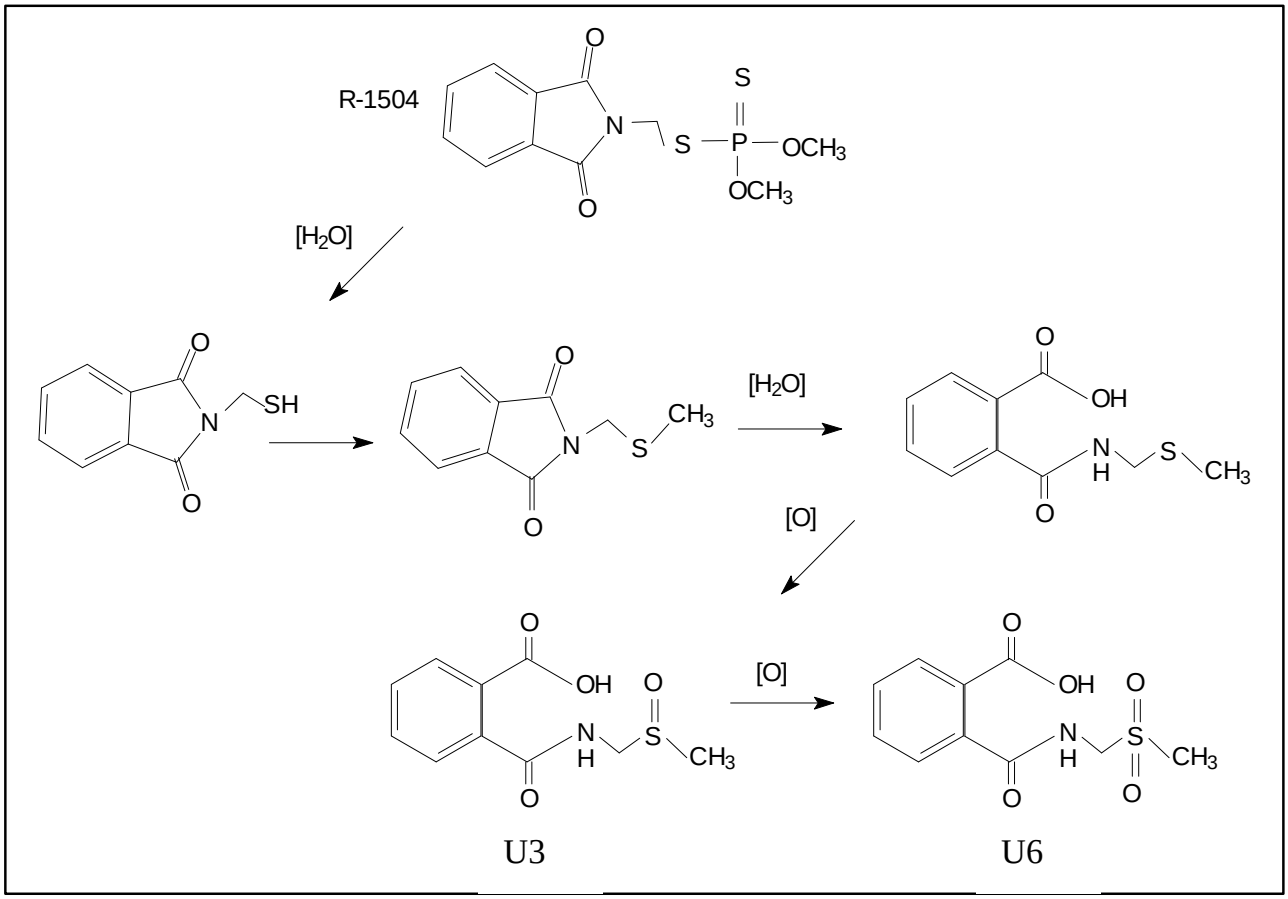
dose levels (excretion was faster after low doses than after high dosage) and to duration of the exposure (higher radioactivity was recovered after acute exposure than after repeated dosage).

Distribution: There was a wide distribution of the test substance and the tissues contained low levels of radioactivity (≤1 % of the administered dose) in all dose groups. The highest activity was in the liver and whole blood and lowest activity in the fat and bone. These data indicate that the test substance and/or its metabolites did not accumulate to an appreciable extent.

Metabolism: two major metabolites were found in rat urine after single acute doses of 1 and 25 mg/kg bw. N-(methylsulfinylmethyl)-phthalamic acid (PaAMS(O)M) (U3) and the corresponding sulfoxide N-methylsulfonylmethyl)-phthalamic acid (PaAMS(O2)M) (U6) accounted in rat urine for 52%-66% and 8%-26% respectively. The proposed metabolic pathway is shown in Figure 1. Phosmet undergoes thiophosphoryl hydrolysis, S-methylation, hydrolysis of the phtalamide ring to the respective phtalamide acid and later oxidation of the sulphur to sulphoxide (U3) and sulphone (U6).

Sulphoxidation to both metabolites is consistent with the action of FAD-containing monooxygenase, a major microsomal enzyme system involved the oxidation of xenobiotics. Sex-related differences in the formation of U3 (greater in females) and U6 (greater in males) can be consistent with current views that the concentration of FAD-containing monooxygenase is related to the presence of steroid sex hormones.

Figure 1: Proposed metabolic pathway for phosmet.



Further investigations (McBain et al. (1968) and Ford et al. (1966)) seemed that phosmet was oxidised to its oxygen analog (phosmet-oxon) *in vivo* as determined by the presence of phosmet-oxon in rat faeces and urine as well as in cockroaches. However both studies are not considered scientifically valid. An *in vitro* metabolism study in rat liver microsomes (Hassler, 2006) confirmed that ^{14}C-phosmet is metabolised when incubated with rat liver microsomes. Phosmet-oxon is rapidly and prominently formed, with a maximum of 20%-25% of the applied dose observed after 1 hour. These results verify that phosmet-oxon is formed in animal metabolism in significant amounts. The toxicity of this metabolite is therefore covered by the studies performed with phosmet and separate testing is not warranted**.**

The investigated data for toxicokinetics with phosmet (absorption, distribution, metabolism and elimination) were already agreed and accepted at ECB level and were included in this document as supplement for the toxicological data.

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

<table>
<tr><th colspan="4">Acute Oral</th></tr>
<tr><th>Method</th><th>Observations</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>The study is pre-guideline but similar to OECD 401
GLP: No
Study acceptable
Sprague-Dawley rats
At least 10/sex/dose
14-days observation
Single doses by oral gavage of 75, 100, 115, 130, 150, 170 mg/kg bw to animals of both sexes and 60 and 175 mg/kg bw to males and females respectively.
Vehicle: 5% polyethylene glycol in corn oil.
Dose volume: 10 ml/kg bw
Purity: 96.1% (Stauffer Chemical material)</td>
<td>Mortality: Most of deaths occurred within first two days.
Table 11.1
<table>
<tr><th rowspan="2">Dose level (mg/kg bw)</th><th colspan="2">Number of deaths</th><th colspan="2">Time of recovery (day)</th></tr>
<tr><th>Male</th><th>Female</th><th>Male</th><th>Female</th></tr>
<tr><td>175</td><td></td><td>10/10</td><td></td><td></td></tr>
<tr><td>170</td><td>10/10</td><td>9/10</td><td></td><td>5</td></tr>
<tr><td>150</td><td>12/15</td><td>9/10</td><td>9</td><td>6</td></tr>
<tr><td>130</td><td>12/15</td><td>6/10</td><td>6</td><td>7</td></tr>
<tr><td>115</td><td>7/15</td><td>5/10</td><td>3</td><td>7</td></tr>
<tr><td>100</td><td>3/10</td><td>2/10</td><td>9</td><td>7</td></tr>
<tr><td>75</td><td>1/10</td><td>2/10</td><td>6</td><td>7</td></tr>
<tr><td>60</td><td>0/10</td><td></td><td>4</td><td>5</td></tr>
</table>
Clinical signs:
Depression, tremors, salivation, exophtalmus, chromodacryorrhea, dyspnea and ~~s~~tains around the ano-genital region were observed at all dose levels. Time of recovering is shown in previous table.
Necropsia:
Red lungs, dark livers, red fluid in the intestine and red spots on the urinary bladder and the intestine.</td>
<td>LD_{50} females: 113 mg/kg bw(98-130)
LD_{50} males: 113 mg/kg bw (101-127)
H301</td>
<td>Mccabe, J., 1978</td>
</tr>
<tr>
<td>OECD 401
GLP: No[a] (see note below)
Study acceptable
Wistar rats
14-days observation
Single doses of 70, 100, 140, 200, 280, 400, 560 mg/kg bw to at least 5 males/dose. Calculated LD_{50} was administered by gavage to 6 females.
Vehicle: carboymethylcellulose (1%).
Dose volume: 10 mL/ kg bw
Purity: 95.4% (General Química material)</td>
<td>Mortality: Mortality occurred between the first and the second day after dosing from dose levels of 100 mg/kg bw onwards.
Table 11.2
<table>
<tr><th>Dose level (mg/kg bw)</th><th>70</th><th>100</th><th>140</th><th>200</th><th>280</th><th>400</th><th>560</th></tr>
<tr><th>Number of deaths (male)</th><td>0/5</td><td>1/5</td><td>2/6</td><td>2/6</td><td>2/5</td><td>4/5</td><td>5/5</td></tr>
</table>
The experiment was repeated with 6 females at calculated LD_{50} of 230 mg/kg bw with 3/6 deaths.
Clinical signs:
Postration and chromodacryorrhea were observed after administration with a dose-related intensity.</td>
<td>LD_{50} males: 230 mg/kg bw(151-347)
LD_{50} both sexes: 230 mg/kg bw
H301</td>
<td>Navarro Aragay, C., 1998</td>
</tr>
</table>

<table>
<tr>
<td>The study is pre-guideline
GLP: No
Study acceptable as additional information due to lack of reported data
Study with male S/D rats (7 rats/group) and male S/W mice (5 mice/group)
14-days observation
Doses administered by stomach tube:
Mice: 10, 21.5, 46.4 y 100 mg/kg bw
Rat: 100, 200, 300, 400 and 500 mg/kg bw
Vehicle: 0.5% aqueous methyl cellulose
Purity: Unknown</td>
<td>No information available about clinical signs, body weights and necropsy findings in rats. All deaths occurred within two days after the treatment.
Table 11.3: Mortality in male rats:
<table>
<tr><td>Dose level (mg/kg bw)</td><td>100</td><td>200</td><td>300</td><td>400</td><td>500</td></tr>
<tr><td>Number of deaths</td><td>0/7</td><td>3/7</td><td>4/7</td><td>5/7</td><td>7/7</td></tr>
</table>
Table 11.4: Mortality in male mice:
<table>
<tr><td>Dose level (mg/kg bw)</td><td>10.0</td><td>21.5</td><td>46.4</td><td>100</td></tr>
<tr><td>Number of deaths</td><td>0/5</td><td>0/5</td><td>2/5</td><td>5/5</td></tr>
</table>
</td>
<td>LD_{50} male mice: 50.1 mg/kg bw (34.4-73.0)
H301
LD_{50} male rats: 245 mg/kg bw (161-367)
H301</td>
<td>Meyding, G. D., 1966</td>
</tr>
<tr><td colspan="4">Acute Inhalation</td></tr>
<tr><td>Method</td><td>Observations</td><td>Results</td><td>Reference</td></tr>
<tr>
<td>The study is pre-guideline but similar to OECD 403 with deviations (temperature, relative humidity, oxygen concentration were not recorded. Gross necropsy findings were not performed. Body weight data of groups of males and females were the only data shown in tabular form)
GLP: No
Study acceptable
Charles River CD rats
5/sex/dose/4 h
14-days observation
Vapour concentration in gas chamber: 0.152 mg/L/4 h (whole body exposure; maximum attainable concentration under the conditions of the test).
Purity: 92.5% (Stauffer Chemical material)</td>
<td>Mortality: No mortality occurred
Clinical signs:
Increase of the activity and eye squint were observed during the exposure.</td>
<td>LC_{50} > 0.152 mg/L /4h</td>
<td>Leong, K., 1977</td>
</tr>
<tr>
<td>The study follows the US EPA 81-3 guideline.
GLP: Yes
Study acceptable
Charles River CD rats
Doses: 0, 0.61, 0.66 and 3.69 mg/l.
5/sex/dose/4 h
14-days observation
Purity: Formulation IMIDAN 70 WP (wettable powder) with 70.2% of phosmet. Co-formulants have no impact on the toxicity.</td>
<td>Table 11.5: Dose levels, MMAD and mortality
<table>
<tr><th>Dose level (mg/l)</th><th>MMAD (µm)</th><th>GSD (µm)</th><th>% less than 1 µm</th><th>Mortality</th></tr>
<tr><td>0</td><td>0</td><td>0</td><td>0</td><td>0/5</td></tr>
<tr><td>0.66</td><td>1.61</td><td>2.00</td><td>27</td><td>0/5</td></tr>
<tr><td>0.61</td><td>2.38</td><td>1.95</td><td>12</td><td>0/5</td></tr>
<tr><td>3.69</td><td>2.03</td><td>2.04</td><td>15</td><td>5/5</td></tr>
</table>
Mortality: Animals at high dose level were killed during exposure (2.5-3.5 h) due to risk of suffering distress.
Clinical signs: In surviving animals those typical for AChE inhibition (lethargy, salivation, cold to touch, tremors, hunched posture, bulging eyes, stained fur). Most of them resolved on day 2 except hunched posture and stained fur
Necropsy: in dead animals, dark or red lungs.</td>
<td>LC_{50} = 1.6 mg IMIDAN 70 WP /L /4h [b]</td>
<td>Mould, A.P., 1995</td>
</tr>
</table>

Acute Dermal			
Method	**Observations**	**Results**	**Reference**
The study is pre-guideline but similar to OECD 402 (only deviation in the number of used animals) GLP: No **Study acceptable** New Zealand Rabbits. 3 animals/sex Doses of 0 and 5000 mg/kg bw. In half of the animals the skin was abraded. 14-days observation since application. Material applied with a protective binder for 24 h and then washed and rewrapped with gauze for 3 days. It is not mentioned if the test substance was moistened before the application. Purity: 96.1% (Stauffer Chemical material)	Mortality: one female died on day 5. Clinical signs: Mild depression and salivation with jaws clamped around caging were observed in the dead female. Necropsia: The rabbit that died on test appeared to have a hemorrhagic pancreas and intestines.	LD_{50} >5000 mg/kg bw	**Mccabe, J., 1978**
Similar to OECD 402 GLP: No **Study acceptable** 5 rat/sex/dose Wistar rats 14-days observation Material was diluted in corn oil at concentration of 200 mg/ml and applied on the skin surface for 24 h. No information about the use of gauzes. Doses of 0 and 1000 mg/kg bw. Purity: 92.7%	Mortality: no deaths. Clinical signs: no clinical signs.	LD_{50}> 1000 mg/kg bw	**Dos Santos, A.E.M.; 1998**

[a] The study has been audited by Quality Assurance Unit of the University of Barcelona in compliance with the GLPs. However the laboratory is not included in the list of laboratories accredited by the Spanish National Entity of Accreditation (ENAC) to meet GLP requirement for toxicological studies.
[b] LC_{50} for phosmet: 1.12 mg/L/4 h (extrapolated from IMIDAN 70 WP)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

LD_{50} for females: 113 mg/kg bw (98-130). LD_{50} for males: 113 mg/kg bw (101-127) based on Mccabe (1978) study with the Stauffer Chemical material.

LD_{50} for males: 230 mg/kg bw (151-347). LD_{50} for both sexes: 230 mg/kg bw based on Navarro Aragay (1998) study with the General Química material.

4.2.1.2 Acute toxicity: inhalation

LC_{50} > 0.152 mg/L /4h (vapour) based on Leong (1977) study

4.2.1.3 Acute toxicity: dermal

LD_{50} > 5000 mg/kg bw based on Mccabe (1978) study in rabbit.

LD_{50} > 1000 mg/kg bw basen on Dos Santos (1998) study in rat.

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

Acute oral toxicity

Two acute oral toxicity studies in rats were carried out with phosmet from two different sources. Stauffer material was used in Mccabe (1978) study in rat with a LD_{50} of 113 (101-127) mg/kg bw for male rats and 113 (98-130) mg/kg bw for female rats. Navarro Aragay (1998) study was performed with the current technical material from General Química with a LD_{50} of 230 mg/kg bw for both sexes.

The study wuth the technical material from Stauffer was conducted prior to the implementation of Good Laboratory Practices (GLP). The new technical material from General Química has been tested in a recent study performed in the Faculty of Pharmacy of the University of Barcelona (Spain) and was audited by the Quality Assurance Unit of this University. In the quality assurance statement, it is reported that the study was performed in complice with the principles of GLP, (OECD) (Directive 87/18/CEE included in Spanish legalisation with RD 822/1993). However, this is not in the list of laboratories accredited by the Spanish National Entity of Accreditation (ENAC) to meet GLP requeriments for toxicological studies.

Besides, there is an acute oral toxicity study in rats and mice (Meyding, 1966). The LD_{50} value in this study was 50.1 mg/kg bw in male mice and 245 mg/kg bw in male rats. This study was considered acceptable only as additional information due to the lack of reported data but it indicates that mouse is a more sensitive species than rat under the same test conditions.

During the last May 2007 ECB meeting no consensus was reached on classification for the oral toxicity as the TC C&L experts were more or less equally divided between the Xn; R22 and T; R25 classification due to a difference in opinion about the influence of impurities on the acute oral toxicity.

There were two relevant impurities (isophosmet and phosmet oxon). The LD_{50} value of isophosmet was 171 mg/kg bw in female Sprague-Dawley rats, which is in the same range as for the active compound. The acute oral toxicity of phosmet oxon is stated to be 46-50 mg/kg bw according to a document of the California Environmental Protection Agency (Spencer, 2003) which is only a factor 2-4 below the LD_{50} of phosmet.

Levels of isophosmet and phosmet oxon are lower in the new technical material from General Quimica than in Stauffer material. However, the difference in the levels of the impurities isophosmet (about 6-10 fold on the basis of the mean values) and phosmet oxon (about 3-5-fold on the basis of the mean values) is very low. There were doubts about if the small differences in the levels of the isophosmet and phosmet oxon impurities were sufficient to explain the discrepancy in acute oral toxicity between the two phosmet materials. It cannot be excluded that the difference in acute oral toxicity is due to differences in other impurities or due to other factors like the test strain used.

Although it is not clear that the differences in acute oral toxicity between both materials are due to the differences in impurities both LD_{50} of 113 and 230 mg/kg bw obtained in rat studies fall within

the dose range for classification as H301, Category 3 according to CLP. This classification is also supported by mice data.

Acute inhalation toxicity

In the available acute inhalation toxicity study (Leong, 1977) rats were caged in groups of 2 or 3 by sex and cages were placed in a glass chamber. Rats were exposed to an atmosphere satured with vapours of phosmet technical during 4 hours, resulting in a LC_{50} greater than 0.152 mg/L, which was the maximum attainable concentration under the conditions of the test. An acute inhalation test with phosmet as respirable aerosol particles is not available.

According to the notifier attempts to experimentally generate respirable particles without causing destructive grinding of the test substance have failed and justified that technical phosmet is an amorphous, crystalline agglomerate that is somewhat analogous to concrete and which must be broken up into smaller pieces to be removed from the container. Notifier argues that the generation of an atmosphere of respirable particles with an MMAD/GSD complying with the provisions of the corresponding testing guidelines is not feasible without destroying the test substance. Besides, the material possesses a negligible vapour pressure of 6.5×10^{-5} Pa (25 °C) and the results of the particle size distribution of phosmet technical demonstrate that only negligible amount of particles in the respirable range.

However, an acute inhalation toxicity test in male and female rats was performed with a wettable powder formulation (Imidan 70 WP) containing 70 % (w/w) phosmet and a LC_{50} (4 hours) of 1.6 mg/l was determined for the product (Mould, 1995) that can be can be extrapolated for a LC_{50} of 1.12 mg/l for phosmet. The only component of this formulation that potentially could induce toxicity by inhalation is phosmet. This LC_{50} warrants classification for the preparation with H332; Category 4. The average values for the mean median aerodynamic diameter of the particles in the atmosphere ranged from 1.61 to 2.38 µm. This indicates that smaller particles sizes can be achieved with phosmet and that generation of atmosferes with respirable particles of phosmet is possible.

According to the Guidance on the Application of the CLP Criteria (version 4.0, November 2013), the use of highly respirable dusts and mists is ideal to fully investigate the potential inhalation hazard of the substance. However, it is acknowledged that these exposures may not necessarily reflect realistic conditions. For instance, solid materials are often micronised to a highly respirable form for testing, but in practice exposures will be to a dust of much lower respirability. Similarly, pastes or highly viscous materials with low vapour pressure need strong measures to be taken to generate airborne particulates of sufficiently high respirability, whereas for other materials this may occur spontaneously. In such situations, specific problems may arise with respect to classification and labelling, as these substances are tested in a form (i.e. specific particle size distribution) that is different from all the forms in which these substances are placed on the market and in which they can reasonably be expected to be used. A scientific concept has been developed as a basis for relating the conditions of acute inhalation tests to those occurring in real-life, in order to derive an adequate hazard classification. This concept, called split-entry approach, is applicable only to substances or mixtures which are proven to cause acute toxicity through local effects and do not cause systemic toxicity (Pauluhn, 2008).

Based on the study with the formuladed product Imidan 70 WP, ECB created a split entry, proposing “Xn, R20” for phosmet ≥ 70%, and no classification for phosmet < 70%. This split entry was, however, not accepted by MS experts during the May 2007 ECB meeting. One argument put forward for not accepting a split entry was that it seemed possible to perform a study with a proper particle size of the substance.

According to the Guidance Document on Acute Inhalation Toxicity Testing (July 21, 2009) corresponding to OECD Document no. 39 for inhalation, achieving the GHS limit concentration of

5 mg/l is technically challenging for most aerosols and greatly exceeds real-world human exposure. If the targeted regulatory limit concentration cannot be achieved by the initial technical procedures, then at least one alternative generation method should be used, ideally using different physical principles but established methodologies. A reasonable attempt should be made to generate the test article, but extreme technical solutions are not recommended. An explanation and supportive data should be provided that explains why the regulatory limit concentration could not be achieved. This guidance also states that if the targeted concentration cannot be attained using the undiluted test article, a suitable non-toxic vehicle should be used and may also be considered to enhance the dustiness of solid test articles (powders). According to this guidance, in the case of solids, an inhalation acute toxicity study in rat is required with a respirable atmosphere (1-4 μm) and if not, a robust consideration should be provided. In this case, justification provided by the Notifier seems not to be very coherent taking into account obtained results with Imidan 70 WP.

For most type of substances, its classification is the starting point for the classification of the preparation. The LC_{50} of the formulation Imidan 70 WP warrants a classification with H332. It would be strange if the substance is not classified for this endpoint.

Since it is not clear that generation of respirable atmosferes with technical phosmet can be discarded, a LC_{50} value of 1.12 mg/l can be extrapolated for the technical phosmet from the LC_{50} value of 1.6 mg/l of the 70% preparation. Therefore, a classification of technical phosmet as H332, category 4, is proposed taking into account data extrapolated from the study with formulation Imidan 70 WP

Acute dermal toxicity

In a dermal toxicity study in rats (Dos Santos, 1998) the LD_{50} was determined to be greater than 1000 mg/kg bw and in the second study (McCabe, 1978) the dermal LD_{50} was found to be greater than 5000 mg/kg bw in rabbits. Available data indicates phosmet does not warrant classification for acute dermal toxicity.

4.2.4 Comparison with criteria

Based on the reported acute oral LD_{50} value of 113 (101-127) mg/kg bw for male rats and 113 (98-130) mg/kg bw for female rats in Mccabe study and the LD_{50} of 230 mg/kg bw in Navarro Aragay study, phosmet should be classified as Acute Tox. 3 - H301 (Toxic if swallowed), according to CLP (oral LD_{50} guidance values for this category from 50 to 300 mg/kg bw). The oral LD_{50} value in mice of 50.1 mg/kg also supports this classification, though this study was considered acceptable only as additional information. Therefore the existing Annex VI entry should be changed accordingly.

The TC C&L experts did not agree about inhalation toxicity classification. LC_{50} (vapour) was calculated to be higher than 0.152 mg/l/4 h (maximum attainable concentration) in the only available study by inhalation in rats with the technical material. According to data submitted by notifier it is not possible to generate respirable atmosferes of the technical material without destructive grinding of the test substance and therefore classification for acute inhalation toxicity should not be required. Nevertheless, results obtained with formulated Imidan 70 WP suggest that generation of toxic by inhalation respirable atmospheres with technical phosmet may be difficult but not impossible. In such situation, a precautory approach considering all data available should be made. In MSCA opinion, phosmet should be classified as Acute Tox. 4 - H332 (Harmful if inhaled) according to CLP (LC_{50} guidance values for this category from 1.0 to 5.0 mg/l for dust/mist). Therefore a new entry in Annex VI entry should be made.

At the TC C&L it was agreed not to classify phosmet for acute dermal toxicity and to the delete the current classification from the Annex I entry: Acute Tox. 4; H312. LD_{50} values for dermal toxicity were above the threshold value of 2000 mg/kg bw for triggering classification and labelling.

Accordingly no acute classification is proposed for the dermal route and the existing Annex VI entry should be delelted accordingly.

4.2.5 Conclusions on classification and labelling

CLP: Acute Tox. 3 - H301 and Acute Tox. 4 - H332

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

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

In one acute oral toxicity study in rats (Mccabe, 1978), deaths occurred from the dose level of 75 mg/kg bw and although reversible, clinical signs as depression, tremors, salivation, exophtalmus, chromodacryorrhea and dyspnea were observed at all dose levels from the lowest dose level of 60 mg/kg bw. In the mouse acute oral toxicity study (Meyding, 1966), deaths ocurred from the dose level of 46.4 mg/kg bw and no information was available about clinical signs.

Besides, in a range acute oral neurotoxicity study in rats (Cappon, 1998a) clinical signs of whole body tremors and gait alterations and biologically significant depression of brain and erytrocite ChE activity (> 20 %) at the dose of 36 mg/kg bw were observed. Biologically significant depression of erytrocite ChE activity (> 20 %) was also observed from the dose of 6 mg/kg bw. No mortalities were observed in this study.

Despite the fact that these effects (i.e., chlolinesterase inhibition, clinical signs) fulfill the criteria for a classification as STOT SE Cat.1 (H370), we do not consider it necessary.

4.3.2 Comparison with criteria

According to the Guidance on the Application of the CLP Criteria (version 4.0, November 2013), *care must be taken not to classify for STOT-SE for effects which are not yet lethal at a certain dose, but would lead to lethalithy within the numeric classification criteria. In other words, if lethalithy would occur at relevant doses then classification for acute toxicity would take precedence and STOT-SE would not be assigned.*

Phosmet is considered by MSCA as toxic to rats and mice after acute oral exposure (H301, Cat. 3). Additional classification and labelling is not justified because effects that mainly suggest specific target organ toxicity (i.e., chlolinesterase inhibition, clinical signs) are not yet lethal at the doses tested, but could lead to lethalithy at relevant doses and are sufficiently covered by classification for acute toxicity yet. It would be a double classification. Therefore no classification for specific target organ toxicity- single exposure is required.

4.3.3 Conclusions on classification and labelling

Phosmet does not warrant classification for specific target organ toxicity following - single exposure (STOT SE). This conclusion is in agreement with the former discussion at ECB level and with the current classification and labelling in Annex VI, Table 3.1 of Regulation (EC) No 1272/2008.

4.4 Irritation

4.4.1 Skin Irritation

Table 12: Summary table of relevant skin irritation studies

Method	Main results	Remarks	Reference
The study is pre-guideline but similar to OECD 404 GLP: No **Study acceptable** 6 male New Zealand White rabbits 3 day observation 0.5 g of test material applied by a semi-occlusive dressing for 24 hours to the intact and abraded skin of 6 rabbits. It is not mentioned if the test substance was moistened before the application. Purity: 96.1% (Stauffer Chemical material)	None of the rabbits died during the study period. No abnormal skin reactions were noted on any treated site during after 24 hour exposure to intact and abraded skin.	Every animal has two points of application: one flank with intact skin and another one with abraded skin.	**Mccabe, J., 1978**

4.4.1.1 Non-human information

See table above.

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

The results of the skin irritation study of Mccabe (1978) indicate that phosmet did not cause any abnormal skin reactions or corrosive effects when applied to the skin of New Zealand rabbits. The overall mean score of oedema and erythema following grading after 24, 48 and 72 hours was 0 in intact and abraded skin. Therefore, phosmet doesn't require classification as skin irritant.

4.4.1.4 Comparison with criteria

At the TC C&L it was agreed not to classify phosmet for acute dermal irritation. Skin irritation scores (0) are below the criteria for triggering classification and labelling according to Regulation (EC) No 1272/2008 (CLP).

4.4.1.5 Conclusions on classification and labelling

The classification criteria for skin irritation were not fulfilled. This is in agreement with the former discussion at ECB level and current classification and labelling in Annex VI, Table 3.1 of Regulation (EC) No 1272/2008.

CLP: Not classified based on available data.

4.4.2 Eye Irritation

Table 13: Summary table of relevant eye irritation studies

<table>
<tr><th>Method</th><th>Main results</th><th>Remarks</th><th>Reference</th></tr>
<tr>
<td>The study is pre-guideline but similar to OECD 405
GLP: No
Study acceptable
9 New Zealand White male rabbits
100 mg of test material was placed in one eye of each animal.
In 3 males, the treated eye was washed after 2 seconds with 0.9% saline, in 3 males after 4 seconds and 3 males remained unwashed.
Purity: 96.1% (Stauffer Chemical material)</td>
<td><u>Table 13.1: Individual and mean eye irritation scores</u>
Conjunctiva, iris and corneal lesions were evaluated to determine grades for ocular lesions, according to method Draize (1965)
<table>
<tr><th></th><th colspan="3">Unwashed eyes</th><th colspan="3">2 sec. wash</th><th colspan="3">4 sec. wash</th></tr>
<tr><td>Time/Rabbit</td><td>1</td><td>4</td><td>7</td><td>2</td><td>5</td><td>8</td><td>3</td><td>6</td><td>9</td></tr>
<tr><td>24 h</td><td>0</td><td>53</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>48 h</td><td>0</td><td>29</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>72 h</td><td>0</td><td>2</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>7 days</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>14 days</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
One rabbit with the unwashed eye exhibited corneal opacity, redness, chemosis and discharge which appeared normal on day 7.</td>
<td>▪ One rabbit was found dead in day 14 but this incidence did not appear compound-related.</td>
<td>Mccabe, J., 1978</td>
</tr>
</table>

4.4.2.1 Non-human information

See table above

4.4.2.2 Human information

There are no human data available relevant for C&L.

4.4.2.3 Summary and discussion of eye irritation

The eye irritation study in rabbits with phosmet resulted in corneal opacity, redness, chemosis and discharge in one animal (unwashed eye) which appeared normal again on day 7. All other animals appeared normal during the course of the study.

4.4.2.4 Comparison with criteria

At the TC C&L it was agreed not to classify phosmet for acute eye irritation. The individual and group mean eye irritation scores and observed eye irritation signs do not meet the criteria for classification as irritating to the eyes according to CLP.

4.4.2.5 Conclusions on classification and labelling

It is concluded that phosmet does not warrant classification as an eye irritant. This is in agreement with the former discussion at ECB level and current classification and labelling in Annex VI, Table 3.1 of Regulation (EC) No 1272/2008.

CLP: Not classified based on available data.

4.4.3 Respiratory tract irritation

No information on respiratory tract irritation is available.

4.5 Corrosivity

Based on the data from the skin and eye irritation studies it can be concluded that phosmet is not corrosive.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 14: Summary table of relevant skin sensitisation studies

Method	Doses and main results	Reference
Guideline: OECD 406 GLP: Yes **Study acceptable** Modified Buehler test (10 applications) Male Hartley albino guinea pigs 10 animals in test and control groups. Purity: 94.3% (Stauffer Chemical Material)	No dermal irritation was observed in a range-finding study. <u>Induction phase (day 1-22):</u> As it can be seen from the dermal penetration study *in vivo*, the active substance phosmet topically administered to rats in an aqueous solution is bioavailable via the dermal route (Jeffcoat, 1987). Ten topical applications for 6 hours apiece in one site on the right flank of three groups: ▪ Test group: 10 animals treated with 0.5 ml saline/ g test material. ▪ Negative control: 10 animals treated with saline. ▪ Positive control: 10 animals treated with 0.1% of 2,4-dinitrochlorobenzene (DNCB) <u>Challenge phase (day 35) and rechallenge phase (day 42)</u> ▪ In challenge and rechallenge test substance and saline was topically applied to different sites in the left flank of the animals of the test group and negative control animals respectively. Topical application of 0.1% of DNCB was applied to animals in positive control group. <u>Results:</u> ▪ There were 4 deaths during the study (two animals in the positive group, one in the test group and one animal in the negative control) but the cause of death appeared to be unrelated to the test substance. ▪ In the test group 1/10 showed postive response during challenge phase and no positive response during the rechallenge phase. Phosmet did not induce hypersensitivity in guinea pigs.	**Mutter, L.C., 1987**

4.6.1.2 Human information

There are no human data available relevant for C&L.

4.6.1.3 Summary and discussion of skin sensitisation

Phosmet does not cause sensitisation by skin contact tested by the Modified Buehler Test (10 applications during the induction phase) in guinea pigs (Mutter, 1987). 1/10 animals of the test group exhibited positive skin response after challenge and 0/10 after rechallenge at 24 hours and 48 hours (100% test material moistened with saline in induction and challenge phases).

In the OECD Guideline 406 it is said: “for water soluble test materials, it is appropriate to use water or a dilute non-irritating solution of surfactant as the vehicle. For other test materials 80% ethanol/water is preferred for induction and acetone for challenge”. The solubility of phosmet in water is low (15.2 mg/l which is < 1g/l). According to OECD Guideline, the physiological saline used as solvent in the study could not be an appropriate vehicle. However, an in *vivo* dermal penetration study in rats with Imidan 50 WP shows that an aqueous solution of the active substance is absorbed by dermal route and is thus systemically available for contact with the immune system.

Therefore, in spite of the low solubility of phosmet in water (15.2 mg/l, which is < 1g/l), the use of saline as a vehicle in the skin sensitisation study is considered adequate. The EPCO meeting 33 (12.-16.09.05) considered that the modified Buehler with 10 inductions, supported by a history of use in humans, was sufficient to demonstrate an absence of sensitising effects (17704/EPCO/BVL/05. rev. 2-1 of 8 May 2006).

4.6.1.4 Comparison with criteria

Effects observed in the skin sensitisation study in guinea pig (modified Buehler Test with 10 applications) are below the criteria for triggering classification and labelling. Phosmet does not warrant classification as being a skin sensitizer according to CLP Regulation.

4.6.1.5 Conclusions on classification and labelling

The classification criteria for skin sensitisation were not fulfilled. This is in agreement with the former discussion at ECB level and current classification and labelling in Annex VI, Table 3.1 of Regulation (EC) No 1272/2008. Therefore, no classification is proposed for phosmet regarding skin sensitisation.

CLP: Not classified based on available data.

4.6.1.6 Respiratory sensitisation

No information on respiratory sensitisation is available.

4.7 Repeated dose toxicity

Table 15: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
28 day dietary range-finding study (feeding) **Study acceptable**	Main effects Statistically significant inhibition of RBC cholinesterase (AChE) activity (>20%) in both sexes at 12.0, 25.7 and 62 mg/kg bw/day Statistically significant inhibition of brain ChE activity (15.7%) at 62 mg/kg bw/day (f). Decreases on body weight (>10%) and food consumption (>10%) from 25.7 mg/kg bw/day. At 62 mg/kg bw/day increased severity of the effects and even some clinical findings such as emaciation, tremors and decrease in general activity in both sexes. **NOAEL = 3.8 mg/kg bw/day (15 ppm)** **LOAEL = 12 mg/kg bw/day (50 ppm)**	B6C3F1 mouse Purity: 95% Dose range of 0, 1.2, 3.8, 12.0, 25.7, 62 mg/kg bw/day (0, 5, 15, 50, 150, 500 ppm)	**Jones, M.S. (1981)**
28 day dietary range-finding study (feeding) **Study acceptable**	Main effects Plasma, RBC and brain AChE inhibition at 6 mg/kg bw/day in both sexes (>20%). Relevant AChE inhibition in erythrocytes in males and females at 3 mg/kg bw/day (21.0 and 24.0 %, respectively). **NOAEL = 1.5 mg/kg bw/day** **LOAEL = 3 mg/kg bw/day**	Beagle dog Purity: 97% Dose range: 0, 1.5, 3, 6 mg/kg bw/day	**Brown, M.A. (2003)**
90 day dietary study (feeding) **Study acceptable with some reservations**	Main effects Statistically significant inhibition of RBC and brain cholinesterase (AChE) activity (>20%) in both sexes at 10 and 50 mg/kg bw/day. Decrease of food consumption (>10%) from 10 mg/kg bw/day and reduction of mean body weight in males at 50 mg/kg bw/day (>10%). Decrease of mean absolute weight and mean relative weight of prostate in males at 50 mg/kg bw/day (>10%). **NOAEL = 2 mg/kg bw/day (20 ppm)** **LOAEL = 10 mg/kg bw/day (100 ppm)**	Albino rat Purity: 98 ± 0.5 % Dose range: 0, 2, 10, 50 mg/kg bw/day (0, 20, 100, 500 ppm)	**Johnston, C.D. (1962)**
90 day dietary study (feeding) **Study acceptable with some reservations**	Main effects Statistically significant inhibition of RBC cholinesterase (AChE) activity (>20%) and brain cholinesterase (AChE) activity (>20%) in both sexes at 14.1 mg/kg bw/day. **NOAEL = 1.88 mg/kg bw/day (75 ppm)** **LOAEL = 14.1 mg/kg bw/day (563 ppm)**	Beagle dog Purity: 98 ± 0.5 % Dose range: 0, 0.25, 1.88, 14.1 mg/kg bw/day (0, 10, 75, 563 ppm)	**Johnston, C.D. (1962)**

Method	Results	Remarks	Reference
2 year dietary study (feeding) **Study not acceptable (additional information)**	Main effects Inhibition on plasma, RBC and brain AChE activity (>20%), ↓body weights and liver vacuolation in both sexes at high dose level. **NOAEL = 4 mg/kg bw/day (40 ppm)** **LOAEL = 40 mg/kg bw/day (400 ppm)**	Albino rat Purity: not indicated Dose range: 0, 2, 4, 40 mg/kg bw/day (0, 20, 40, 400 ppm)	**Johnston, C.D. (1966)**
2 year dietary study (feeding) **Study acceptable with some reservations**	Main effects Inhibition on RBC and brain AChE activity (>20%) at high dose level for males/females and inhibition of brain activity AChE (15.23%) at 40 ppm. **NOAEL = 0.5 mg/kg bw/day (20 ppm)** **LOAEL = 1 mg/kg bw/day (40 ppm)**	Beagle dog Purity: not indicated Dose range: 0, 0.5, 1, 10 mg/kg bw/day (0, 20, 40, 400 ppm)	**Johnston, C.D. (1966)**
21 day dermal study **Study acceptable**	Main effects Inhibition on plasma and RBC activity (>20%) at high dose level. **NOAEL = 100 mg/kg bw/day** **LOAEL = 1000 mg/kg bw/day**	New Zealand White rabbit Purity: 94.3%-95.2% Dose range: 0, 10, 100, 1000 mg/kg bw/day	**Henwood, S.M. (1988)**
21 day dermal study **Study acceptable**	Main effects Inhibition on brain AChE activity (>20%) at high dose level. **NOAEL = 22.5 mg/kg bw/day** **LOAEL = 60 mg/kg bw/day**	Sprague Dawley rats Purity: 71.2% Dose range: 0, 15, 22.5, 60 mg/kg bw/day	**Hilaski, R.J. (1999)**

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Oral 28-day toxicity (mice)

Title	Four week dietary range-finding study in mice with Imidan Tecnical
Author (s) (year):	Jones, M.S. (1981)
Administration	Dietary (oral via corn oil) for 28 days
Guideline	None, this is a range finding study to determine the appropriate levels for a subsequent 2-year oncogenicity study in mice
Species	B6C3F1
GLP	Yes
Purity:	95%
Groups	10 mice/sex/dose level
Dose levels	0, 5, 15, 50, 150, 500 ppm equivalent to 0, 1.2, 3.8, 12, 25.7 and 62 mg/kg bw/day
Study acceptable	

Executive Summary

In a 28 day feeding study, phosmet (Imidan Technical, purity: 95%) was administered to B6C3F1 mice at concentrations of 0, 5, 15, 50, 150 or 500 ppm (10 mice/sex/dose level). The mean daily intakes of Imidan Technical were 0, 1.2, 3.8, 12.0, 25.7 and 62 mg/kg bw/day, respectively. Animals were observed twice daily for mortality and signs of toxicity. A weekly examination for clinical signs included palpation for masses. Body weights and food consumption were measured weekly (not on day 28).

On the day of necropsy blood was collected from the heart of animals anaesthetised with sodium pentobarbital and analysed for total erythrocyte count, total and differential leukocyte count, haematocrit, haemoglobin concentration and platelet count. Differential leukocyte counts were performed manually. Red blood cell and plasma cholinesterase activities were also measured.

Gross pathological examination was performed on all animals, and liver and kidneys of all animals were weighed. Liver, kidneys, lung, heart, spleen and any gross lesions were fixed and examined histopathologically. Brain cholinesterase activity was measured in 5 animals/sex/dose level.

Quantitative continuous data were compared between the dose groups and the control group by a one-way analysis of variance and the appropriate T-test (for equal and unequal variances) using Dunnett's multiple comparison tables. The fiducial limit of 0.05 was employed to delineate the critical level of significance in all statistical comparisons.

Results

Mortality: There were no unscheduled deaths during the course of the study.

Clinical observations: decrease of activity at 62 mg/kg bw/day in all animals. At this dose level was also observed emaciation (2/10 in males and females), listless (10/10 males and 4/10 females) and tremors (1 female).

Food consumption: statistically significant decrease on day 7 in both sexes at 62 mg/kg bw/day (50.0% in both sexes) and at 25.7 mg/kg bw/day in males (33.3%). The day 14 there was a statistically significant decrease at 62 mg/kg bw/day (66.7% in both sexes) and at 25.7 mg/kg bw/day (33.3% in both sexes). The day 22 there was a statistically significant decrease in both sexes at 62 mg/kg bw/day (66.7% in males and 71.4% in females) and at 25.7 mg/kg bw/day (50.0% in males and 42.9% in females).

Mean body weights: statistically significant decrease in animals of both sexes dosed with 62 mg/kg bw/day from day 7 to day 22 (weight loss during this period was in the interval 23.1%-26.1% in males and 10.0%-21.0% in females). Males, dosed with 25.7 mg/kg bw/day also decreased their body weight (statistically significant) from day 7 to day 22 (weight loss during this period was in the interval of 8.7%-12.0%), in accordance with decreased food consumption. An occasional decrease of body weight (statistically significant) was observed in males dosed with 3.8 mg/kg bw/day on days 7 and 14 (7.7%-8.0%), with no explanation.

Plasma cholinesterase (Table 16): Males showed significant decrease at 25.7 and at 62.0 mg/kg bw/day. There were quality problems with the control group and other dose group (12 mg/kg bw/day).

Red blood cell cholinesterase activity (Table 16): Statistically significant decrease at 12, 25.7 and 62 mg/kg bw/day (depression >20%) in both sexes.

Brain cholinesterase activity (Table 16): Statistically significant decrease at 62 mg/kg bw/day in females (15.7%).

Table 16: Cholinesterase tests: variations with respect to controls at necropsy time

Parameter	**Dose Level [mg/kg bw/d]**					
	0	**1.2 (5 ppm)**	**3.8 (15 ppm)**	**12 (50 ppm)**	**25.7 (150 ppm)**	**62 (500 ppm)**
Cholinesterase Activity						
Males						
Plasma AChE						
Mean value	#	4571	3682	#	**1719***	**1397***
Standard deviation		680	523		**265**	**224**
No. animals		9	6		**9**	**9**
RBC AChE						
Mean value	8224	9376*	7902	**5748***	**2558***	**1812***
Standard deviation	1503	756	459	742	738	405
No. animals	9	10	9	10	10	9
% change from control	-	+14.0	-3.9	**-30.1**	**-68.9**	**-78.0**
Brain AChE						
Mean value	88.6	85.2	86.4	#	86.8	79.0
Standard deviation	2.5	8.6	7.1	-	6.1	6.0
No. animals	5	5	5	-	5	5
% change from control	-	-3.8	-2.5	-	-2.0	-10.8
Females						
Plasma AChE						
Mean value	#	8439	5213	#	#	1595
Standard deviation		483	2752			129
No. animals		8	8			9
RBC AChE						
Mean value	8553	7523	9624	**6530***	**4968***	**2382***
Standard deviation	732	529	2251	924	827	630
No. animals	8	8	10	10	10	9
% change from control	-	-12.0	+12.5	-23.6	-41.9	**-72.1**
Brain AChE						
Mean value	94.2	86.0	98.6	#	#	**79.4***
Standard deviation	5.0	8.8	5.1	-	-	2.9
No. animals	5	5	5	-	-	5
% change from control	-	-8.7	+4.7	-	-	**-15.7**

* significantly different from controls at $p < 0.05$
\# values invalid due to quality control problems
RBC red blood cells

Haematology: the most relevant observed effects were a statistically significant increase at 62 mg/kg bw/day of the platelet count in both sexes (19.3 in males and 21.4% in females) and a statistically signicant decrease of the mean corpuscular volume in females (2.8%).

Organ weight modifications: At 62 mg/kg bw/day there was a statistically significant decrease of the mean absolute weight of kidney (21.4%) and liver (13.2%) in males, an increase in the relative weight of liver in both sexes (15.7 in males and 16.4% in females) and of the relative weight of kidney in females (6.6%).

At 25.7 mg/kg bw/day was observed a statistically significant decrease in the mean absolute weight of liver in males (10.0%) and a significant increase in females (12.0%). Moreover it was observed an increase in the relative weight of liver in both sexes (6.0% in males and 7.5% in females) and reduction in the mean absolute weight of the kidney in males (9.5%).

Conclusion

The NOAEL in this study was established in 3.8 mg/kg bw/day due to the red RBC cholinesterase inhibition at 12.0 mg/kg bw/day.

Dose levels of 0, 5, 25 and 100 ppm were selected for the 2-year carcinogenicity study in mice with Imidan technical (Katz et al., 1984).

Oral 28-day toxicity (dogs)

Title	**Phosmet: Dose range-finding oral (feeding) toxicity study in the dog**
Author (s) (year):	Brown, M.A. (2003)
Administration	Dietary (oral) for 28 days
Guideline	Not available
Species	Beagle dogs
GLP	Yes
Purity:	97%
Groups	3 dogs/sex/dose level
Dose levels	0, 1.5, 3.0 and 6 mg/kg bw/day
	Study acceptable

Executive Summary

In a 28-day feeding study, phosmet (purity: 97 %) was administered to three male and three female pure-bred beagle dogs for a period of 4 consecutive weeks each at dose levels of 0, 1.5, 3.0 and 6 mg/kg bw/day. The mean daily intakes of phosmet were 0, 1.6, 3.2 and 6.4 mg/kg bw/day for males and 0, 1.6, 3.4 and 6.4 mg/kg bw/day for females.

Phosmet was dissolved in an appropriate amount of acetone which was then mixed with an appropriate amount of microgranulated diet and subsequently mixed with the remaining microgranulated feed. Control animal diet was prepared in the same way but without the test item.

Animals were observed at least twice daily for viability, clinical signs from commencement of the pretest period. Body weights were measured weekly from the pretest period and before necropsy. Food consumption was measured daily from commencement of the pretest period. The daily ration was weighed before and after feeding.

Twice during pretest (on days -8 and -2) and once on day 28, blood samples were obtained prior to feeding from the jugular vein of all animals for plasma and erythrocyte acetylcholinesterase activity determinations. The dogs were fasted overnight but allowed access to water *ad libitum*. Blood samples were analysed on the day of collection.

A gross pathological examination was performed on all animals. Organ weights of selected organs were recorded. Tissues and organs were collected from all animals but not histopathologically examined. Brain cholinesterase activity was measured in 3 animals/sex/dose level.

At sacrifice, two samples of cerebellar tissue were obtained for brain cholinesterase determination.

For body weights and organ weights if the variables could be assumend to follow a normal distribution the Dunnett- test (many to one t-test) was applied for the comparison of the treated groups and the control groups for each sex. The Steel-test (many of rank test) was applied instead of Dunnett-test when the data could not be assumed to follow a normal distribution.

For acetylcholinesterase data, quantitative data was analysed by a one way analysis of variance (ANOVA) when the variances were considered homogeneous according to Bartlett. Alternatively, if the variances were considered heterogeneous ($p \leq 0.05$), a non-parametric Kruskal-Wallis test was used. Treated groups were then compared to the control groups using Dunnett's test if the ANOVA was significant at the 5% level and by the Dunn's test in the case of a significant Kruskal-Wallis test ($p \leq 0.05$)

Results

Mortality: There were no premature deaths.

Clinical observations: No clinical signs related to administration of the test item were noted during treatment.

Mean body weights and food consumption: Unaffected.

Plasma cholinesterase (Table 17): In animals administered with 6 mg/kg bw/day, statistically significant reductions in mean plasma cholinesterase activities of up to approximately 23.0 % (males) or 34.6 % (females) less than pre-test activities were noted on day 28. These reductions were considered to be biologically relevant (*i.e.* cholinesterase inhibition above the trigger of 20 %). Plasma cholinesterase activities were also reduced by up to approximately 4 % in males administered with 1.5 mg/kg bw/day and females with 1.5 or 3 mg/kg bw/day. This level of inhibition is not considered to be biologically relevant as it is significantly below the trigger of 20 %.

Red blood cell cholinesterase activity (Table 17): RBC cholinesterase activities were reduced compared to pre-test by up to approximately 71.4 % (males) and 77.6 % (females) at 6 mg/kg bw/day and 21.0 % (males) and 24.0 % (females) at 3 mg/kg bw/day. These reductions reached statistical significance except at 3 mg/kg bw/day in females and were considered to be biologically relevant (*i.e.* above the trigger of 20 %). RBC cholinesterase activities were also significantly reduced by approximately 13.8 % in males administered 1.5 mg/kg bw/day. This level of inhibition was not considered to be biologically relevant in the absence of similar observations in females at 1.5 mg/kg bw/day (a mean increase of 0.3 % was noted in females) and also given that the inhibition was below the trigger of 20 %.

Brain cholinesterase activity (Table 17): Brain cholinesterase activities were approximately 41 % (males) or 55 % (females) lower than control values in animals administered 6 mg/kg bw/day. These reductions were considered to be biologically relevant (*i.e.* above the trigger of 20 %). Levels of brain cholinesterase were not decreased in animals administered 1.5 mg/kg bw/day or in males administered 3 mg/kg bw/day. A mean reduction of 17 % was evident in female animals administered 3 mg/kg bw/day. However, this was not considered to be biologically relevant with a degree of inhibition below 20 % and the absence of inhibition of brain cholinesterase activity in males at 3 mg/kg bw/day in which a mean increase of 18 % activity was observed.

Table 17: Plasma and RBC AChE variations between pretest and day 28 values and brain AChE variations between values of dosed groups and controls

Dose	**Change in Cholinesterase Activity [%]**					
	Plasma		**RBC**		**Brain**	
[mg/kg bw/day]	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
0	-0.2%	2.4%	8.7%	-3.7%	-	-
1.5	-4.3%	0.3%	-13.8%*	0.3%	19%	11%
3	-0.7%	-4.0%	-21.0%**	-24.0%	18%	-17%
6	-23.0%**	-34.6%**	-71.4%**	-77.6%**	-41%	-55%

* $p < 0.05$, one way analysis ANOVA and Dunnett's test

** $p < 0.01$, one way analysis ANOVA and Dunnett's test

No macroscopical abnormalities were noted at necropsy.

No haematology data available.

Organ weights were considered to be unaffected by administration of the test item.

Conclusion

Based on relevant cholinesterase inhibition in RBCs of males and females at 3 mg a.s./kg bw/day (21.0 and 24.0 %, respectively) the NOAEL of the study is deduced to be 1.5 mg/kg bw/day.

Oral 90-day toxicity (rats and dogs)

Title	**An evaluation of safety on Imidan in the rat and the dog (1962) and and evaluation of safety on Imidan in the rat and the dog – final supplement (1963)**
Author (s) (year):	Johnston, C.D. (1962)
Administration	Dietary (oral) for 90 days
Guideline	At the time the study was conducted, guideline was not compulsory. Deviations with respect guideline OECD 408: Rat: the number of animals is lower than 20 per group, there is no ophthalmological examination, sensory reactivity to stimuli and FOB was not investigated, some haematology parameters are missing, all biochemistry parameters and urinalysis are missing and some organ hispatology examinations are missed. Dog: there is no ophthalmological examination, food consumption was not determined, sensory reactivity to stimuli was not investigated, biochemistry parameters are missing, some haematology parameters, organ weights (epididymides and parathyroid) and some hispatology data are missing.
Species	Albino rats and Beagle dogs
GLP	No
Purity:	98%
Groups	2 groups of 15 rats/sex/dose level 4 dogs/sex/dose level
Dose levels	Rats: 0, 20, 100 and 500 ppm equivalent to 0, 2, 10 and 50 mg/kg bw/day Dogs: 0, 10, 75 and 563 ppm equivalent to 0, 0.25, 1.88, 14.1 mg/kg bw/day.
	Study acceptable with some reservations

Part I: Oral 90-day toxicity (rats)

Executive Summary

In a feeding study, phosmet (Imidan (R-1504), purity 98 ± 0.5 %) was administered for at least 13 weeks to groups of male and female (purchased from the Charles River Breeding Laboratories) albino rats at concentrations of 0, 20, 100 and 500 ppm (mean achieved dose 0, 2, 10 and 50 mg/kg bw/day in males and females). The rats were divided randomly into two groups (15 animals/sex/dose level). The group 2 levels were started approximately four weeks after group 1 levels in order to elevate each dose level. Five animals/sex/dose level of group 1 were sacrificed at week 14, the remaining animals at week 24. Animals of group 2 were sacrificed at week 19.

Animals were examined for clinical signs of toxicity weekly. Body weights and food consumption were recorded weekly.

At week 4, 9 and 13 haemograms (including determination of haemoglobin, microhaematocrite and white and differential cell counts) were obtained on 5 males and 5 females from the control and

high dose rats of group 1 and from similar numbers of mid and low-dose animals if the data from the high dose suggested this might be desirable. Haemograms of the group 2 rats were made at week 6 and 10. At week 2, 4 and 8, red blood cell and plasma cholinesterase assays were performed on 5 males and 5 females from each dose level of group 1. Blood from similar numbers of group 2 rats was assayed at week 3 and 11. In addition, cholinesterase assays were carried out on blood collected from sacrificed rats and the enzyme activity in the brain of each animal was estimated. 5 animals/sex/dose level of group 1 were sacrificed at week 14, the remaining animals at week 24. Animals of group 2 were sacrificed at week 19. The study was terminated with no further haematology or cholinesterase determination.

Cholinesterase activity was measured using the Delta-pH (Δ pH/h) electrometric method which is based on the hydrolysis of acetylcholine and production of acetic acid which in turn decreases the pH of the reaction mixture.

At week 14, 5 male and 5 female rats for each dose level of group 1 were sacrificed and gross autopsy was performed on each animal. Selected organs were weighed. Various tissues and organs of control and high dose rats, and selected tissues and organs of low and mid dose animals were examined histopathologically.

No statistical analysis for comparison between groups was performed in this study.

Results

There were no noticeable differences between the treatment and control groups as far as general appearance and behaviour were concerned.

Mortality: One death occurred in the group 1 (one male at 50 mg/kg bw/day in week 13).

Clinical observations: No manifested clinical signs at any dose level.

Food consumption: It was followed until week 24 in group 1 and until week 19 in group 2. On week 14 there were only significant decreases in food consumption in males of group 1 at 50 mg/kg bw/day (13.5%) and at 10 mg/kg bw/day (30.0%). These reductions in males of group 1 persisted in week 24, at 50 mg/kg bw/day (18.0%) and at 10 mg/kg bw/day (15.8%).

Mean body weights: At 50 mg/kg bw/day, there was a loss of weight in males at week 14 (15.4%) in group 1 and in males of group 2 at week 11 (7.8%)

Plasma cholinesterase (Table 18): Activity was about 35% to 50 % inhibited at 50 mg/kg bw/day, but only 10% to 15 % inhibited at 10 mg/kg bw/day. The plasma ChE activity of the 2 mg/kg bw/day rats was not affected.

Red blood cell cholinesterase (Table 18): A nearly complete inhibition of the red blood cell (RBC) cholinesterase (ChE) activity was noted in rats at 50 mg/kg bw/day and about 50 % inhibition was seen in rats at 10 mg/kg bw/day, while no effect on RBC ChE activity was noted at 2 mg/kg bw/day.

Brain cholinesterase (Table 18): Brain ChE inhibition determination in females of group 1 indicated 82% in the high dose, 39% in the mid dose and 11% in the low dose group and in males of the same group of 75% in the high dose, 42% in the mid dose and 6% in the low dose group

Table 18: Cholinesterase (ChE) activity as per cent of controls

Time Point	Dose Level [mg/kg bw/day]					
	Group 1			Group 2		
[week]	2	10	50	2	10	50
	Plasma [% of controls]					
Males						
2, 3[a]	100	100	100	100	92	38
4	88	100	64	n.a.	n.a.	n.a.
8, 11[b]	90	87	60	92	77	46
14	93	85	52	n.a.	n.a.	na
Females						
2, 3[a]	100	100	100	100	90	37
4	88	100	64	n.a.	n.a.	n.a.
8, 11[b]	90	87	60	100	72	17
14	93	85	52	n.a.	n.a.	n.a.
	RBC [% of controls]					
Males						
2, 3[a]	100	56	19	100	44	0
4	100	48	0	n.a.	n.a.	n.a.
8, 11[b]	100	40	4	88	38	3
14	100	58	0	n.a.	n.a.	n.a.
Females						
2, 3[a]	100	56	12	100	39	0
4	91	35	13	n.a.	n.a.	n.a.
8, 11[b]	89	36	0	93	46	0
14	100	63	0	n.a.	n.a.	n.a.
	Brain [% of controls]					
Males						
13	94	58	25	n.a.	n.a.	n.a.
Females						
13	89	61	18	n.a.	n.a.	n.a.

a week 2 in group 1, week 3 in group 2
b week 8 in group 1, week 11 in group 2
RBC red blood cells
n.a. not applicable (not measured)

Haematology: The mean values of the parameters examined for haematological investigations for all dose groups were within the normal range and were comparable to the control values. The white cell counts of one male at 50 mg/kg bw/day and another one at 10 mg/kg bw/day were increased at week 9. Variation of the individual values of the 10 mg/kg bw/day females of group 1, the control males of group 2 and 50 mg/kg bw/day females of group 2 was high. Since only single rats were involved and since more recent data (10 and 13 week interval) were consistent in every rat examined it can be concluded that these effects are not test substance-related.

Necropsy findings: There were no apparent treatment-related findings at necropsy. No treatment-related microscopic abnormalities were observed in any dose group, except some slight liver cell damage changes noted in males at high dose and in one male at low dose.

Organ weights: There were no pronounced changes in the relative organ weights of the treated animals, except for a slight decrease in the mean relative prostate weight (23.4% in group 1 and 10.6% in group 2) and mean absolute prostate weight (26.9%) in group 1 at the high dose group and

a slight increase of the relative kidney weight of females at mid and high dose of group 1 (3.8% and 6.4% respectively).

Histopathology: No treatment related microscopic abnormalities were observed in any dose group, except some slight liver cell damage changes noted in males of the high dose group and in one male of the low dose group.

Conclusion

Based on the results of this 90-day feeding study, the NOAEL was 2 mg/kg bw/day for male and female rats. This NOAEL was mainly based on brain and RBC ChE inhibition in males and females at 10 mg/kg bw/day.

Part II: Oral 90-day toxicity (dogs)

Executive Summary

In a 90-day feeding study, phosmet (Imidan (R-1504), purity: 98 ± 0.5 %) was administered to male and female beagle dogs (4 dogs/sex/dose level) at concentrations of 0, 10, 75 or 563 ppm equivalent to 0, 0.25, 1.88 and 14.1 mg/kg bw/day. The animals were observed daily for mortality, morbidity and clinical signs of toxicity, and were examined weekly in detail. Body weights were recorded weekly. Dogs were fed with 200 g of a dry dog meal and 100 g of a commercial beef preparation. Test substance was incorporated in the dry food. It was not reported, if all food was eaten.

At weeks 0, 3-4, 7-10, and 13, blood was collected for haematological and clinical chemistry determinations from all dogs. Blood cholinesterase assays were performed at weeks –1, 0, 1, 2, 3-4, 6-7, 10 and 13 on all dogs. Also at week 14, one male and one female dog each from the high and mid dose group were put on control feed in order to determine the regeneration rate of the blood cholinesterase. Cholinesterase determinations were carried out on the blood of these four recovery dogs and on the blood of two control animals at weeks 14, 16, 17 and 18. Following the week 18 cholinesterase determination, the animals from the mid dose were returned to compound, but the entire dog study was terminated at week 20 without further clinical or biochemical determinations. The urine of all animals was examined at beginning of treatment and at week 4, 8 and 13.

Cholinesterase activity was measured using the Delta-pH (Δ pH/h) electrometric method which is based on the hydrolysis of acetylcholine and production of acetic acid which in turn decreases the pH of the reaction mixture.

At week 14, one male and one female from each dose group were sacrificed and examined *post mortem*. Sections of various tissues and organs from all animals were preserved and examined by microscopy. Brain samples were taken and brain cholinesterase activity was measured. The animals remaining after week 14 were also sacrificed, a gross autopsy was performed on each animal and selected organs were weighed.

No statistical analysis for comparison between groups was performed in this study.

Results

Mortality: No mortalities.

Clinical observations: No test item-related clinical signs were observed during the study.

Food consumption: Not evaluated.

Mean body weights: All the dogs gained weight except two, one male at 1.88 mg/kg bw/day (reduction of 12.8% on week 14 with respect to the weight on week 0) and one female at 0.25 mg/kg bw/day (reduction of 6.5% on week 13 with respect to the weight on week 0). The dog at the mid dose was sacrifice on week 14 and the female at the low dose decrease the body weight during weeks 14-20.

Plasma cholinesterase (Table 19): There was only an inhibition greater than 20% at the high dose level on week 10. Determination of cholinesterase activity for the recovery group showed that regeneration of the plasma enzyme was essentially complete within 2 – 4 weeks.

Red blood cell cholinesterase (Table 19): The red blood cell (RBC) cholinesterase activity of the high dose groups of both sexes was markedly decreased as early as the second to the fourth weeks and completely inhibited from week 6 onwards (99.65% for both sexes at week 13). Low dose showed inhibition greater than 20% at week 13 but was considered not dose related due to the results of the mid dose. The RBC cholinesterase was slower to recover and returned to only 20 – 40 % of pre-treatment activity in 4 weeks in the high dose group.

Table 19: Cholinesterase (ChE) activity variations in plasma and RBCs with respect to controls

Time Point	**Dose Level [mg/kg bw/d]**		
	0.25	**1.88**	**14.1**
[week]	**Plasma [% of controls]**		
Males/Females			
-1	9.03	9.25	6.69
0	4.85	10.55	8.46
1	9.53	12.81	3.42
2	7.14	1.79	-13.39
4	32.58	28.08	-3.37
6	23.19	19.50	-13.11
10	17.04	12.90	-27.87
13	25.67	31.63	-12.56
[week]	**RBC [% of controls]**		
Males/Females			
-1	-9.70	-3.43	-4.93
0	-1.74	-19.13	6.52
1	-5.03	-8.97	-33.48
2	12.19	14.34	-71.33
4	-21.96	-14.19	-91.22
6	-15.99	-20.35	-83.14
10	-16.58	-16.32	-95.60
13	-23.59	-16.90	-99.65

RBC red blood cells

Brain cholinesterase (Table 20): Brain cholinesterase activity at high dose was markedly inhibited (>95 % inhibition), but the brain cholinesterase activity of the mid and low dose dogs was not affected.

Table 20: Cholinesterase (ChE) activity variation in brain tissue with respect to controls.

Time Point	**Dose Level [mg/kg bw/d]**			
	0	**0.25**	**1.88**	**14.1**
[week]	**Brain [Δ pH/h (% of controls)]**			
Males				
14	0.52 (0)	0.52 (0)	0.55 (+5.8%)	0.04 (-92.3%)
Females				
14	0.54 (0)	0.55 (+1.9%)	0.54 (0)	0.01 (-98.1%)

Haematology: There were no test item-related effects in haematology, clinical chemistry or urinalysis parameters.

Necropsy: findings: All treated dogs were examined and appeared normal and comparable to controls.

Organ weights: At the high dose group in males and females an increase in the relative weight of kidney (12.2% and 60% respectively) and adrenals (19.4% and 10.5% respectively) was observed. At the mid dose group an increase in the relative weight of kidney was observed in males (32.8%). However, the organ weights were only determined for one male and one female per dose level and a high organ weight variability was observed.

Histopathology: At gross and histopathological examination, no treatment-related effects were recorded at all dose levels. None of the changes observed were attributable to the ingestion of the test substance.

Conclusion

The NOAEL in the 90-day feeding study in dogs was 1.88 mg/kg bw/day for males and females as the ChE inhibition for the mid and low dose was not dose-related.

Oral 2-year toxicity

Title	**Imidan – Safety evaluation by 2-year feeding studies in the rat and the dog**
Author (s) (year):	Johnston, C.D. (1966)
Administration	2-year oral dietary (daily administration)
Guideline	OECD 451 with deviations (dog and rat): Some haematology parameters are missed: mean corpuscular volume, red blood cell count, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count. Some paramters of chemical chemistry are missed: potassium calcium, sodium, phosphorus, chloride, creatinine, total cholesterol etc. Urinalysis was only performed qualitevely. Gross pathology/histopathology at least for all animals in the control/high dose group for the following organs are missing: brain, testes, ovaries, epididymides, nose, pharynx, larynx, parathyroid. Only 25 rats/sex/group were used.
Species	Albino rats Beagle dogs
GLP	No
Purity:	Not indicated
Groups	25 rats/sex/dose level 3 dogs/sex/dose level
Dose levels	Rats: 0, 2, 4 and 40 mg/kg bw/day (0, 20, 40 and 400 ppm) Dogs: 0, 0.5, 1, 10 mg/kg bw/day (0, 20, 40 and 400 ppm)
	Study is not acceptable (rat) (additional information**)** due to the high mortality occurred from week 80 onwards in this study. Method OECD no. 35 of evaluation of chronic toxicity and carcinogenesis stipulates that survival of all groups should be not less than 50% at 24 months for rats. The survival in the study was lower than 50% in control and 20 ppm groups at week 85. At week 94 only the 200 ppm group had a survival greater than 50%. At the end of study (week 104), the survival was 20%, 24%, 32% and 38%. **Study is acceptable with some reservations (dog)**

Part I (rat)

Executive Summary

In a 2 year feeding study, phosmet (Imidan (RP4-RCT-116), purity not indicated) was administered to weanling albino rats from Charles River Laboratories (25 rats/sex/dose level) at concentrations of 0, 20, 40 and 400 ppm corresponding to 0, 2, 4 and 40 mg/kg bw/day.

Parameters evaluated weekly were the body weight and food consumption, and daily examined for general physical condition. Hemograms, consisting of hemoglobin and hematocrit determinations, and total and differential white cell counts were obtained from 5 males and females per group at 14, 26, 39, 52, 78 and 104 weeks. At the same intervals, plasma and RBC cholinesterases were carried out. Finally, at termination, all rats were sacrificed and some organs were weighed and others tissues preserved of microscopic examination.

No statistical analysis for comparison between groups was performed in this study.

Results

Mortality: There was a great mortality at all dose levels (manifested on week 80 onwards) and the author of the study stated that was due to respiratory infections. Mortality appeared not to be compound-related. Phosmet had no adverse effects on the survival rats.

Table 21: Mortality at 13-Weel Intervals.

	Doses (ppm)							
	0		**20**		**40**		**400**	
Number of animals	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Weeks								
0-13					2		1	
14-26								
27-39	1	1	1					1
40-52	1		1	1	2		1	
53-65	4		3	1			2	
66-78	1		2	4	1	1		
79-91	7	5	8	2	11	8	8	8
92-104	5	3	5	7	3	11	9	4
Total deaths	19	9	20	15	19	20	21	13
Survival rate (%)	34	74	20	40	16	48	34	20

Mean body weight: The mean body weight was comparable to the controls, except when receiving 40 mg/kg bw/day from week 8 to 96 that was 17%-13% lower than controls.

Food consumption: It was comparable between groups.

Haematology: Hematological data remained unaffected in all dosed groups.

Cholinesterase activity: Noticeable decrease of the plasma (34%-69% for males and 50%-74% for females) and RBC ChE activity (76%-80% in males and 72%-80% in females) in the group treated with 40 mg/kg bw/day throughout the treatment period. Brain AChE activity was also depressed at sacrifice (65%-82% in males-females respectively).

Necrospy: No consistent changes in gross appearences or ogans examined at autopsy were found at terminal sacrifice.

Histopathology and organ body weights: The most common histopathological finding in those unscheduled sacrificed rats was an increase in lung weights and mucus accumulation in the respiratory indicating a bronchopneumonia. The absolute weight of pituitary, prostate, spleen, kidney decreased and the heart, lungs, thyroid absolute weight increased. Moderate liver cell vacuolation was observed at 40 mg/kg bw/day.

As can be observed in Table 22, pituitary adenomas were the most frequent, but there is no increase of dose-response attributable to phosmet.

Table 22: Number and Type of tumours produced in rat exposed for 2 years to Imidan

Dose (mg/Kg bw/day)	**0**	**2**	**4**	**40**	**0**	**2**	**4**	**40**
	Males				**Females**			
Transitional Cell Carcinoma						1		
Pituitary Adenoma	3	1	6	3	5	3	5	6
Fibroadenoma					5			3
Sarcoma		2	1					
Carcinoma Mammary gland								1
Interstitial Cell tumour of testis		1				1		

Conclusion

Taking into account that the study was not performed in accordance to modern guidelines, there are some data gaps that could hide other effects. Therefore, the conclusion of this study is that, again, the RBC and brain AChE depression is the critical effect, and the Imidan produced an important depression at 40 mg/kg bw/day. Besides at this dose level mean bodyweights were reduced and moderate liver vacuolation was also observed. NOAEL can be established at 4 mg/kg bw/day.

Part II (dog)

Executive Summary

In a 2 year feeding study, phosmet (Imidan (RP4-RCT-116), purity not indicated) was administered to male and female beagle dogs (3 dogs/sex/dose level) at concentrations of 0, 20, 40 and 400 ppm corresponding to 0, 0.5, 1 and 10 mg/kg bw/day.

The animals were examined weekly as to general physical condition. Body temperature, respiration rate and heart rate (by stethoscope) were recorded. Body weights were recorded weekly. Food consumption was not recorded. All surviving animals were examined ophthalmoscopically at week 104. Haematological and clinical chemistry measurements were carried out initially and at weeks 14, 26, 40, 52, 78 and 104 weeks for all dogs. Cholinesterase activity was determined at week 14, 26, 39, 52, 78 and 104. Cholinesterase activity was measured using the Delta-pH (Δ pH/h) electrometric method which is based on the hydrolysis of acetylcholine and production of acetic acid which in turn decreases the pH of the reaction mixture. Qualitative urinalyses were conducted at weeks 13, 26, 40, 53, 75 and 104. Detailed neurological examinations were conducted on all surviving animals at week 104. Marked or unusual variations from normal sensory or motor activities, reflexes, attitude or postural reactions, and eye structure appearances were noted. Electrocardiograms, heart rate and blood pressure values were obtained on each survivor at week 104. At scheduled necropsy, macroscopic examinations were performed and selected organs of all animals were weighed. Various tissues and organs were processed for microscopic examination. Brain samples were taken and brain cholinesterase activity was determined.

No statistical analysis for comparison between groups was performed in this study.

Results

Mortality: All animals survived the 104 weeks of the study in generally satisfactory condition except one male from the high dose group which was sacrificed at week 52 due to its poor condition. Findings in this animal prior to and at autopsy do not refer to test substance-related effects.

Clinical observations: The incidence pattern of clinical findings noted during the course of this study did not indicate dose-dependence with the exception of lacrimation. The body temperature values, respiration rates and heart rates were comparable to the control values throughout the study. Ophthalmological examinations did not show any findings which could be related to the test substance.

Mean body weight: During the 2 years of treatment with phosmet no significant influence on body weight was observed.

Food consumption: Not evaluated.

Plasma cholinesterase (Table 23): The plasma cholinesterase was marginally decreased in activity in one or two animals of the high dose group.

Red blood cell cholinesterase (Table 23): Animals receiving 10 mg/kg bw/day of the test substance showed a clear inhibition of the RBC cholinesterase during all the time of the study (61.9%).

Brain cholinesterase (Table 24): Brain cholinesterase determination showed inhibition in animals of the high dose group above the trigger value of 20 % (value of toxicological relevance). There was also a decrease of 15.23% at 1 mg/kg bw/day.

Haematology: Haematological values were within the normal ranges for all dogs that survived the study, except for isolated instances of elevated sedimentation rates in several dogs. Except for two isolated instances at week 78 of very high serum glutamic-oxalacetic and glutamic-pyruvic transaminases (one in the controls and one in the 0.5 mg/kg bw/day group), the values of the various tests were within normal ranges and any trends up or down were found in all groups.

Necropsy findings: No treatment-related effects were recorded in animals fed with phosmet at all dose levels. The changes seen in single organs were not related to test substance administration due to sporadic nature. Gross examination disclosed oedema of the entire gastrointestinal tract with evidence of haemorrhage in the stomach. In addition, the adrenals appeared enlarged, the spleen granular in texture and the pituitary gland haemorrhagic. The histopathological investigations of the animals at all dose levels did not show substance-related effects.

Organ weights: Remained within the normal range, except isolated instances of increased or decreased weights were noted in various groups but no indication of a trend with diet level of test substance was noted.

There were no relevant changes in urinalysis parameters. Neurological examinations did not reveal any differences compared to control. Blood pressure values, electrocardiograms and heart rates taken on the surviving dogs were in the normal ranges.

Table 23: Cholinesterase (ChE) activity variation in plasma and RBCs with respect to controls

Time Point	Dose Level [mg/kg bw/d]		
	0.5	**1**	**10**
[week]	**Plasma [% of controls]**		
Males/Females			
0	1.40	-4.20	-3.08
14	-0.14	-6.10	-13.05
26	5.72	-5.27	-8.73
40	-0.79	-11.30	-13.14
52	3.85	-2.88	-8.68
78	5.94	4.35	-2.78
104	24.83	11.91	-1.54
[week]	**RBC [% of controls]**		
Males/Females			
0	4.85	8.50	-2.43
14	-5.08	-4.16	-83.60
26	-1.41	2.35	-85.41
40	6.35	2.76	-81.22
52	9.21	-12.58	-61.17
78	-5.42	-3.16	-78.33
104	7.19	-3.39	-61.92

RBC red blood cells

Table 24: Cholinesterase (ChE) activity variaton in brain tissue with respect to controls

Time Point	Dose Level [mg/kg bw/d]		
	0.5	**1**	**10**
[week]	**Brain [Δ pH/h (% of controls)]**		
Males/Females			
105 or 106	0.37 (-9.05%)	0.34 (-15.23%)	0.17 (-57.53%)

Conclusion

The high dose level of 10 mg/kg bw/day was characterized by moderate to marked cholinesterase inhibition. Inhibition of brain ChE (15.23%) was observed at 1 mg/kg bw/day for males and females. The NOAEL was 0.5 mg/kg bw/day for males and females based on brain ChE inhibition at 1 mg/kg bw/day.

4.7.1.2 Repeated dose toxicity: inhalation

28-day inhalation toxicity

No sub-acute inhalation toxicity studies were conducted as phosmet is not regarded to be a volatile substance and oral toxicity studies are available.

90-day inhalation toxicity

No sub-chronic inhalation toxicity studies were conducted as phosmet is not regarded to be a volatile substance and oral toxicity studies are available.

4.7.1.3 Repeated dose toxicity: dermal

Percutaneous 21-day toxicity (rabbits)

Title	**21-day dermal toxicity study with Imidan Technical in rabbits.**
Author (s) (year):	Henwood, S.M. (1988)
Administration	21-day dermal application
Guideline	EPA guideline 82-2. Deviations of OECD 410 guideline: Purity was not reported Comments: A satellite group may have been treated with the high dose level for 3 weeks and observed for reversibility, persistence, or delayed occurrence of toxic effects for 14 days post-treatment.
Species	Hra:(NWZ) SPF Rabbits
GLP	Yes
Purity:	94.3%-95.2%
Groups	5 rabbits/sex/dose level
Dose levels	Rats: 0, 10, 100 and 1000 mg/kg bw/day
	Study acceptable

Executive Summary

In a 21-day dermal study, phosmet (Imidan Technical, purity: 94.3%-95.2%) was administered to male and female New Zealand White rabbits (5 animals/sex/dose level) at concentrations of 0, 10, 100 and 1000 mg/kg bw/day for 6 hours/day and 5 days/week using semi-occlusive dressing.

In a dose range-finding study, animals were assigned at random to 6 groups (1 animal/sex/group) that had 0, 10, 100, 250, 500 or 1000 mg/kg bw applied to the dorsal dermal surface. Animals were treated 5 days/week with a 6-hour exposure period per day for 15 days. There were no test material-related *ante mortem* observations or effects on body weights. Mild dermal irritation was seen at all dose levels tested. Lower plasma and red blood cell cholinesterase concentrations may have been test substance-related, but were not conclusive. The dose levels for the 21-day dermal toxicity study were selected based on these results.

The area of exposure was approximately 10 % of the total body surface area. The fur was clipped from the dorsal area of the trunk approximately 24 hours before study initiation and as needed thereafter. The appropriate amount of test substance was thoroughly mixed with 4 ml mineral oil just before application. Control animals received 4 ml mineral oil. The test substance was held in contact with the skin for an approximate 6-hour period with a gauze dressing secured with tape. At the end of the exposure period, the collars and overwrap dressing were removed and the exposure area was washed with tepid tap water.

All animals were checked at least twice daily for moribundity and mortality, and at least once daily for obvious clinical signs of toxicity. Dermal irritation was scored immediately before each application of the test or control material and on the day of necropsy. Individual body weights were recorded at study initiation, weekly thereafter, and at study termination. Food consumption was recorded weekly. At study termination blood was collected from the marginal ear vein of all surviving animals for haematological and clinical measurements. At necropsy, terminal body weight and selected organ weights were recorded. All animals were examined macroscopically and histopathologically.

Standard one-way analysis of variance (ANOVA) was used to analyse the following data for each sex: initial body weight, food consumption, clinical chemistry and hematology (except erythrocyte morphology), organ weight, organ-to-body weight percentages and organ-to-brain weight ratios.

Levene's test is done before ANOVA to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.001$, the following transformations are used to stabilize the variance: log10, square, square root, reciprocal, angular, or rank.

ANOVA was performed on the homogeneous or ranked data. If the ANOVA is significant, Dunnett's t-test (for one control) is used for pairwise comparison between groups. When no transformation establishes variance homogeneity at $p \leq 0.001$, the data are also examined by nonparametric techniques. These statistics include the Kruskal-Wallis H-test ANOVA and, if the test is significant, the Nemenyi-Kruskal-Wallis test for multiple comparisons or the Wilcoxon-Mann-Whitney tow-sample rank test. All group comparisons found to be statistically significant at the 5.0% two-tailed probability level are indicates with and asterisk (*).

Results

Mortality: One control male was found dead on day 4 and one control female was sacrificed in moribund condition on day 17. No mortality was recorded in the treated groups.

Clinical observations: In general, animals appeared healthy throughout the study. There were only a few clinical observations and they were considered incidental and not test substance-related. Treated rabbits at all dose levels exhibited dermal irritation during the study. However, the severity of irritation did not appear to be dose or sex-related.

Mean body weight: No test substance-related effects on body weights for treated rabbits.

Food consumption: There were not statistical differences between control and treated groups.

Haematology: Urea nitrogen was significantly higher in males treated with 100 mg/kg bw (25.4%) when compared to the corresponding control values. This elevated urea nitrogen concentration was considered to be within normal biological variation, not related to the test substance and not of toxicological significance as it occurred only at intermediate dose level and only in one sex.

Plasma cholinesterase (Table 25): Plasma cholinesterase was lower (decrease >20%) in males and females treated with 1000 mg/kg bw. The difference was only statistically significant for females at this dose level.

Red blood cell cholinesterase (Table 25): The RBC cholinesterase activity was also lower in males and females treated with 1000 mg/kg bw. Although the difference was only significant for males, inhibition was above the trigger value of 20% for both males (25%) and females (22%).

Table 25: Cholinesterase activity in plasma and RBCs with respect to controls on week 3

Parameter	Dose Level [mg/kg bw]			
	0	**10**	**100**	**1000**
Males				
Plasma AChE				
Mean value [mu/mL]	468	519	431	349
Standard deviation	64.2	34.1	95.8	68.1
No. animals	4	5	5	5
% change from control	-	+10.8%	-7.9%	-25.4%
RBC AChE				
Mean value [mu/mL]	1817	1788	1729	**1371***
Standard deviation	61.4	145.7	171.4	138.2
No. animals	4	5	5	5
% change from control	-	-1.6%	-4.8%	**-24.5%**
Females				
Plasma AChE				
Mean value [mu/mL]	488	465	415	**317***
Standard deviation	60.7	55.4	39.4	119.0
No. animals	4	5	5	5
% change from control	-	-4.7%	-15.0%	**-35.0%**
RBC AChE				
Mean value [mu/mL]	1731	1801	1595	1349
Standard deviation	133.0	271.4	206.5	249.5
No. animals	4	5	5	5
% change from control	-	+4.0%	-7.9%	-22.1%

* significantly different from control, $p < 0.05$.
RBC red blood cells
ChE cholinesterase activity

Organ weights (Table 26): Male rabbits treated with 10, 100 or 1000 mg/kg bw/day had a statistically significant lower absolute left and right kidney weights. Also male rabbits at 100 and 1000 mg/kg bw/day showed a statistically significant decrease in the right and left kidney-to-brain weight ratios. At 10 mg/kg bw/day only showed a statistically significant decrease in the right kidney-to-brain ratio. The lack of similar weight changes in females suggests this finding to be incidental.

Table 26: Statistically significant weight variations in males with respect to controls ($p<0.05$)

Dose (mg/kg bw/d.)	Left Kidney	Right Kidney	Left kidney to brain	Right kidney to brain
10	-16.40%	-19.08%	n.s.	-17.18%
100	-17.41%	-18.29%	-19.89%	-20.83%
1000	-18.46%	-20.59%	-18.78%	-20.82%

n.s. not statistically significant

Necropsy: Macroscopic and microscopic findings were few in number and there were no important differences between control and treated animals.

Conclusion

Although there were statistical increases in some haematological and chemistry parameters in females treated at 100 mg kg bw/day these results were not considered as biological relevance. With regard to lower kidney weight data, there were not remarkable macroscopic and microscopic findings associated.

Based on the inhibition of RBC-cholinesterase activity at 1000 mg/kg bw/day (>20%), the NOAEL was established at 100 mg/kg bw/day.

Percutaneous 21-day toxicity (rats)

Title	**A 21-day dermal toxicity study of Imidan in rats**
Author (s) (year):	Hilaski, R.J. (1999)
Administration	21-day dermal application
Guideline	EPA guideline 82-2. Deviations from OECD 410: No historical control data is available; nevertheless an expanded control study was initiated following the Imidan study to increase the control database for RBC, plasma and brain cholinesterase. A satellite group may have been treated with the high dose level for 3 weeks and observed for reversibility, persistence, or delayed occurrence of toxic effects for 14 days post-treatment. The purity of the test substance was 71.2%.
Species	Sprague-Dawley rats
GLP	Yes
Purity:	71.2%
Groups	10 rats/sex/dose level
Dose levels	Rats: 0, 15, 22.5 and 60 mg/kg bw/day
	Study acceptable

Executive Summary

In a 21-day dermal toxicity study, phosmet (Imidan 70-WSB, purity: 71.2% active) was administered to 3 groups of Sprague-Dawley rats (10 rats/sex/group). The dose levels were 15, 22.5 and 60 mg/kg bw/day. A vehicle control group (10 animals/sex) was administered deionised water only in an identical manner. Duration and frequency of treatment was 6 hours per day, 5 days a week for a total of 15 applications in 21 days. In addition, a second (expanded) control group of 20 animals/sex was run immediately after the first control group and under the same conditions (using the same equipment and sampling methods) with the objective of increasing the control database for cholinesterase inhibition since no historical control data were available.

The dose levels were selected by the Sponsor on the basis of available data from a range-finding study and other related previous studies. Test article suspensions in deionised water were made twice each week. During administration, the Imidan preparations were continuously stirred with a magnetic stirring bar to ensure homogeneity. Concentrations of the dosing mixtures were confirmed in an analytical trial.

The skin area of exposure was approximately 10% of the total body surface area. The fur was clipped from the dorsal area of the trunk approximately 24 hours before study initiation and as needed thereafter. The appropriate amount of test substance was thoroughly mixed with deionised water. Control animals received deionised water. The test substance was held in contact with the skin for an approximate 6-hour period with a gauze bandaging secured with non-irritating tape. At

the end of the exposure period, the dosing application, the gauze and the taper were removed and the site was rinsed with tepid water and gently dried with disposable paper towels.

All animals were checked at least twice daily for moribundity and mortality, and at least once daily for obvious clinical signs of toxicity. Dermal irritation was scored immediately before each application of the test or control material and on the day of necropsy. Individual body weights were recorded within 3 days of arrival, at least twice per week during acclimation, and at day 1, 8, 15 and 21. Food consumption was recorded once during acclimation and weekly during the study. Haematology and clinical chemistry analyses were conducted on all rats.

Cholinesterase (ChE) activity in plasma and erythrocytes (RBC) was measured in all animals on the day prior to study initiation, at day 8 and at the day of sacrifice. Blood samples for ChE activity evaluations were taken from the jugular vein in all animals except 6 females (2 each from doses of 15, 22.5 and 60 mg/kg bw/day) that required the samples to be collected via the orbital sinus. The animals were not fasted prior to blood collection for the ChE assay.

At study termination, all animals were euthanized by carbon dioxide inhalation. The animals were examined carefully for external abnormalities including palpable masses. The skin was reflected from a ventral midline incision and any subcutaneous masses were identified and correlated with *ante mortem* findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities and the organs removed, examined, and placed in fixative. All tissues were fixed in neutral buffered formalin. All animals of each dose group were subjected to a macroscopic pathologic examination.

Kidney, liver and brain were weighed. Weight ratios (relative-to-body weights) were calculated. Paired organs were weighed together. Kidney, liver, skin (normal and application site), brain and gross lesions from control and high dose group animals were processed and microscopical examination of stained paraffin sections was performed.

Brain cholinesterase activity was measured in all animals at terminal sacrifice. The brain was removed, weighed (whole organ), bisected, and the right half individually weighed and snap frozen as soon as possible after euthanasia to avoid possible decreases in cholinesterase activity. One half of the brain was used for the cholinesterase assay, while the other half was used for histopathological examination.

For each endpoint and time period, Levene's test was used to assess homogeneity of group variances. If the test was not significant ($p>0.01$), Dunnett's test was used to compare each treated group to the control group. If Levene's test was significant ($p<0.01$), comparisons with the control group were made using the Welch t-test with a Bonferroni correction. Results of all comparison were reported at the 0.05 and 0.01 significance levels.

Results

Mortality: All animals survived.

Clinical signs: Not relevant clinical signs observed. No dermal reactions were noted.

Mean body weight: There was no test substance-related effect on body weight gain for any sex or group. With a few exceptions at single observations, all animals gained weight at each interval throughout the study.

Food consumption: With the exception of a few individuals, food consumption remained fairly steady throughout the study.

Haematology: The only statistical significant parameter was reduction of hemoglobin in males at 15 mg/kg bw/day compared to control group but this alteration was not observed in the rest of levels.

Cholinesterase activity

The range of cholinesterase (ChE) values (Table 27 and Table 28): In order to expand the control data base, an expanded control study immediately following the 21 day dermal study with phosmet was conducted providing further control ChE data from animals of the same age and strain under the same test conditions.

For purposes of data evaluation ChE values from treated groups were compared to both the concurrent control and the expanded control group. Several of the values from the concurrent controls exceeded the upper limit of the expanded control data base.

Plasma cholinesterase: Significant decrease with respect to concurrent controls in females at day 22 at 60 mg/kg bw/day (37.50%) Compared to second expanded group controls there was a singnificant decrease (day 22) at 22.5 and 60 mg/kg bw/day in males (33.33% at both doses).

Red blood cell cholinesterase: Decrease of 21.43% at 60 mg/kg bw/day in females at day 22 with respect to concurrent controls.

Brain cholinesterase activity: Significant decrease with respect to concurrent controls at day 22 in males and females at 60 mg/kg bw/day (35.81% and 61.18% respectively). Compared to second expanded group controls, there was inhibition at day 22 in males and females at the high dose level (24.60 and 53.17% respectively). Brain ChE levels, significantly reduced at 22.5 mg/kg bw/day and 15 mg/kg bw/day (20.39% and 12.50%) with respect to concurrent controls in females but they were not considered being biologically relevant, since the values were not reduced when compared second expanded control group. Only in the 60 mg/kg bw/day groups (males and females) individual brain ChE activity values were consistently below the second expanded group control values.

Table 27: Cholinesterase (ChE) activity: comparison with concurrent control

Time Point		**Dose Level [mg/kg bw/day]**			
		0	**15**	**22.5**	**60**
Plasma [mM/L/min]					
Males					
Acclimation	Mean Value	0.2	0.2	0.2	0.2
	S.D.	0.12	0.11	0.07	0.07
	N	10	10	9	9
Day 8	Mean Value	0.2	0.2	0.2	0.2
	S.D.	0.05	0.08	0.05	0.06
	N	10	10	10	10
Day 22	Mean Value	0.2	0.3	0.2	0.2
	S.D.	0.09	0.08	0.06	0.07
	N	10	10	10	10
Females					
Acclimation	Mean Value	0.2	0.3	0.2	0.3
	S.D.	0.09	0.10	0.15	0.13
	N	10	10	9	10
Day 8	Mean Value	0.4	0.4	0.3	0.3
	S.D.	0.15	0.13	0.12	0.12
	N	10	10	9	7
Day 22	Mean Value	0.8	0.6	0.7	**0.5****
	S.D.	0.26	0.10	0.17	0.13
	N	10	10	10	10

RBC [mM/L/min]					
Males					
Acclimation	Mean Value	1.1	1.1	1.0	1.1
	S.D.	0.16	0.15	0.17	0.16
	N	10	10	9	9
Day 8	Mean Value	1.3	1.3	1.2	1.2
	S.D.	0.15	0.13	0.22	0.14
	N	10	10	10	10
Day 22	Mean Value	1.2	1.3	1.1	1.2
	S.D.	0.23	0.26	0.25	0.13
	N	10	10	10	10
Females					
Acclimation	Mean Value	1.2	1.2	1.2	1.2
	S.D.	0.25	0.24	0.18	0.17
	N	10	10	9	10
Day 8	Mean Value	1.4	1.2	1.2	**1.0***
	S.D.	0.16	0.25	0.30	0.21
	N	10	10	9	7
Day 22	Mean Value	1.4	1.3	1.2	1.1
	S.D.	0.29	0.21	0.22	0.22
	N	10	10	10	10
Right Brain [mM/g/min]					
Males					
Day 22	Mean Value	1.48	1.38	1.37	**0.95****
	S.D.	0.120	0.104	0.099	0.097
	N	10	10	10	10
Females					
Day 22	Mean Value	1.52	**1.33****	**1.21****	**0.59****
	S.D.	0.080	0.113	0.164	0.100
	N	10	10	10	10

* significantly different from control by the (Dunnett`s test or Welch t-test) criteria, p<0.05

** significantly different from control by the (Dunnett`s test or Welch t-test) criteria, p<0.01

RBC: red blood cells; S.D: Standard deviation; N: number of animals

Table 28: Cholinesterase (ChE) activity: comparison with second expanded group (day 22)

		Second expanded control group	**15 mg ai/kg bw/day**	**22.5 mg ai/kg bw/day**	**60 mg ai/kg bw/day**
Males					
Plasma Cholinesterase (mM/L/min)	Mean Value	0.3	0.3	**0.2****	**0.2****
	S.D.	0.06	0.08	0.06	0.07
	N	20	10	10	10
Red Blood Cell Cholinesterase (mM/L/min)	Mean Value	1.0	**1.3****	1.1	1.2
	S.D.	0.22	0.26	0.25	0.13
	N	20	10	10	10
Right Brain Cholinesterase (mM/g/min)	Mean Value	1.26	**1.38****	**1.37****	**0.95****
	S.D.	0.078	0.104	0.099	0.097
	N	20	10	10	10

		Second expanded control group	15 mg ai/kg bw/day	22.5 mg ai/kg bw/day	60 mg ai/kg bw/day
Females					
Plasma Cholinesterase (mM/L/min)	Mean Value	0.6	0.6	0.7	0.5
	S.D.	0.20	0.10	0.17	0.13
	N	20	10	10	10
Red Blood Cell Cholinesterase (mM/L/min)	Mean Value	1.1	1.3	1.2	1.1
	S.D.	0.25	0.21	0.22	0.22
	N	20	10	10	10
Right Brain Cholinesterase (mM/g/min)	Mean Value	1.26	1.33	1.21	**0.59****
	S.D.	0.079	0.113	0.164	0.100
	N	20	10	10	10

* Significantly different from control by the (Dunnett`s test or Welch t-test) criteria, $p<0.05$

** Significantly different from control by the (Dunnett`s test or Welch t-test) criteria, $p<0.01$

S.D: Standard deviation; N: number of animals

Necropsy and organ weights: Males treated at the highest dose level showed statistical increases in right half brain/brain weight (5.83%) and in the kidney/body weight data (12%). In females there were statistical increases in the weight of brain (8.19%) and the brain/right half weight (9.88%) and statistical decrease in liver/brain weight percentage (11.26%) were observed at the lowest dose level tested. Since only one brain weight parameter was increased in the male high dose group, the only brain weight parameters increased in females occurred in the low dose group, and the brains from animals in the high dose group of both sexes were histologically normal, the brain changes were considered spurious and not related to test article administration. Changes in kidney and liver were not considered test-article related. With regard to macroscopic and microscopic alterations distended uterus was observed at highest dose level tested.

Conclusion

It was observed that the main adverse effect was the inhibition of brain ChE-activity. This inhibition was clear at highest dose level. Nevertheless, at mid dose level an inhibition of 20.39% was observed in females when it was compared with concurrent control group. When it was compared with the second expanded control group, this inhibition at mid dose level was not observed; only a percentage of inhibition of 3.97% was recorded in females. Accordingly, the NOAEL was established on 22.5 mg/kg bw/day.

4.7.1.4 Repeated dose toxicity: other routes

No further repeated dose toxicity studies with Phosmet, performed on other routes, are available.

4.7.1.5 Human information

There are no human data available relevant for C&L.

4.7.1.6 Other relevant information

No other relevant information is available.

4.7.1.7 Summary and discussion of repeated dose toxicity

In the available dataset phosmet-related repeated dose toxicity was largely driven by acetylcholinesterase activity (brain, plasma, RBC) inhibition, which was the key effect for NOAEL setting.

In a dietary 28-day dose range-finding study, male and female mice (10 animals/sex/dose level) were treated with phosmet at dose levels of 0, 5, 15, 50, 150 or 500 ppm for 4 weeks equivalent to 0, 1.2, 3.8, 12, 25.7 and 62 mg/kg bw/day. A significant decrease in food consumption was observed for the 25.7 and 62 mg/kg bw/day in males and females when compared to controls. Mean body weight was significantly decreased in the 62 mg/kg bw/day males and females and in the 25.7 mg/kg bw/day males. The 62 mg/kg bw/day animals of both sexes were not as responsive to external stimuli as the controls. All other clinical observations were comparable among groups. Red blood cell (RBC) cholinesterase (ChE) activity was inhibited (>20%) in the 12, 25.7 and 62 mg/kg bw/day animals of both sexes. Brain ChE activity was inhibited in the 62 mg/kg bw/day females (15.7%). At necropsy, there was a decrease (>10%) in the absolute liver and kidney weights of the 25.7 mg/kg bw/day and 62 mg/kg bw/day males. However, in the 25.7 mg/kg bw/day females, absolute liver weight was greater than controls. The relative liver weights were increased in the 25.7 and 62 mg/kg bw/day males and females (>10% at the high dose) while the relative kidney weights were increased in the 62 mg/kg bw/day females (6.6%). The NOAEL was considered to be 3.8 mg/kg bw/day.

In a 28 day dose range-finder feeding study in dogs, phosmet was administered to males and females (3 dogs/sex/dose level) at dose levels of 0, 1.5, 3.0 and 6 mg/kg bw/day. The mean daily intakes of phosmet were 0, 1.6, 3.2 and 6.4 mg/kg bw/day for males and 0, 1.6, 3.4 and 6.4 mg/kg bw/day for females. No effects on body weights and food consumption and no clinical signs of toxicity were evident. In animals administered with 6 mg/kg bw/day, relevant reductions in mean plasma, RBC and brain ChE activities in males and females were noted on day 28. These reductions are considered to be biologically relevant (*i.e.* ChE inhibition above the trigger of 20 %). Based on relevant ChE inhibition in RBCs in males and females at 3 mg /kg bw/day (21.0 and 24.0%, respectively) the NOAEL of the study was deduced to be 1.5 mg/kg bw/day.

In a 90-day oral toxicity study male and female rats (15 animals/sex/dose level) were fed with phosmet at 0, 20, 100 or 500 ppm equivalent to 0, 2, 10 and 50 mg/kg bw/d for 14 weeks. Significant reduction of food consumption occurred in males of group 1 at 10 and 50 mg/kg bw/day on week 14 (30% and 13.5% respectively). Significant decreased body weight was observed at high dose level in group 1 (15.4% on week 14). There was also a decrease in the mean relative prostate weight (23.4% in group 1 and 10.6% in group 2) and mean absolute prostate weight (26.9%) in group 1. RBC ChE activity was decreased by >20 % at 10 and 50 mg/kg bw/day, while plasma ChE was inhibited at the high dose level only. Brain ChE was inhibited at terminal sacrifice in the 10 and 50 mg/kg bw/day dose groups above the trigger value of 20%. No evident test substance-related effect in haematology and clinical chemistry parameters (except ChE activity) was noted. There were no apparent findings at necropsy and histopathology. The NOAEL was considered to be 2 mg/kg bw/day.

In a 90-day oral toxicity groups of 4 male and 4 female Beagle dogs were treated with dietary concentrations of 0, 10, 75 or 563 ppm of phosmet equivalent to 0, 0.25, 1.88 and 14.1 mg/kg bw/day for 14 weeks. All of the animals gained body weight, except for one dog given the middle and one given the low dose. Haematological and clinical chemical parameters were not affected by the treatment, with the exception that inhibition of RBC ChE activity (99.65% in males/females) was observed at high dose. Brain ChE activity in all animals at terminal sacrifice was also depressed (>20%) at high dose. The levels of RBC and brain ChE were normal in the other dose groups. At autopsy, no pathological changes attributable to the test substance were noted. The NOAEL was 1.88 mg/kg bw/day.

In a 2-year feeding study, groups of 3 male and 3 female Beagle dogs received phosmet in the diet at concentrations of 0, 20, 40 or 400 ppm equivalent to 0, 0.5, 1 and 10 mg/kg bw/day. One male at the high dose group was killed *in extremis* at week 52 but no other death during the course of the study occurred. At termination, brain ChE activity was reduced at 1 mg/kg bw/day in both sexes (15%) and at 10 mg/kg bw/day RBC ChE activity decreased in both sexes (62%) and also brain ChE activity was depressed by 58 % in both sexes. No apparent test substance-related effects on body weight gain, urinalysis parameters, ophthalmoscopy parameters and organ weights for any group or sex were noted. No evident test substance related effect in haematology and clinical chemistry parameters (except ChE activity) was noted

In a 21 day dermal study, phosmet was administered to male and female rabbits at dose levels of 0, 10, 100 or 1000 mg/kg bw/day. In general terms, animals appeared healthy throughout the study. One male in the control group was found dead and one female in the control group was sacrificed because of health problems which did not appear to be treatment-related. There were no test substance-related dermal observations or effects on body weight, food consumption, organ weight or macroscopic and microscopic pathologic observations of treated animals when compared to controls. Of the clinical pathology variables tested, the only differences considered to be treatment-related were lower plasma and RBC ChE activities in rabbits treated at 1000 mg/kg bw/day (>20%). Based on the results of this study, the NOEL for phosmet in rabbits was 100 mg/kg bw/day.

In a 21 day dermal study phosmet was administered to male and female rats at dose levels of 0, 15, 22.5 or 60 mg/kg bw/day. In addition an expanded control study was initiated immediately following the phosmet study to increase the control data base for RBC, plasma and brain ChE activity since no historical control data were available using current instrumentation. Not relevant clinical signs were observed. No apparent test substance related effects on body weight gain, food consumption for any group or sex were noted. No evident test substance-related effect in haematology and clinical chemistry parameters (except ChE activity) was noted. Test substance-related changes in ChE activity were observed during the study. Statistically significant reductions in plasma (37.5%) and RBC ChE activity (21.43%) were found for 60 mg/kg bw/day females with respect to concurrent controls at day 22. Statistically significant reductions in brain ChE activity, when compared to both concurrent and expanded control groups, occurred in males and females receiving phosmet at 60 mg/kg bw/day. Males in the 60 mg/kg bw/day group had brain ChE activity reduced 35.81% and 24.60% when compared to concurrent and expanded control groups respectively. Females receiving phosmet at 60 mg/kg bw/day showed brain ChE reductions of 61.18% and 53.17% when compared to concurrent and expanded control groups, respectively. There were no apparent test substance-related changes in the macroscopic examinations, organ weights and histopathology endpoints in either sex. Overall evaluation of the RBC, plasma and brain ChE activity with the concurrent and the expanded control groups showed that 22.5 mg/kg bw/day was the NOAEL.

The dermal toxicity studies indicated a lower toxicity by the dermal compared to the oral route.

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

Rat is the species on which the oral cut-off values for repeated exposure according to Regulation 1272/2008 are based on. Haber´s rule is used to adjust the standard guidance values, which are for studies of 90-day duration, for studies of longer or shorter durations. Table 29 and Table 30 include the equivalent guidance value for category 1 and 2 considering the time duration of each study. For the time being, there is no agreed EU position on how to apply the guidance values for classification of tested species other than rats. The current practice of RAC is to apply the guidance values for rats to other species as well.

A substance is classified with STOT RE under CLP when it has produced or has been shown to have the potential to produce significant toxicity in humans or be harmful to human health following repeated exposure by the oral, dermal or inhalation routes. This can be on the basis of human data or evidence from studies in animals that cause such effects at or below given guidance values. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included under this classification.

Table 29 and Table 30 cover the main effects after repeated oral and dermal exposure observed from subacute, subchronic and chronic studies relevant classification after the comparison with the specific guidance levels for repeated dose toxicity (RDT).

Table 29: Main effects after oral exposure from subacute, subchronic and chronic studies of relevance for RDT classification and comparation with guidance levels

Species, route, duration and author	Dose levels (mg/kg bw/day)	Main effects for repeated exposure toxicity[a]	Resulting classification according to CLP (cut-off values)
MOUSE			
B6C3F1 mouse Oral (feeding) 28 days **(Jones, 1981)**	1.2, 3.8, 12.0, 25.7 and 62	**12.0 mg/kg bw/day:** ↓RBC ChE (M: 30.1% and F: 23.6%) on week 4. **25.7 mg/kg bw/day:** ↓RBC ChE (M: 68.9% and F: 41.9%) en week 4. **62 mg/kg bw/day:** ↓RBC ChE (M: 78% and F: 72.1%) and ↓brain ChE (F: 15.7%) on week 4. Clinical signs as emaciation, tremors and decreased activity in both sexes.	**STOT RE 1** (≤ 30 mg kg bw/day)
B6C3F1 mouse Oral (diet) 2 years **(Katz et al., 1984)**	M: 1, 4 and 14 F: 1.2, 5 and 18	**M: 4 mg/kg bw/day and F: 5 mg/kg bw/day [b]:** ↓brain ChE in M: 22% and F: 31% (month 12) and in F: 14% (month 24). **M: 14 mg/kg bw/day and F: 18 mg/kg bw/day:** ↓brain ChE in M: 31% and F: 34% (month 12) and in F: 22% (month 24). Increased incidence of convulsions (spontaneous, handle-induced for this strain of mice) in males and histopathological findings in liver observed in males (mild vacuolative degeneration).	**STOT RE 2** (≤ 12.5 and ≥ 1.25 mg kg bw/day
RAT			
Albino rat Oral (feeding) 90 days **(Johnston, 1962)[c]**	2, 10 and 50	**10 mg/kg bw/day:** ↓RBC ChE (M: 42% and F: 37%) on week 14 and ↓brain ChE (M: 42% and F: 39%) on week 14. **50 mg/kg bw/day:** one death and ↓RBC ChE (100% in both sexes) on week 13 and ↓brain ChE (M: 75 and F: 82%) on week 14.	**STOT RE 1** (≤ 10 mg kg bw/day)
Sprague Dawley rat Subchronic (13-weeks) neurotoxicity (feeding) **(Cappon, 1999)**	M: 1.5, 2.7, 9.4 F: 1.6, 3.1, 11	**M: 1.5 mg/kg bw/day and F: 1.6 mg/kg bw/day [d]:** ↓RBC ChE on week 13 (M: 19%) and on week 7 (F: 42%). ↓Brain ChE regions at termination on week 13 in females: bainsteam (21%), olfactory (36%) and hippocampus (**33%**). **M: 2.7 mg/kg bw/day and F: 3.1 mg/kg bw/day:** ↓RBC ChE (M: 27% and F: 54%) on week 13 and ↓brain ChE (M: 17% and F: 19%) on week 7. ↓Brain ChE olfactory (F: 27%) a termination on week13. **M: 9.4 mg/kg bw/day and F: 11 mg/kg bw/day:** ↓RBC ChE (M: 70% and F: 86%) on week 13 and ↓brain ChE (M: 43% and F: 68%) on week 7. Significant ↓brain ChE in all brain regions was observed in both sexes at termination on week 13.	**STOT RE 1** (≤ 10 mg kg bw/day)
Sprague Dawley rat Oral carcinogenicity 2 years (diet) **(Chang, 1991)**	M: 1.1, 1.8, 9.4, 23 F: 1.1, 2.1, 10.9, 27	**M: 1.8 mg/kg bw/day and F: 2.1 mg/kg bw/day**: ↓RBC ChE: M: 16% and F: 15% (month 24). **M: 9.4 mg/kg bw/day and F: 10.9 mg/kg bw/day**: ↓RBC ChE: M: 64% and F: 70% (month 24) and ↓brain ChE M: 20% and F: 27% (month 12) and M: 12% and F: 19% (month 24). **M: 23 mg/kg bw/day and F: 27 mg/kg bw/day:** ↓RBC ChE (M: 77% and F: 78%) and ↓brain ChE (M: 34 and F: 43%) in month 12 (time of termination).	**STOT RE 2** (≤ 12.5 and ≥ 1.25 mg kg bw/day)

Alpk APfSD rat Oral development study in rat (dosing from days 7-16 of gestation, 10 days of duration) (gavage) **(Hodge, 1991)**	5, 10 and 15	**15 mg/kg bw/day:** clinical symptoms indicative for an organophosphorous ester (shaking, piloerection and salivation).	**STOT RE 1** (≤ 90 mg kg bw/day)
CD rat Oral 2-generation study (diet) (70+ days) **(Meyer and Walberg, 1990)**	M: 0, 1, 4.2, 16.4 F: 0, 1.8, 7.3, 25.5	**M: 4.2 mg/kg bw/day and F: 7.3 mg/kg bw/day:** ↓RBC ChE: M: 37% and F: 48% for P0 and M: 48% and F: 59% for P1. Brain ChE not measured. **M: 16.4 mg/kg bw/day and F: 25.5 mg/kg bw/day:** Clinical signs indicative for an organophosphorous ester in both P0 and P1. ↓RBC ChE: M: 74% and 81% for of P0 and M: 85% and F: 80% for P1. Brain ChE not measured. Mild to moderate centrilobular hepatocellular vacuolisation in P1 males.	**STOT RE 1** (≤ 12.9 mg kg bw/day)
DOG			
Beagle dog Oral (feeding) 28 days **(Brown, 2003)**	1.5, 3 and 6	**3 mg/kg bw/day:** ↓RBC ChE (M: 21% and F: **24**%) at necropsy time. **6 mg/kg bw/day:** ↓RBC ChE (M: 71.4% and F: 77.6%) and ↓brain ChE (M: 41% and F: 55%) at necropsy time.	**STOT RE 1** (≤ 30 mg kg bw/day)
Beagle dog Oral (feeding) 90 days **(Johnston, 1962)**[c]	0.25, 1.88 and 14.1	**14.1 mg/kg bw/day:** ↓RBC ChE (99.65%) for males/females on week 13 and ↓brain ChE (M: 92.3% and F: 98.1%) on week 14.	**STOT RE 2** (≤ 100 and ≥ 10 mg kg bw/day)
Beagle dog Oral (feeding) 2 years **(Johnston, 1966)**[c]	0,5, 1 and 10	**1 mg/kg bw/day:** ↓Brain ChE (15.23%) in males/females on week 105/106. **10 mg/kg bw/day:** ↓RBC ChE (61.92%) on week 104 and ↓brain ChE on week 105-106 (57.53%) in males/females.	**STOT RE 1** (≤ 1.25 mg kg bw/day)
RABBIT			
New Zealand Rabbit Oral range finding development study (dosing from days 7-19 of gestation, 13 days of duration) (gavage) **(Pinto, 1991)**	5, 10 and 15	**15 mg/kg bw/day**: mortality and clinical signs in two animals (including the one found dead on day 13) showed clinical signs of an organophosphorous compound (shaking, constricted/dilated pupils and salivation in both animals and high stepping gait in the one that survived) shortly after dosing, one on the day of its death (day 13), the other one during days 9 to 18 of gestation.	**STOT RE 1** (≤ 69.2 mg kg bw/day)
New Zealand Rabbit Oral development study (dosing from days 7-19 of gestation, 13 days of duration) (gavage) **(Moxon, 1991)**	2, 15 and 15	**15 mg/kg bw/day**: two deaths than can be treatment related and clinical signs (unsteady gait, salivation, increased breathing rate and shaking).	**STOT RE 1** (≤ 69.2 mg kg bw/day)

[a] In studies with statistical data effects were statistically significant except for those cholinesterase inhibitions put in bold.
[b] Experts in PRAPeR 86 agreed that inhibition at the low and mid doses, only observed at the interim kill not dose related and not associated with convulsions, was not an adverse effect. The MSCA in Classification&Labelling agrees with this statement. However, a dose dependent statistically significant ↓brain AChE was observed in month 24 in females (14%) at 5 mg/kg bw/day and it is considered relevant.
[c] Statistical data not available for cholinesterase inhibitions.
[d] EFSA did not take into account these cholinesterase reductions at 25 ppm (M: 1.5 mg/kg bw/day and F: 1.6 mg/kg bw/day) during the peer review of phosmet and established a NOAEL of 25 ppm. However, the Spanish Competent Authority in Classification&Labelling regards that AChE inhibition cannot be ruled out at this dose level. Therefore, this effect is considered relevant for repeated dose toxicity (RDT) classification.

Table 30: Main effects after dermal exposure from subchronic studies and comparation with guidance levels for RDT classification in different species

Species, route, and author	Dose levels (mg/kg bw/day)	Main effects for repeated exposure toxicity[a]	Resulting classification according to CLP (cut-off values)
RAT			
Sprague-Dawley Rat Dermal 21 days **(Hilaski, 1999)**	15, 22.5 and 60	**60 mg/kg bw/day**: ↓brain ChE in M: 35.81% and F: 61.18% with respect to controls and in M: 24.60% and F: 53.17% with respect to second expanded group.	**STOT RE 1** (≤ 85.7 mg kg bw/day)
RABBIT			
New Zealand Rabbit Dermal 21 days **(Henwood, 1988)**	10, 100 and 1000	**1000 mg/kg bw/day**: ↓RBC ChE (M: 24.5% and F: **22.1**%) on week 3.	No classification (≥ 857.1 mg kg bw/day)

[a] In studies with statistical data effects were statistically significant except for those cholinesterase inhibitions put in bold.

The main sensitive adverse effect after oral and dermal repeated exposure of phosmet was brain and erythrocyte AChE inhibition. A statistically significant inhibition of brain, peripheral nerve or erythrocyte AChE ≥ 20% with respect to the concurrent control group or with respect to the 'pre-exposure' values in the treated group is considered toxicologically relevant ('adverse'). Even statistically significant inhibition of less than 20% or statistically insignificant inhibitions above 20% indicates that a more detailed analysis of the data should be undertaken (JMPR, 1998; US EPA, 2000; Nielsen et al., 2008; EFSA, 2013). Inhibition of brain and erythrocyte AChE by 20% or more was obtained from subacute, subchronic and chronic studies after oral and dermal phosmet exposure as shown in Table 29 and Table 30. Besides this neurochemical effect, some clinical signs typical of an organophosphorous compound were also observed in some studies after oral exposure Cholinesterase inhibition and some clinical signs occurred below cut-off values for classification as STOT RE 1 as it can be observed in the previous tables. Furthermore, in a randomised double blind study for effects in plasma and RBC AChE in humans (Cameron, 1999) inhibition of RBC AChE was observed after single oral dose at dose levels from 1 mg/kg bw.

Phosmet did not result in pathology or histhopathology of the nervous system. However, according to CLP regulation (chapter 3.9.1.1), classification after repeated dose exposure covers all significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included. In MSCA opinion, critical effects of significant inhibition of brain and erythrocite cholinesterase and clinical signs associated with neurotoxicity are considered adverse and toxicollogicaly relevant effects. The central question is whether these adverse effects observed after repeated dose exposure in several species are covered by acute toxicity. Data suggest that the doses which elicited these functional adverse effects in oral and dermal acute and repeated toxicity testings (Table 31 and Table 32) are considered to be sufficiently different to justify an additional classification for repeated dose toxicity. Similar adverse effects after repeated exposure are observed at lower doses than for single exposure. Therefore, classification for STOT RE 1 is required for phosmet.

Table 31: Main effects in oral acute toxicity studies

Species, route and author	Dose levels (mg/kg bw)	Main effects[a]
MOUSE		
Male S/W mice Acute oral (gavage) **(Meyding, 1966)**	10, 21.5, 46.4 and 100	Mortality from **46.4 mg/kg bw** **LD_{50} (male): 50.1 mg/kg bw**
RAT		
Male S/D rat Acute oral (gavage) **(Meyding, 1966)**	100, 200, 300, 400 and 500	Mortality from **200 mg/kg bw** **LD_{50} (male): 245 mg/kg bw**
Sprague Dawley rat Acute oral (gavage) **(Mccabe, 1978)**	60, 75, 100, 115, 130, 150, 170 and 175	From **60 mg/kg bw** onwards: Depression, tremors, salivation, exophtalmus, chromodacryorrhea, dyspnea and stains around the ano-genital region. From **75 mg/kg bw** onwards there was mortality. **LD_{50}: 113 mg/kg bw(m-f)**
Wistar rat Acute oral (gavage) **(Navarro Aragay, 1998)**	70, 100, 140, 200, 280, 400, 560	Postration and chromodacryorrhea were observed after administration with a dose-related intensity. From **100 mg/kg bw** onwards mortality occurred. **LD_{50}: 230 mg/kg bw(both sexes)**
Sprague Dawley rat Oral range finding acute neurotoxicity study (gavage) **(Cappon, 1998a)[b]**	1.5, 3, 6, 9 and 36	**36 mg/kg bw:** related clinical signs as whole body tremors, gait alterations and salivation. ↓RBC ChE of 42%-94% for males and 53%-94% for females from two hours after dose administration onwards. ↓Brain ChE 48 h after treatment of 28% for males and 38% for females. **9 mg/kg bw:** ↓RBC ChE of 20%-43% for males and 9%-48% for females from two hours after dose administration onwards **6 mg/kg bw:** ↓RBC ChE of 13%-45% for males and 14%-41% for females from two hours after dose administration onwards
Sprague Dawley rat Oral acute neurotoxicity study (gavage) **(Cappon, 1998b)**	3, 4.5 and 22.5	**22.5 mg/kg bw:** ↓RBC ChE (75%-88%) three hours post-dosing. These reductions persisted in females on day 7 (**25%)** and 15 (40%). ↓Brain ChE (61%-70%) three hours post dosing that persisted on day 7(15%-20%) and on day 15 (9%-17%).
HEN		
White Leghorn Hens Oral acute delayed neurotoxicity (diet) **(Sprague, 1982)[b]**	Acute oral tests: 1130-2250	Severe motor incoordination and diarrhoea increasing dose levels. **LD_{50}: 2020 mg/kg bw[c]**
	ChE inhibiton tests: 4.2, 16.4, 65.6, 131, 525, 1050 and 2050	From **65.6 mg/kg bw:** significant inhibition of brain ChE (≥20%).
	Acute delayed neurotoxicity test: 20, 200, 2050	At **200 mg/kg bw** signs of diarrhea, feather loss and noticeably non-vocal 1-2 days after each treatment and transient motor incoordination for up to 4 days after treatment. At **2050 mg/kg bw** these effects occurred with greater severity and in some cases hens were not able to stand in their cages. Behavioural depression was evident in most hens up to 6 days after treatment and these hens were described as "listless"
Lohman Brown Hens Oral acute delayed neurotoxicity (gavage) **(Johnson, 1997)[b]**	Acute oral tests: 0, 323, 420, 546, 710, 923 and 1200	**323-1200 mg/kg bw**: unsteadiness, subdued behaviour, recumbency and salivation during the first 4 days. **LD_{50}: 577 mg/kg bw**
	Acute delayed neurotoxicity tests: 600	**600 mg/kg bw:** unsteadiness, recumbency, subdued behaviour and weakness for 4 days after administration of phosmet (atropine was administered prior dosing and even further dosing depending on observed symptoms). ↓Brain ChE (63%) after 48 h.

HUMAN		
Human Randomised double blind study for effects in plasma and RBC AChE (diet) **(Cameron, 1999)**[a]	Males: 1, 2 and 4 mg/kg bw Females: 2 mg/kg bw	P-values <0.05 using error variance from ANOVA Student's t-distribution were observed in RBC AChE of males at 1 mg/kg bw dose level (12 and 24 h), 2 mg/kg bw (8 h) and 4 mg/kg bw (1 h) and in plasma ChE of males at 2 mg/kg bw (8 h). The test for a linear trend with dose was not found to be significant at any of the time points. Only RBC AChE significant pairwise comparisons after Bonferroni adjustment were observed for males at 1 mg/kg bw/day at 24 h and for 2 mg/kg bw/day at 8 h. These decreases observed in males were considered incidental and therefore not relevant.

[a] In studies with statistical data effects were statistically significant except for those cholinesterase inhibitions put in bold.
[b] Statistical data not available for cholinesterase inhibition.
[c] Pre-treatment with atropine and 2-PAM reduced toxicity. Besides, half of the hens at the high dose level were treated with atropine and 2-PAM after dosing.

Table 32: Main effects in dermal acute toxicity studies

Species, route, duration and author	Dose levels (mg/kg bw)	Main effects
RAT		
Wistar rat Acute dermal **(Dos Santos, 1998)**	1000	No effects.
RABBIT		
New Zealand rabbit Acute dermal **(Mccabe, 1978)**	5000	**5000 mg/kg bw:** one female died on day 5. Mild depression and salivation with jaws clamped around caging were observed in the dead female

The EFSA Panel on Plant Protection Products and their Residues (PPR) provided a scientific Opinion (EFSA, 2013) on the identificaction of pesticides to be included in the cumulative assessment groups (CAGs) based on their toxicological profile from the basis of datasets of oral toxicity studies evaluated in the Draft Assessment Reports (DARs) for pesticides having effects on thyroid or nervous system. It was decided that data collection needed to be re-evaluated with the aim of identifying adverse effects of pesticide active substances, among others, on the nervous system. The PPR panel regarded re-evaluated data provided from an external scientific report by the Danish Technical University (DTU) published by the EFSA in 2012 and a further revision of the DTU data published in 2013 (ANSES/ICPS/RIVM). The PPR panel recognized inhibition of brain and erythrocyte cholinesterase as a neurochemical effect which represents a level of grouping for neurotoxic substances based on mechanism of action rather that on phenomenological effects. Accordingly, phosmet was grouped in the acute and chronic CAGs for the nervous system based on neurochemical endpoints (inhibition of brain AChE). It has to be noted that a chronic LOAEL of 1 mg/kg bw/day for phosmet is established in this document (EFSA, 2013) based on brain AChE inhibition.

Besides, recently an EFSA publication (EFSA, 2013) has suggested the association between environmental exposures to organophosphate pesticides such as phosmet and neurodevelopmental and neurobehavioural effects in humans.

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

Adverse effects such as brain and erythrocite cholinesterase inhibition and some clinical signs associated with neurotoxicity were observed after oral and dermal repeated dose exposure with phosmet. These findings were seen below the cut-off values for classification as STOT RE 1 and at lower dosages than for single exposure. Therefore, classification as STOT RE 1 is warranted for phosmet.

CLP: STOT RE 1 (H372): Causes damage to nervous system through prolonged or repeated exposure.

4.8 Germ cell mutagenicity (Mutagenicity)

Table 33: Summary of phosmet genotoxicity *in vitro* studies

TEST	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE/ ACCEPTANCE
Bacterial plate incorporation mutation assay GLP: No Purity of 95.7%	*S. typhimurium* TA98, TA100, TA1535, TA1537 S9 from livers of rats and mice induced with Aroclor 1254.	1st and 2 nd exp: 156, 313, 625, 1250 and 2500 µg/plate in all strains (±S9).	Positive in TA100 (±S9).	Toxicity was not observed due to Phosmet approached the limit of solubility.	Majeska, J.B., 1986 YES
Bacterial plate incorporation mutation assay GLP: No Purity of 99%	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and *E.coli* WP2 hcr S9 from livers of rats induced with Aroclor 1254.	10, 50, 100, 500, 1000 and 5000 µg/plate (±S9)	Positive dose related increases in TA100 (±S9). E.coli WP2 hcr strain gave a dose related increase in the number of revertants with S9 but not significantly	Toxicity from 1000 µg/plate in TA1535 and TA1537 (±S9). Results were not confirmed in a second experiment.	Shirasu, Y., et al., 1979 YES
Bacterial plate incorporation mutation assay. GLP: No Purity of the test substance was not given	Strains of *S. typhimurium* and *Escherichia coli* WP2	Without S9	Positive in TA100 without S9. Mutagenic potency of phosmet: 0.34 revertants/nmole	Study considered as additional information due to lack of information	Shirasu, Y., et al., 1984 With reserves
Bacterial plate incorporation mutation assay GLP:No Purity of 99.63%	*S. typhimurium* TA97, TA98, TA100, TA1535 and TA1538 S9 from livers of male rats induced with Delor 103	10, 100, 500 and 1000 µg/plate in all strains (±S9)	Positive in TA100 and TA97 (±S9)	Toxicity was not observed in any strain. The results were not confirmed in an independent experiment.	Vlcková et al., 1993 YES
In vitro mammalian gene mutation assay GLP:No Purity of 95.7%	L5178Y mouse lymphoma cells S9 from livers of rats induced with Arochlor 1254.	Without S9: 1st exp : 20, 40, 60, 80 and 100 µg/ml. 2nd exp.: 40, 60, 70, 80 and 100 µg/ml. With S9: 1st exp.: 4, 4.5, 5, 6 and 8 µg/ml 2nd exp. 8, 10, 15, 20 and 40 µg/ml	Positive without S9 only at highest concentrations. Negative with S9	Toxicity at the two highest dose level without S9. No toxicity in the1st exp.with S9. Toxicity from dose level of 10 µg/ml in the 2nd exp with S9.	Hertzel, K.M., 1986 YES
Saccharomyces cerevisiae, gene mutation assay GLP:No Purity of 99.63%	D7 strain	0.5, 1, 2, and 2.5%	Positive.	Survival dose-related decreased Study considered not acceptable due to lack of information	Vlcková et al., 1993 NO

TEST	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE/ ACCEPTANCE
Saccharomyces cerevisiae, mitotic recombination assay GLP:No Purity of 99.63%	D7 strain	0.5, 1, 2, and 2.5%	Positive	Survival dose-related decreased Study considered not acceptable due to lack of information	Vlcková et al., 1993 NO
In vitro mammalian chromosome aberration assay GLP:No Purity of 95.7%	L5178Y mouse lymphoma cells S9 from livers of rats induced with Arochlor 1254	Without S9: 40, 50, 60, 80 and 100 µg/ml. With S9: 8, 10, 15, 20 and 40 µg/ml	Negative with and without S9	50 methaphases were meausured intead of 200 per concentration. With regard to mitotic index, the highest concentration suppressed the mitotic activity by approximately 50 per cent with S9 but not without S9. In the non-activated assay significant increase in the number of chromosomal aberrant cells was observed at 100 µg/ml only in one culture.	Snyder R.D, 1986a YES
In vitro mammalian chromosome aberration assay. GLP:No Purity of the test substance was not given	Culture of lymphocytes of human peripheral blood	Without S9: 0.02, 2 and 20 µg/ml.	Positive at all dose level tested	Study considered not acceptable due to lack of information	Kurinnyi A.I., Pilinskaya M.A., 1977 NO
Rec assay with *Bacillus subtilis* GLP:No Purity of 99%	H17 Rec+ and M45 Rec-	20, 100, 200, 500, 1000 and 2000 µg/plate	Negative		Shirasu, Y. et al., 1979 With reserves
In vitro Sister Chromatid Exchange Assay in Mammalian Cells GLP:No Purity of 95.7%	L5178Y mouse lymphoma cells S9 from livers of rats induced with Arochlor 1254	Without S9: 40, 50, 60, 80 and 100 µg/ml. With S9: 8, 10, 15, 20 and 40 µg/ml	Questionable increase at all dose levels tested with and without S9, but there is not doubling of SCE frequency and this effect was not confirmed by an independent experiment	Toxicity observed at the highest dose levels (±S9) Results were not confirmed in a second independent study	Snyder R.D, 1986a YES
In vitro DNA breaks study GLP: No Purity of 95.7%	Diploid human fibroblast cells	Ranged from 1 to 0.25mg/ml (±S9)	Negative		Snyder, R.D., 1986b With reserves

Table 34: Summary of Phosmet genotoxicity *in vivo* studies in somatic cells

TEST	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE/ ACCEPTANCE
In vivo mammalian micronucleus test GLP:Yes Purity of 95.5%	Bone marrow cells from male and female CD®-1 mice.	Single dose level of 17 mg/kg (LD50) by oral gavage. Bone marrow samples at 24, 48 and 72h.	Negative	One dose level instead of three was tested. 1000 PCE intead of 2000 PCE were scored. Very slight cell toxicity at the dose level tested.	Gibbs J.A., 1986 YES
In vivo mammalian micronucleus test GLP:No The material was Imidan 500 PM with a content of phosmet of 499 g/kg.	Bone marrow cells from Swiss male mice	Dose level of 13.86 mg/kg/day (80% of LD_{50}), two consecutive days, by intraperitonial route. Bone marrow samples at 48 hours after the first application.	Negative.	The ratio PCE/NCE in treated group was slightly greater than in negative control group. No statically significant increase in the frequency of MNPCE was recorded, even.	Pestana C.B., 1999 YES
In vivo mammalian chromosome aberration assay. GLP:No Purity of the test substance was not indicated	Bone marrow cells from white non-linear male mice.	Single dose levels of 5, 10, 20 and 50 mg/kg, by gavage. Bone marrow samples at 20 hours	Positive result at 20 mg/kg.	Study considered not acceptable due to lack of information	Kurinnyi A.I., 1975 NO
In vivo UDS assay GLP:Yes Purity of 96.4%	Hepatocytes from male Alderley Park (Alpk AP_fSD) rat.	Single dose levels of 50 and 32 mg/kg, by gavage. Liver samples at 2 and 16h after treatment.	Negative	60 cells per animal instead of 100 were scored but 5 animals intead of 3 were used in the treatment groups. No toxicity was observed (individual data were not shown)	Mackay, J.M., 1996 YES
In vivo UDS assay GLP:Yes Purity of 96.1%	Hepatocytes from male Sprague-Dawley rat.	Single dose levels of: 58 and 180 mg/kgbw (2 hour time point). 58 and 108 mg/kgbw (14 hour time point). By gavage.	Negative	Toxicological signs at all groups at 2 h, and at 14h at 108 and 180 mg/kg bw	Proudlock R.J., 1998 YES
In vivo DNA alkylation assay GLP:No Purity of 99%	Liver and kidney from AB Jena/Halle male mice	Single dose level of 20 mg/kg, by intraperitoneal injection. Liver and kidney samples at 6 and 24h	Negative	No toxicological signs were reported.	Dedek W. et al., 1984 With reservations

4.8.1 Non-human information

The genotoxicity of phosmet has been investigated in a battery of *in vitro* and *in vivo* assays, including gene mutation, chromosomal aberration and DNA damage as endpoints. It should be noted that phosmet reaches bone marrow as well as the liver and kidney according to toxicokinetics data. Phosmet genotoxicity *in vitro* and *in vivo* studies are summarised in Table 33 and Table 34 respectively.

4.8.1.1 *In vitro* data

Five *in vitro* studies were available for evaluating the genotoxicity of phosmet: two studies on bacterial mutagenicity, a gene mutation study in mammalian cells study, one assay on chromosome aberrations and one *in vitro* DNA breaks study.

Another three studies, published as scientific literature (one on bacterial mutagenicity, one mutation assay in bacteria and *Saccharomyces cerevisiae*, and one assay on chromosome aberrations) were included for assessment purposes.

***In vitro* genotoxicity testing - Bacterial assay for gene mutation**

Title	**Imidan, mutagenicity evaluation in *Salmonella typhimurium***
Author (s) (year):	Majeska, J.B. (1986)
Guideline	Materials and methods used in this study complied with requirements of US EPA guidelines and OECD guideline 471 adopted 26 May 1983.
System	*S. typhimurium* TA98, TA100, TA1535, TA1537 S9 from livers of rats or mice induced with Aroclor 1254.
GLP	Yes
Purity:	95.7%
Dose levels	156, 313, 625, 1250 and 2500 µg /plate in all strains (±S9).
	Study acceptable

Executive Summary

Imidan was evaluated for its ability to induce mutations in the histidine operon of *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537. Dimethylsulphoxide (DMSO) was used to solve the test substance. One preliminary assay was performed (plate incorporation assay). 20, 39, 78, 156, 313, 625, 1250, 2500, 5000 and 10000 µg/plate were evaluated in triplicate without and with S9 activation (S9 from rat liver; 20 or 50 µL) in *S. thyphimurium* strain TA 100. In main assay, 156, 313, 625, 1250 and 2500 µg/plate were evaluated (plate incorporation assay) in triplicate in the presence and absence of S9 activation (from rat and mouse liver; 20 or 50 µL). All test strains were used.

Concurrent negative/solvent and positive controls were included. At doses higher than or equal to 625 µg/plate, the test substance precipitated upon addition to the top agar. This appeared in the pre-experiment with TA 100 at doses equal to 10 mg/plate as visible chemical residue on the plates with and without S9 mix. No relevant increase in the number of histidine (his+) revertants was observed in the bacterial strains TA 98, TA 1535 and TA 1537 tested either with or without activation by S9 mix. However, in two independent experiments slight but dose-related increases in the number of revertants were observed in strain TA 100 (factors 2.0 to 4.1). The increases were observed with and without metabolic activation.

Conclusion

Imidan (phosmet) was mutagenic in *Salmonella typhimurium* strain TA100, directly or in the presence of Araclor 1254 induced rat or mouse liver S9 metabolic activation system.

Title	**Mutagenicity evaluation of Japanese-made Phosmet in microbial assay**
Author (s) (year):	Shirasu, Y. *et al*. (1979)
Guideline	The guideline did not exist when the study was performed. Materials and methods used in this study complied with requirements of US EPA guidelines and OECD guideline 471 adopted 26 May 1983.
System	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and *E.coli* WP2 hcr S9 from livers of rats induced with Aroclor 1254.
GLP	No
Purity:	99%
Dose levels	10, 50, 100, 500, 1000 and 5000 μg/plate (±S9)
	Study acceptable

Executive Summary

Phosmet was tested for mutagenicity in the reverse mutation assay in bacteria with and without metabolic activation (S9 mix from male Sprague-Dawley rats treated with Aroclor 1254). The following *Salmonella typhimurium* strains were used in this assay: TA 1535, TA 1537, TA 1538, TA 98 and TA 100. In addition, the *Escherichia coli* strain WP2 hcr was tested. The testing was performed according to the standard plate assay.

The test was performed at doses ranging from 10 to 5000 μg/plate with and without metabolic activation. Dimethylsulphoxide (DMSO) was used to solve the test substance. Concurrent negative/solvent controls and positive controls were included and demonstrated the sensitivity of the test system.

No relevant increase in the number of histidine (his^+) or thryptophan (trp^+) revertants was observed in the bacterial strains TA 98, TA 1537, TA 1535 and TA 1538 tested with or without metabolic activation by S9 mix. However, dose-related increases in the number of revertants were observed in strain TA 100. The increases were observed either with or without activation by S9 mix.

WP2 hcr strain gave a not significant dose-related increase in the number of revertants in the presence of S9 mix.

Conclusion

Imidan (phosmet) was mutagenic for tester strain TA100, directly and in the presence of an Aroclor 1254 induced rat liver S9 metabolic activation system.

Further studies on genotoxicity in bacterial

The bacterial mutagenicity assays reported in the two scientific publications (Shirasu et al, 1984; Vlcková et al, 1993) were non-GLP compliant and were not conducted under any valid testing guideline.

Shirasu et al., (1984), reported a positive result for phosmet (no information on batch or purity is given) in strain TA 100 (0.34 revertants/mol) without S9 mix when testing 228 pesticides by means of screening mutagenicity studies (Ames test). Metabolic activation system was not used. This study is considered only acceptable as additional information due to lack of data reported.

Vlcková et al. (1993) test was carried out at doses of 10, 100, 500 and 1000 µg/plate. Metabolic activation was provided by adding Delor 103 induced male rat liver microsomal enzymes supplemented with cofactors. Positive results for phosmet (purity: 99.63%) were reported in TA 100 and TA 97 with and without S9 mix. This effect was greater in TA100 than in TA97 and was concentration-dependent. No increase in the number of revertants was found in *S. typhimurium* strains TA 1535, TA 1538 and TA 98. These results indicate that phosmet induces both frameshift (TA97) and base substitutions mutations (TA 100). This study was considered acceptable.

***In vitro* gene mutation in mammalian cells**

Title	**Mutagenicity evaluation in mouse lymphoma multiple endpoint test forward mutation assay**
Author (s) (year):	Hertzel, K.M. (1986)
Guideline	Materials and methods used in this study complied with requirements of US EPA guidelines and OECD guideline 476 adopted 21 July 1997
System	L5178Y mouse lymphoma cells S9 from livers of rats induced with Aroclor 1254
GLP	Yes
Purity:	95.7 %
Dose levels	Without S9: 1st exp : 20, 40, 60, 80 and 100 µg /ml. 2nd exp.: 40, 60, 70, 80 and 100 µg /ml. With S9: 1st exp.: 4, 4.5, 5, 6 and 8 µg /ml 2nd exp. 8, 10, 15, 20 and 40 µg /ml
	Study acceptable

Executive Summary

Phosmet (Imidan, EHC-0829-03, T12820) was examined for genetic activity in the L5178Y TK+/- mouse lymphoma multiple endpoint test in the absence and presence of metabolic activation. DMSO (dimethylsulfoxide) was used as solvent. Point mutation is measured in this test system by examining the induction of trifluorothymidine (TFT) resistance by forward gene mutation at the thymidine kinase (TK) locus. The test was performed with and without metabolic activation (S9 mix from the liver of Aroclor 1254 induced male Sprague-Dawley rats). Two range-finding tests and two independent mutagenicity experiments were carried out.

Culture medium was used as negative control while ethylmethanesulphonate (EMS; 0.50 µL/mL) was used as a positive control without metabolic activation and N-nitrosodimethylamine (DMN; 0.02 mg/ml) with metabolic activation.

In the first non-activated assay, the dose levels tested were 20, 40, 60, 80 and 100 µg/ml. The relative population growth ranged from 84 to 16%. A dose-related increase in the average mutant frequency was observed. It was significantly greater than negative control at 80 and 100 µg/ml although at these dose levels there was toxicity (21 and 16% population growth respectively).

Accordingly, the second non-activation assay Imidan was tested at dose levels of 40, 60, 70, 80 and 100 µg/ml. The relative population growth ranged from 39 to 3%. A dose-related increase in the average mutant frequency was observed. It was significantly greater than negative control at 70, 80

and 100 µg/ml although at these doses level there was toxicity (16, 9 and 3% population growth respectively). Positive control gave a satisfactory response.

In the first activated assay, Imidan was tested at dose levels of 4, 4.5, 5, 6 and 8 µg/ml. The relative population growth ranged from 109 to 50%. Accordingly, Imidan was tested in a second activated assay. The dose levels selected to determine mutagenicity were 8, 10, 15, 20 and 40 µg/ml. The relative population growth ranged from 24 to 8%. This toxicity was greater compared with the second range finding toxicity study. No increase in mutant frequency was found in both experiments with metabolic activation.

The positive controls demonstrated in both assays sensibility of the test system.

Conclusion

In the absence of metabolic activation in the mouse lymphoma multiple endpoint test phosmet was mutagenic with respect to gene mutation *in vitro* under the experimental conditions chosen, but only at highly cytotoxic concentrations (relative suspension growth 3 to 20 %). No mutagenic effect was noted in the presence of metabolic activation.

Gene mutation in yeast cells

The scientific publication (Vlcková et al, 1993) also includes the genotoxic potencial of phosmet in the *Saccharomyces cerevisiae* D7 strain (gene mutation and mitotic recombination). The doses tested were 0.5, 1, 2 and 2.5%. The number of revertants at the isoleucines *locus* was dose-related increased and statistically significant from dose level of 1% (7-fold increased at the highest dose level). Mitotic crossing-over at the adenine locus was found at the two highest dose levels, but it was only significant at the highest concentration. The number of convertants at the tryptophan *locus* per 10^5 surviviors was slightly, but not significantly increased.

Chromosome aberration *in vitro*

Title	**Mutagenicity evaluation in mouse lymphoma multiple endpoint test cytogenetic assay**
Author (s) (year):	Snyder, R.D. (1986a)
Guideline	Materials and methods used in this study complied with requirements of US EPA guidelines and OECD guideline 473 adopted 21 July 1997.
System	L5178Y mouse lymphoma cells S9 from livers of rats induced with Aroclor 1254
GLP	Yes
Purity:	95.7 %
Dose levels	Without S9: 40, 50, 60, 80 and 100 µg/ml. With S9: 8, 10, 15, 20 and 40 µg/ml
	Study acceptable

Executive Summary

Imidan (Phosmet) was examined for genetic activity in the L5178Y $TK^{+/-}$ mouse lymphoma multiple endpoint test in the absence and presence of metabolic activation. A chromosome damaging effect was examined by staining the cells and evaluation of the metaphases.

Cells were exposed to the test substance (95.7 % purity) using dimethylsulfoxide (DMSO) as solvent for 4 hours at concentrations ranging from 40 to 100 µg/ml in the non-activation assay and

for 4 hours at concentrations ranging from 8 to 40 µg/ml in the presence of metabolic activation S9-mix from the liver of Aroclor 1254 induced male Sprague-Dawley rats. For both assays, cells were harvested 24 hours after the end of treatment. Following harvest, cells were fixed on slides, stained and examined for structural and numerical chromosomal aberrations and for cytotoxic effects. 50 cells from each duplicate culture were analysed instead of 200 cells recommended. Cytotoxicity was determined by means of relative cell suspension growth and by means of mitotic index (number of mitosis per 500 cells).

The solvent DMSO was used as negative control while ethylmethanesulphonate (EMS; 0.50 µl/ml) was used as a positive control without metabolic activation and N-nitrosodimethylamine (DMN; 0.02 µl/ml) with metabolic activation.

In the main experiment (without S9 mix) at the time of harvest, severe toxic effects were observed at 80 and 100 µg/ml by means of cell suspension growth (average relative growth: 26 and 20 %, respectively). In the presence of metabolic activation cell suspension growth was reduced at all concentration (relative cell growth ranged from 48 to 20 % as compared to solvent controls). This decrease was dose-related.

The mitotic index was not affected in the absence of metabolic activation but was reduced in the presence of metabolic activation at all concentrations tested as compared to the untreated and solvent control.

When tested directly, Imidan (phosmet) did not mean an increase in either structural or numerical aberrations. Only an increase in the number of chromosomal aberrant cells was observed at 100 µg/ml in one culture. In the presence of metabolic activation no significant increase in chromosomal aberrations was observed. The significant elevation in the numerical aberration in one of the replicates at 40 µg/ml can be attributed to random sampling error.

Conclusion

Imidan (phosmet) did not induced structural chromosome aberrations with and without S9 under the conditions of this study.

Further study on chromosomal aberrations

In other scientific publication (Kurinnyi and Pilinskaya, 1997), Imidan was assayed for the potential to induce chromosomal aberrations in a culture of lymphocytes of human peripheral blood. Purity of the test substance and evaluation criteria were not given and metabolic activation was not used. The dose levels tested were 0.02, 2 and 20 µg/l. It was reported a significant increase ($p < 0.05$) in the frequency of chromosomal aberration at all dose levels. Aberrations of the chromatid type (mainly single fragments) predominated. At the same time, for all concentrations of Imidan, the frequency of chromatid and chromosomal interchanges exceeded the level in the control. This study is not considered acceptable due to lack of data reported. On the basis of the magnitude of the induced effect, Imidan posseses low cytogenetic activity.

DNA effects

Rec assay with Bacillus subtillis

A scientific publication (Shirasu et al., 1979), phosmet (purity of 99%) was tested for DNA damaging potential in a rec-assay using the recombination wild (H17) and deficient (M45) strains of *Bacillus subtilis*. This study is pre-guideline. Besides, there is no available OECD Guideline. The cells were exposed to phosmet dissolved in DMSO at concentrations ranging from 20 µg/disk to 2000 µg/disk without activation. After the incubation period (overnight) the width of the inhibition zones were measured. Negative controls (Kanamycinsulfate) and positive controls (Mitomycin-C) were used concurrently to demonstrate the sensitivity of the test system.

Phosmet did not affect the width of inhibition zones at any concentration tested. The sensitivity of the test system used was demonstrated by the respective effects of the negative and positive controls on the inhibition zones of M45 and H17.

Conclusion

Under the conditions of this study, phosmet did not affect DNA repair as determined in the *B. subtilis* rec-assay.

***In vitro* Sister Chromatid Exchange (SCE) Assay in Mammalian Cells**

Title	**Mutagenicity evaluation in mouse lymphoma multiple endpoint test cytogenetic assay**
Author (s) (year):	Snyder, R.D. (1986a)
Guideline	Materials and methods used in this study complied with requirements of US EPA guidelines and OECD guideline 473 adopted 21 July 1997.
System	L5178Y mouse lymphoma cells S9 from livers of rats induced with Aroclor 1254
GLP	Yes
Purity:	95.7 %
Dose levels	Without S9: 40, 50, 60, 80 and 100 µg/ml. With S9: 8, 10, 15, 20 and 40 µg/ml
	Study acceptable

Executive Summary

In this study (Snyder, 1986a), Imidan (phosmet) was evaluated for its ability to increase sister chromatid exchanges (SCE) in L5178 mouse lymphoma cells in the absence and presence of metabolic activation. Effects on sister chromatid exchanges were examined by staining the cells and evaluation of the metaphases.

Cells were exposed to the test substance (95.7% purity) using dimethylsulfoxide (DMSO) solvent for 4 hours at concentrations ranging from 40 to 100 µg/mL in the non-activation assay and for 4 hours at concentrations ranging from 8 to 40 µg/mL in the presence of metabolic activation S9-mix from the liver of Aroclor 1254 induced male Sprague-Dawley rats. For both assays, cells were harvested 24 hours after the end of treatment. Two hours prior to harvest, cultures were exposed to colcemid. These test conditions had been selected on the basis of preliminary range-finding tests which are also described in the original report. Following harvest, cells were fixed on slides, stained and examined for structural and numerical chromosomal aberrations and for cytotoxic effects. 50 cells from each duplicate culture were analyzed instead of recommended 200 cells. Cytotoxicity was determined by means of relative cell suspension growth and by means of mitotic index (number of mitosis per 500 cells).

The solvent DMSO was used as negative control while ethylmethanesulphonate (EMS; 0.50 µl/ml) was used as a positive control without metabolic activation and N-nitrosodimethylamine (DMN; 0.02 µl/ml) with metabolic activation.

Based upon these findings of a range finding study, concentrations of 40, 50, 60, 80 and 100 µg/ml in the absence of S9 mix and of 8, 10, 15, 20 and 40 µg/ml in the presence of S9 mix were selected and evaluated in the cytogenetic assay. In the main experiment (without S9 mix) at the time of harvest, severe toxic effects were observed at 80 and 100 µg/ml by means of cell suspension growth

(average relative growth: 26 and 20 %, respectively). In the presence of metabolic activation cell suspension growth was reduced at all concentration (relative cell growth ranged from 48 to 20 % as compared to solvent controls). This decrease was dose-related.

The mitotic index was not affected in the absence of metabolic activation but was reduced in the presence of metabolic activation at all tested concentrations as compared to the untreated and solvent control.

There were increases in SCEs at each concentration tested which were statistically significant when tested in the absence and presence of S9 mix. However, no clear dose-relationship could be established.

Conclusion

Imidan (phosmet) induced a statistically significant increase over solvent controls in chromatid exchange in the absence or presence of metabolic activation in the mouse lymphoma multiple test *in vitro*, but there is not doubling of the SCE frequency and this effect was not confirmed by independent experiment. Thus, the result is questionable.

***In vitro* DNA breaks study**

Title	**Effect of Imidan on human fibroblast DNA**
Author (s) (year):	Snyder, R.D. (1986b)
Guideline	No guideline exists for this test method
System	Diploid human fibroblast cells S9 from liver of rat Aroclor 1254
GLP	Yes
Purity:	95.7 %
Dose levels	Ranged from 0.25, 0.50 and 1 mg/ml (± S9)
	With reserves

Executive Summary

The objective of this study (Snyder, 1986b) is to assess the potential of phosmet to induce DNA stand break in diploid human fibroblast cells *in vitro*. There is no available OECD Guideline. The purity of the test compound was 95.7%. The human fibroblasts were exposed one hour to the test substance in the absence or presence of metabolic activation (S9 from liver of rat Aroclor 1254). Three dose levels were used (1, 0.50 and 0.25 mg/ml). Higher doses could not be employed because of the insolubility of the compound.

In four independent experiments, the human fibroblasts were exposed to the test substance with one to four concentrations in a range of 0.25 to 1.0 mg/ml in the absence or presence of S9 mix. Higher concentrations were not used due to the limit of solubility. No information was provided regarding dose selection or cytotoxicity. Exposure time for all test groups was one hour.

Concentrations of Imidan (phosmet) in the range of 0.25 to 1.0 mg/ml did not slow the sedimentation of DNA nucleoids either in the presence or absence of a metabolic activation system. This indicates that no DNA strand breaks were induced by these treatments.

Conclusion

Under the test conditions phosmet did not induce DNA strand breaks in human diploid fibroblasts either in the presence or absence of metabolic activation.

4.1.1.2 *In vivo* data

The mutagenic potential of phosmet has been assessed by four *in vivo* studies in somatic cells: two studies about mammalian micronucleus and two studies to assay the potencial of phosmet to induce unscheduled DNA synthesis (UDS) in hepatocytes.

Another two studies, published as scientific literature (one of chromosomal aberrations in mice and other of DNA alkylation in mice), were included for assessment purposes.

***In vivo* studies in mammalian somatic cells**

***In vivo* mammalian erythrocyte micronucleus test**

Title	**Report of a micronucleus test in the mouse**
Author (s) (year):	Gibbs, J.A. (1986)
Guideline	Guidelines 84-2, 84-4 Study is similar to OECD guideline 474.
System	Bone marrow cells from male and female CD-1 mice.
GLP	Yes
Purity:	95.5 %
Dose levels	Single dose level of 17 mg/kg by oral gavage.
	Study acceptable

Executive Summary

In a bone marrow micronucleus assay using Charles River CD-1 mice, phosmet (technical Prolate) was administered by gavage (solvent 1 % methyl cellulose in distilled water) to groups of male and female animals at target doses of 0, 15, 20 or 30 mg/kg bw (3 animals/sex) and 45 mg/kg bw (1 male) in a dose range-finding assay, and at 17 mg/kg bw (6-7 animals/sex/group) in the main micronucleus assay.

In the range-finding study, all mice treated with 30 mg/kg bw and one mouse treated with 45 mg/kg bw died shortly after the single administration. At 20 mg/kg bw only one female died and at 15 mg/kg bw all animals survived until scheduled necropsy. The cell toxicity examination revealed a reduction in polychromatic cell number in mice dosed at 15 or 20 mg/kg bw after 48 and 72 hours. The single female dosed at 20 mg/kg bw (72 hours value) died prematurely.

Negative control groups were treated with the vehicle only (1 % methyl cellulose in distilled water, 10 ml/kg bw), and positive control groups were treated with cyclophosphamide (75 mg/kg bw, 10 ml/kg bw). Mouse bone marrow was sampled at 24, 48 and 72 hours after dosing for the vehicle and the phosmet dosed groups. A single sampling time of 24 hours after dosing was used for the cyclophosphamide positive control group. Slides of bone marrow cells were prepared and 1000 (range-finding study 500) polychromatic erythrocytes (PCEs) were evaluated per animal (5 male and 5 female mice per test group) and investigated for micronuclei. The normochromatic cells with and without micronuclei occurring per 1000 polychromatic erythrocytes were also determined. The ratio of poly- to normochromatic erythrocytes (PCE ratio) was determined to assess inhibition of erythropoiesis.

In the main micronucleus test, no mortalities occurred and no clinical signs of toxicity were noted. There was slight cell toxicity, evidenced by a small reduction in the number of PCEs after 24 hours for males and females treated with phosmet at 17 mg/kg bw indicating that phosmet reaches the target, *i.e.* the bone marrow. This conclusion is supported by the results of the ADME study with oral treatment.

There were no significant increases in micronucleated PCEs at any of the three sampling time points for animals treated with phosmet (17 mg/kg). The micronuclei rate was comparable to the negative control throughout for both male and female mice.

Positive control treatment induced the appropriate response indicating that the test system was able to detect genotoxic compounds.

Conclusion

The observations and findings of the study indicate that phosmet does not exhibit *in vivo* genotoxic potential. No increase in the incidence of micronuclei in bone marrow polychromatic erythrocites was observed. Data were negative at all three samples times and in both sexes in CD-1 mice.

***In vivo* mammalian erythrocyte micronucleus test**

Title	**Micronucleus test for Imidan 500 PM**
Author (s) (year):	Pestana, C.B. (1999)
Guideline	OECD 474. Deviation: one dose level was tested.
System	Bone marrow cells from male Swiss mice.
GLP	No
Purity:	The test material was Imidan 500 PM with a content of phosmet of 499 g/kg
Dose levels	Dose level of 13.86 mg/kg bw/day, two consecutive days, by intraperitoneal application.
	Study acceptable

Executive Summary

In a bone marrow micronucleus assay using Swiss mice, Imidan 500 PM was administered intraperitoneally to groups of male animals at target doses of 10, 15, 20 or 50 mg/kg bw (3 animals/group) in a dose range-finding assay. All animals treated at 20 and 50 mg/kg bw/day died while at 10 and 15 mg/kg bw/day all survived. According to the results of the range-finding study, the dose of 13.86 mg/kg bw/day (80% id LD_{50}) was used in the micronucleus assay.

For the range-finding study, all mice were dosed twice (at 0 and 24 h) and observed for a period of at least 72 hours after the first dose was administered.

Mouse bone marrow for micronuclei examination was prepared 48 hours after first dosing for the control and the Imidan dosed groups. To assess cytotoxicity, 400 bone marrow erythrocytes per animal were analysed. After staining of the preparations, 2000 polychromatic erythrocytes (PCEs) were evaluated per animal (10 male mice per test group) and investigated for micronuclei.

In the micronucleus test, no mortalities occurred and no clinical signs of toxicity were noted. There was slight cell toxicity, evidenced by an increase in the PCE/NCE ratio in both positive control and Imidan treatment groups indicating that phosmet reached the target, *i.e.* the bone marrow.

The ratio PCE/NCE in treated group was slightly greater than in negative control group. No statistically significant increases in micronucleated PCEs were noted in the Imidan treatment group when compared to the negative control group.

Positive control treatment induced the appropriate response indicating that the test system was able to detect genotoxic compounds.

Conclusion

Imidan 500 PM did not induce micronuclei in polychromatic erythrocytes of male Swiss mice when treated intraperitoneally at a cytotoxic dose of 13.86 mg/kg bw/day. This corresponds to a dose of 6.92 mg/kg bw/day of the active ingredient phosmet.

In vivo mammalian bone marrow chromosome aberration test

Title	**Comparative study of the effect of certain organophosphorous pesticides**
Author (s) (year):	Kurinnyi, A.I. (1975)
Guideline	This study is a pre-guideline
System	Bone marrow cells from white non-linear male mice.
GLP	No
Purity:	Purity of the test substance was not indicated
Dose levels	Single dose level of 5, 10, 20 and 50 mg/kg bw, by gavage
	Study not acceptable due to lack of information

Executive Summary

The ability of Phthalophos (Imidan, batch and purity not indicated) to cause chromosomal damage *in vivo* was investigated in a chromosome bone marrow test using "white non-linear" male mice. Groups of 5 mice received a single dose of 5, 10, 20 or 50 mg/kg bw of the test compound by gavage. No mortality and no signs of intoxication were reported. All animals were sacrificed 20 hours after dosing and bone marrow cells were prepared for cytogenetic metaphase analysis. Approximately 2 hours before sacrifice, colchicine was given to the animals to stop cell division at metaphases. Control animals were used but it was not indicated neither the vehicle for the test substance nor whether the control animals were treated with the vehicle or not. No positive control group was used.

A statically significant increase in the number of chromosomal aberrations was reported after treatment with 20 mg/kg bw Imidan (2 ± 0.46 % cells with aberrations) as compared to the control group (0.9 ± 0.29 %). In the low (5 and 10 mg/kg bw) and the high dose groups (50 mg/kg bw) no relevant increase was reported.

Due the deficiencies in reporting, test design, (*e.g.* no positive control) and evaluation criteria for aberrations (e.g. Types of aberrations, incl. or excl. Gaps), this study is not acceptable for the evaluation of the mutagenic potential of Imidan (phosmet).

Unscheduled DNA synthesis in rat hepatocytes *in vivo*

Title	**Phosmet: *in vivo* rat liver unscheduled DNA synthesis assay**
Author (s) (year):	MacKay, J.M. (1996)
Guideline	There is not available OCDE guideline
System	Hepatocytes form male Alderley Park (Alpk AP_fSD) rat
GLP	Yes
Purity:	96.4%
Dose levels	Single dose levels of 32 and 50 mg/kg bw, by gavage
	Study acceptable

Executive Summary

Phosmet was assessed for its potential to induce DNA damage and unscheduled DNA synthesis in the liver of male Alpk AP_fSD rats. In a preliminary study, the dose of 50 mg/kg bw was determined as the maximum tolerated dose (MTD) for male rats based on patterns of clinical signs and lethality noted over a four day observation period. None of the animals died at this dose level and no micropathological changes were observed in the liver. However, the selected dose level was high enough to ensure that phosmet reached the target organ, *i.e.* the liver. Accordingly, a single oral dose was given to groups of 5 male rats at dose levels of 32 or 50 mg/kg bw in the UDS test. Two sampling time points, 2 and 16 hours post-dose, were used and two independent experiments were carried out at each time point. A concurrent negative control group was treated with the vehicle, corn oil, and a positive control group was treated with dimethylhydrazine dihydrochloride (DMH) at 30 mg/kg bw.

No treatment-related adverse reactions were observed for rats dosed with phosmet. Mean viabilities of the hepatocyte cultures ranged from 75 to 79 %.

After processing of cultured hepatocytes for autoradiography, unscheduled DNA repair was assessed by comparing the labelling levels of hepatocyte nuclei from treated animals with control values and with the accompanying cytoplasmic labelling levels.

The values recorded did not indicate any DNA repair induced in the rat liver following treatment with phosmet up to the maximum tolerated dose of 50 mg/kg bw.

No significant increases in mean net nuclear grain counts were noted at either dose level or time point investigated as compared to the vehicle control. The sensitivity of the test system was clearly demonstrated by marked increases in DNA repair induced by the positive control substance DMH.

Conclusion

Under the conditions of test, phosmet did not induce DNA damage with subsequent DNA repair as measured by unscheduled DNA synthesis in the rat liver *in vivo*.

Unscheduled DNA synthesis in rat hepatocytes *in vivo*

Title	**Rat liver DNA repair (UDS) test**
Author (s) (year):	Proudlock, R.J. (1998)
Guideline	OCDE guideline 486
System	Hepatocytes from male Sprague-Dawley rat
GLP	Yes
Purity:	96.1
Dose levels	Single dose levels of 54 and 180 mg/kg bw, by gavage (2 hour time point) Single dose levels of 54 and 108 mg/kg bw, by gavage (14 hour time point)
	Study acceptable

Executive Summary

Phosmet (technical) was assessed for its potential to induce DNA damage and unscheduled DNA synthesis in the liver of male Sprague-Dawley rats. A single oral dose was given to groups of at least 5 male rats at dose levels of 54 or 180 mg/kg bw (2 hour time point) and 54 or 108 mg/kg bw (14 hour time point). A concurrent negative control group was treated with the vehicle (1% methyl cellulose) and a positive control group was treated with dimethylnitrosamine at 4 mg/kg bw (2 hour

time point) or 2-acetylaminofluorene at 50 mg/kg bw (14 hour time point). Four animals were assessed at each experimental point with the exception that only two animals from the positive control group were assessed at each time point.

One rat died at 2 h expression and three rats died at 14h expression, therefore, other group of six animal was treated at 108 mg/kg bw, at 14 h expression, where one animal died. Clinical signs as body tremors, increased respiratory rate and salivation were observed in all groups the first two hours and at 14h in groups treated with 108 and 180 mg/kg bw. Mean viabilities of the hepatocyte cultures ranged from 81 to 98 %.

After processing of cultured hepatocytes for autoradiography, unscheduled DNA repair was assessed by comparing the labelling levels of hepatocyte nuclei from treated animals with control values and with the accompanying cytoplasmic labelling levels.

At 2 and 14 hours post-dose, neither the nuclear grain nor the resulting net grain counts were enhanced due to the *in vivo* treatment of the animals with phosmet. No dose level of phosmet revealed UDS induction in the hepatocytes of treated animals as compared to vehicle control.

The sensitivity of the test system was clearly demonstrated by marked increases in DNA repair induced by the positive control substances DMH.

Conclusion

Under the conditions of test, phosmet did not induce DNA damage with subsequent DNA repair as measured by unscheduled DNA synthesis in the rat liver *in vivo*.

***In vivo* DNA alkylation assay**

Title	**A comparative study of guanine N7-alkylation in mice *in vivo* by the organophosphorous insecticides Trichlorphon, Dimethoate, Phosmet and Bromophos**
Author (s) (year):	Dedek, W. et al. (1984)
Guideline	There is not available OCDE guideline
System	Liver and kidney from AB Jena/Halle male mice
GLP	No
Purity:	99%
Dose levels	Single dose level of 20 mg/kg bw, by intraperitoneal injection. Liver and kidney samples at 6 and 24h.
	Additional information

Executive Summary

Following single intraperitoneal administration of ^{14}C-methyl-labelled phosmet to male mice (strain AB Jena/Halle) at a dose level of 20 mg/kg bw (dissolved in propylene glycol). The dose selected was chosen in order to avoid symptoms of severe acute intoxications. DNA from liver and kidneys was isolated and analysed spectrophotometrically.

The extent of methylation ranged from 0.2 to 0.4 µmol 7-MeG/mol guanine in liver and kidney. This result was within the limit of detection (0.1-0.3 µmol 7-MeG/mol guanine). Phosmet has a limited solubility in water and possibly due to this fact it cannot penetrate in the target site of DNA bases.

Conclusion

Phosmet did not induce DNA adducts (N-7 methylation of guanidine) in mouse liver or kidney after intraperitoneal administration *in vivo.*

4.8.2 Human information

For further details on phosmet-related toxicity in humans, please refer to paragraph 4.11.2.

4.8.3 Other relevant information

No other relevant information is available.

4.8.4 Summary and discussion of mutagenicity

The genotoxicity of phosmet has been investigated in a battery of ***in vitro*** and ***in vivo*** assays, including gene mutation, chromosomal aberration, and DNA damage as endpoints. It should be noted that phosmet reaches bone marrow as well as the liver and kidney according to toxicokinetic data. Phosmet genotoxicity ***in vitro*** and ***in vivo*** studies are summarised in Table 33 and Table 34 respectively.

With respect to gene mutation, phosmet was positive in bacterial (TA97 and TA100 ±S9) strains. In the cultured mammalian cells (-S9), forward mutation at KT *locus* in mouse lymphoma cells (L15784) was positive. In the first non-activated assay a dose-related increase in the average mutant frequency was observed and, at doses of 80 and 100 µg/ml it was significantly greater than control; in the second non-activation assay a dose-related increase in the average mutant frequency was observed and, at doses of 70, 80 and 100 µg/ml it was significantly greater than control. However, this result was not confirmed ***in vivo*** where negative results were obtained in UDS (Hertzel, 1986).

In a chromosome aberration study ***in vitro*** with mouse lymphoma cells, negative results were obtained (±S9) (Snyder, 1986a). In culture of lymphocytes of human peripheral blood (Kurinnyi and Pilinskaya, 1977) phosmet induced chromosome aberrations (-S9), the test substance was not tested with S9. This study was not acceptable due to lack of data reported.

An ***in vivo*** study in somatic cells shows that phosmet was not genotoxic in mouse bone marrow micronucleus test. In the preliminary assay decreased PCE to total erythrocytes were observed at doses ≥ 15 mg/kg. The main test used 17 mg/kg as the treatment level. No increase in micronucleated polychromatic erythrocytes jointed a decreased PCE to total erythrocytes were seen (Gibbs, 1986). In another ***in vivo*** micronucleus *test* using Imidan 500 (content of phosmet of 499 g/kg) there was no evidence of genotoxic activity in bone marrow cells from treated Swiss male mice (Pestana, 1999). Furthermore, in a chromosome aberration assay, phosmet did not show a clear clastogenic potential in bone marrow cells from male mice where a positive result was found at 20 mg/kg but not at 50 mg/kg. This study (Kurinnyi, 1975) was considered not acceptable due to lack of data reported and it has not been taken into account in the risk assessment of genotoxicity.

In relation to DNA damage ***in vitro***, negative results are obtained in tests with *Bacillus subtilis* and in a DNA breaks study in diploid human fibroblast cells; but a clear positive response is observed in the sister chromatid exchange assay in mouse lymphoma cells (±S9). However, this result is not confirmed ***in vivo*** where negative results are obtained in UDS and DNA alkylation assays.

In conclusion, phosmet is genotoxic ***in vitro*** based on the positive responses observed in the *Salmonella* test and in an ***in vitro*** mammalian gene mutation assay. The only ***in vivo*** study that was positive was reported by Kurinnyi (1975) in a bone marrow in mice. However the increase in chromosome aberrations observed was not dose-dependent and the study was not considered acceptable. Besides the results in one ***in vitro*** chromosome aberration assay and in two ***in vivo***

micronucleus tests were negative. Furthermore, negative results were reported in an ***in vivo*** UDS assay and DNA alkylation assay.

The weight of evidence suggests that phosmet has a genotoxic potential ***in vitro*** but not ***in vivo***. Therefore, based on the available data classification regarding mutagenicity is not necessary.

4.8.5 Comparison with criteria

According to CLP classification of a substance as mutagen Category 1B is based on the following criteria.

- Positive result (s) from *in vivo* heritable germ cell mutagenicity test in a mammals; or
- Positive result (s) for *in vivo* somatic cell mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or
- Positive result from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cell of exposed people.

Classification into category 2 according to CLP is required for substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans based on:

- Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:

  Somatic cell mutagenicity tests in vivo, in mammals.

  Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

Note: Substances which are positive *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

In conclusion, phosmet is genotoxic *in vitro* based on the positive responses observed in the *Salmonella* test and in an *in vitro* mammalian gene mutation assay. The only *in vivo* study that was positive was reported by Kurinnyi (1975) in a bone marrow in mice. However, the increase in chromosome aberrations observed was not dose-dependent and the study was not considered acceptable. Besides, the results in one *in vitro* chromosome aberration assay and in two *in vivo* micronucleus tests were negative. Furthermore, negative results were reported *in vivo* UDS assay and DNA alkylation assay.

Based on the results of all studies provided, the weight of evidence suggests no *in vivo* genotoxic potential for phosmet. Therefore, phosmet does not warrant classification for mutagenicity according to CLP criteria.

4.8.6 Conclusions on classification and labelling

CLP: A classification is not required.

4.9 Carcinogenicity

Table 35: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
2-year combined dietary chronic toxicity / oncogenicity study **Adequate for the assessment of carcinogenicity in rats.**	Main effects Decreased ChE activities (plasma, brain and RBC) at 200 and 400 ppm. Increased incidence of liver fatty changes from 200 ppm. Decreased RBC ChE activity at 40 ppm. Not carcinogenic. No evidence of oncogenicity up to the maximum tolerated dose. **LOAEL = 1.8 mg/kg bw/day (40 ppm)**	Sprague-Dawley rat Purity: 94.3% Dose range: 20, 40, 200, 400 ppm equivalent to: 1.1, 1.8, 9.4, 23 mg/kg bw/day in males 1.1, 2.1, 10.9 27 mg/kg bw/day in females (400 ppm in chronic part only)	**Chang, J.C.F. et al. (1991)**
2-year dietary oncogenicity study **Accepetable with some reservations**	Main effects Convulsions and signs of liver toxicity in form of vacuolic degeneration at 100 ppm in males. Statistically significant inhibition of brain cholinesterase at the termination of the study in females at 25 ppm (14%) and 100 ppm (22%). Carcinogenicity: statistically significant increase at 100 ppm of liver cell adenomas in males that showed a significant trend. **[a]LOAEL = 5 mg/kg bw/day**	B6C3F1 mouse Purity: 94.7% Dose range: 5, 25, 100 ppm equivalent to: 1.0, 4, 14 mg/kg bw/day in males and 1.2, 5, 18 mg/kg bw/day in females	**Katz et al. (1984), subsequent addenda and Haseman et al. (1999)**

[a] Experts in PRAPeR 86 agreed that inhibition at the low and mid doses, only observed at the interim kill not dose related and not associated with convulsions, was not an adverse effect. The MSCA in Classification&Labelling agrees with this statement. However, a dose dependent statistically significant ↓brain AChE was observed in month 24 in females (14%) at 5 mg/kg bw/day and in our opinion it is considered relevant.

4.9.1 Non-human information

4.9.1.1 Carcinogenicity: oral

Long-term (2 years) oral toxicity in the rat (can be a combined long-term and carcinogenicity study)

Title	2-year chronic toxicity/oncogenicity study with R-1504 in rats
Author (s) (year):	Chang, J.F.C. et al. (1991)
Administration	Two-year chronic toxicity/oncogenicity study
Guideline	EPA FIFRA guideline 83-5. No deviations with respect to OECD 453 guideline.
Species	Sprague-Dawley rats from Charles River Laboratories, USA
GLP	Yes
Laboratory	Ciba-Geigy Corporation, Farmington, CT, USA
Purity:	94.3%
Groups	20, 60 and 70 rats/sex/dose level depending on the group
Dose levels	0, 20, 40, 200 and 400 ppm equivalent to: Males: 0, 1.1, 1.8, 9.4 and 23 mg/kg bw/day Females: 0, 1.1, 2.1, 10.9 and 27 mg/kg bw/day
	EPCO Expert Meeting 33 (12-16 September 2005) concluded that this study was adequate for the assessment of carcinogenicity in rats treated with phosmet.

Executive Summary

In a 2-year chronic toxicity-oncogenicity study, phosmet (R-1504 (Imidan), purity: 94.3%) was administered in the diet to Sprague-Dawley rats at concentrations of 0, 20, 40 and 200 ppm (70 animals per sex for the control group and 60 animals for each of the other groups). 20 animals per sex from the control group and 10 animals per sex from each of the other groups were terminated after 12 months for interim evaluation. Aproximatelly, 50 animals per sex from all mentioned groups were evaluated after 24 months of treatment. An additional high dose satellite group (20 animals/sex) for chronic toxicity evaluation was treated at 400 ppm for 12 months only. The mean achieved daily doses were 1.1, 1.8, 9.4 and 23 mg/kg bw/day in males and 1.1, 2.1, 10.9 and 27 mg/kg bw/day in females fed with 20, 40, 200 and 400 ppm, respectively.

All animals were observed at least twice daily for general apereance, behaviour, clinical signs and mortality. Moribund animals were sacrified.

All animals were given a detailed physical examination each week. Palpation for the presence of tissue masses was performed. Ophthalmoscopic examinations were performed at pre-test on all animals, at 12 months on animals scheduled for interim sacrifice and at 24 months on all surviving animals.

Body weights and food consumption were determined weekly during the first 13 weeks of treatment and once every 4 weeks thereafter. Termination body weights were recorded on all animals.

Blood for haematology and clinical chemistry analyses was collected at 6, 12, 18 and 24 months in 10 rats/sex/group. Serum and red blood cell (RBC) cholinesterase (ChE) activity was measured in 10 rats/sex/group after 6, 12, 18 and 24 months and brain ChE activity in brain samples collected from 10 rats/sex/group scheduled for 12 months sacrifice and those designated for clinical

chemistry at 24 months termination. Urinalysis was performed at 6, 12, 18 and 24 months in 10 rats/sex/group.

Necropsies were performed on all rats that died prematurely or were killed *in extremis* and on all surviving animals at the scheduled necropsies after 12 and 24 months of treatment. Selected organs were weighed in rats sacrificed as scheduled after 12 and 24 months. All scheduled sacrifice rats were subjected to gross pathological assessment followed by histopathological examination.

Statistical analysis of results was performed as appropriate. The specifical statistical methods used to analyze the test parameters were one-way analysis of variance followed by Dunnett's "t" test (body weights, body weight gain, food consumption, feed efficiency, hematology values, clinical chemistry and organ weights), Duncan's multiple range test (urynalisis), chi-square (urynalisis) and Fisher's exact test with Bonferroni correction (treatment-related neoplastic and non-neoplastic lesions). All tests were two-tailed. The probability of Type I error (alpha) was set at 0.05. Statistical test results that reached the 0.01 level of significance were also noted.

Results

Mortality:

Survival at terminal scheduled sacrifice was lower than 50% in all groups treated with phosmet (Table 36). However, survival rates of treated animals were comparable to or greater than the controls in both sexes. Male control rats showed the poorest survival rate. In the opinion of the notifier, the shortened life span is considered to be associated with the "fat rat" syndrome of obesity. Further, poor survival is generally seen in long-term studies with Sprague Dawley rats [OECD Guidance document 116 on the conduct and design of chronic toxicity and carcinogenicity studies, supporting test guidelines 451, 452 and 453, 2nd edition 2012. Section 3.3.1].

Table 36: Summary of adjusted survival rate

	Males Dose level (ppm)					**Females** Dose level (ppm)				
	0	**20**	**40**	**200**	**400**	**0**	**20**	**40**	**200**	**400**
Initiation	70	60	60	60	20	70	60	60	60	20
Adjusted survival at 12-month sacrifice	65/70 (93%)	51/60 (85%)	54/60 (90%)	54/58 (93%)	18/20 (90%)	67/70 (96%)	55/59 (93%)	57/60 (95%)	58/60 (97%)	20/20 (100%)
12-months sacrifice	20	10	10	10	18	20	10	10	10	20
Adjusted survival at 24-month sacrifice	10/50 (20%)	12/50 (24%)	16/50 (32%)	18/48 (38%)	-	16/50 (32%)	17/49 (35%)	16/50 (32%)	21/50 (42%)	-

The validity criteria in OECD 453 Guideline for long-term studies are "survival of all groups should be no less than 50% at 24 months for rats". However, for assessing long-term/carcinogenicity studies, it is crucial to know the time point at which survival falls below 50 %. If the latter occurs after week 94 of treatment in the highest dose groups then the study is not considered to be unduly compromised [(Reporting Table, Phosmet; 17112/EPCO/BVL/04, rev. 1-1 (07.06.2005)].

Survival in the high dose groups falls below 50% between weeks 98 to 99 and between weeks 100 to 101, in males and females respectively. In the mid dose males, the criterion is almost met i.e. survival falls below 50% between weeks 92 to 93. However, for females, this criterion is met also in the mid dose group (survival falls below 50% between weeks 94 to 95). Then, the median age of all unscheduled deaths was not adversely different from controls in all groups of treated animals of both sexes, i.e. the time span until death was comparable to or greater than the controls.

Table 37: Summary of time points at which survival falls below 75% and 50%

% survival	Dose level [ppm]			
	0	20	40	200
	Time point [weeks]*			
Males				
<75%	72-73	68-69	75-76	81-82
<50%	84-85	83-84	92-93	98-99
Females				
<75%	77-78	71-72	72-73	89-90
<50%	92-93	87-88	94-95	100-101

*between the indicated weeks the survival fell below 50% or 75%, respectively

Poor survival in long term studies conducted with Sprague-Dawley rats is a common issue.

The notifier presented historical control data concerning mortality for Sprague-Dawley rats from 24 studies conducted in six different laboratories between 1991 and 1997 of approximately 104 weeks duration using either dietary, gavage or subcutaneous. This compilation was prepared by Charles River Laboratories, the predominant worldwide supplier of the CD Sprague-Dawley rat (Giknis and Clifford, 2001). The range of survival, in that historical control, was 17.1%-62.9% and 20%-61.4% for males and females respectively. In other words, a survival rate lower than 50% was often observed for Sprague Dawley rats in studies conducted at about the same time as the Chang study. In fact, a control survival rate of 50% or higher was obtained in only six out of 23 studies for males and in two out of 24 studies for females. Thus, the historical control survival data shows that control survival in the phosmet study was within the range typically seen in contemporaneous chronic studies with Sprague-Dawley rats.

Table 38: Historical control data: Mortality in Sprague-Dawley Rats

Study no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15[1)]**	**16[1)]**	**17[1)]**	**18[1)]**	**19[1)]**	**20**	**21**	**22**	**23**	**24**	**Chang et al., 1991 controls**
Initiation date	'94-'96	'94-'96	'92	'94	'96	'92	'92	'93	'92	'94	'96	'96	'95	'95	'96	'96	'96	'96	'97	'97	'97	'97	'91	'94-'96	'88
Duration (weeks)	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
	Males																								
Total no. of animals	130	115	60	110	54	50	52	50	60	70	70	70	70	60	50	50	50	50	60	70	60	60	60	-	50[1)]
Number surviving	56	45	25	25	13	10	13	17	20	12	31	36	19	20	25	26	18	16	30	44	31	22	19	-	10
% survival	43.1	39.1	41.7	22.7	24.1	20.0	25.0	34.0	33.0	17.1	44.3	51.4	27.1	33.3	50.0	52.0	36.0	32.0	50.0	62.9	51.7	36.7	31.7	-	**20.0**
	Females																								
Total no. of animals	130	115	60	110	54	50	52	50	60	70	70	70	70	60	50	50	50	50	60	70	60	60	60	200	50[2)]
number surviving	44	41	27	41	13	15	15	24	20	14	20	24	21	17	25	24	19	17	29	43	21	19	27	68	16
% survival	33.8	35.7	45.0	37.3	24.1	30.0	29.4	49.0	33.3	20.0	28.6	34.3	30.0	28.3	50.0	48.0	38.0	34.0	48.3	61.4	35.0	31.7	45.0	34.0	**32.0**

[1)] Animals from Crl:CD® (SD) BR IGS colonies
[2)] 70 animals at initiation, 20 sacrificed scheduled after 12 months on study

Therefore, it can be conclude that there were no adverse effects upon survival associated with exposure to phosmet.

Clinical signs: There were no specific clinical observations or neurological damage, central or peripheral, detected at any of the tested dose levels. There were no treatment-related ocular abnormalities.

Body weight gain: It was reduced in males and females at 400 ppm and in females at 200 and 40 ppm. Reductions persisted throughout the study at 400 ppm but were confined to the early phase of the study at 200 and 40 ppm.

These reductions were statistically significant in females at 400 ppm from week 4 to week 12 and at 200 and 40 ppm at week 4. In males, reduction is only statistically significant at week 4 at 400 ppm. Only in females at 400 ppm reductions were greater than 10%.

Table 39: Mean cumulative body weights change (g)

Males:	**0 ppm**	**20 ppm**	**40 ppm**	**200 ppm**	**400 ppm**
Week 4	241	241	238	231	222*
Week 7	345	351	347	337	328
Week 12	437	447	448	433	412
Week 25	558	570	573	560	530
Week 53	671	704	679	678	625
Week 77	724	753	734	725	Not applicable
Week 104	659	747	694	690	Not applicable
Females	**0 ppm**	**20 ppm**	**40 ppm**	**200 ppm**	**400 ppm**
Week 4	115	112	106*(-7.8%)	105* (-8.7%)	85*(-16%)
Week 7	152	153	147	148	129*(-15%)
Week 12	188	189	180	188	168** (-10.6)
Week 25	247	252	229*	254	233
Week 53	351	366	326	357	313 (-10.8%)
Week 77	396	430	388	409	Not applicable
Week 104	437	447	392	449	Not applicable

* Significantly different from control by Dunnett´s "t" test: $p<0.05$
** Significantly different from control by Dunnett´s "t" test: $p<0.01$

Food consumption: The average mean daily food consumption was generally similar to the controls. Occasionally, treated animals consumed more food than the controls, especially females at 200 and 400 ppm in the early study period. Feed efficiency, as expected, decreased with time (age) in all groups, including the controls.

Plasma cholinesterase activity: Statistically and/or biologically significant reductions (>20%) in cholinesterase (ChE) activities in serum were seen in both sexes at 200 and 400 ppm throughout the study. At 40 ppm there was a reduction in the activity after 6 months in females (24%) and after 18 months in males (36%) and females (20%). Also at 20 ppm after 18 and 24 months in females (21% and 22% respectively) and after 18 months in males (26%).

RBC cholinesterase activity: There were reductions greater than 20% in both sexes at 400 and 200 ppm throughout the study. There were also statistically significant reductions at 40 ppm in almost all points (less at 12 months) and fullfiled almost the criteria to be biologically relevant after 6 months in both sexes (19% inhibition). Variations at 20 ppm were considered to be of no biological relevance.

Brain cholinesterase activity: There were statistically and biologically significant reductions (>20%) in both sexes at 400 ppm after 12 months (termination) and at 200 ppm after 12 months. Variations at 200 ppm after 24 months were statistically significant in both sexes and fullfilied almost the criteria to be biologically relevant in females (19% inhibition).

Table 40: Cholinesterase (ChE) activity per cent as controls

Time Point [months]		**Dose Level [ppm]**				
		0	**20**	**40**	**200**	**400#**
ChE Activity in Serum [I.U./L]						
Males						
6	Mean value	654	669	621	498	**393****
	Standard deviation	192	214	260	93	62
	No. animals	10	10	10	10	10
	% of control	100	102	95	76	**60**
12	Mean value	705	704	636	511	**358****
	Standard deviation	196	255	161	140	56
	No. animals	10	10	10	10	10
	% of control	100	100	90	72	**51**
18	Mean value	1352	1002	860	**625***	na
	Standard deviation	923	276	311	171	-
	No. animals	9	10	10	10	-
	% of control	100	74	64	**46**	-
24	Mean value	886	873	935	729	na
	Standard deviation	276	207	241	352	-
	No. animals	9	10	10	10	-
	% of control	100	99	106	82	-
Females						
6	Mean value	3630	3524	**2744***	**1891****	**947****
	Standard deviation	1292	909	762	571	236
	No. animals	10	10	10	10	10
	% of control	100	97	**76**	**52**	**26**
12	Mean value	2138	1957	1834	**1130****	**692****
	Standard deviation	469	531	498	302	172
	No. animals	10	10	10	10	10
	% of control	100	92	86	**53**	**32**
18	Mean value	3054	2401	2455	**1677****	na
	Standard deviation	1206	729	695	445	-
	No. animals	10	10	10	10	-
	% of control	100	79	80	**55**	-
24	Mean value	2288	1795	1967	**1197***	na
	Standard deviation	1077	576	481	623	-
	No. animals	10	10	10	10	-
	% of control	100	78	86	**52**	-
ChE Activity in Red Blood Cells [I.U./protein]						
Males						
6	Mean value	1706	**1431***	**1388****	**507****	**452****
	Standard deviation	267	215	131	188	159
	No. animals	10	9	10	9	10
	% of control	100	**84**	**81**	**30**	**26**
12	Mean value	1344	1154	1206	**447****	**310****
	Standard deviation	203	193	291	153	103
	No. animals	10	10	10	9	10
	% of control	100	86	90	**33**	**23**

Time Point [months]		Dose Level [ppm]				
		0	**20**	**40**	**200**	**400#**
18	Mean value	1426	1286	**1198***	**416****	na
	Standard deviation	240	187	204	143	-
	No. animals	10	10	10	9	-
	% of control	100	90	**84**	**29**	-
24	Mean value	1838	1616	**1536***	**658****	na
	Standard deviation	418	221	156	187	-
	No. animals	9	10	10	10	-
	% of control	100	88	**84**	**36**	-
Females						
6	Mean value	1454	1424	**1180****	**338****	**127****
	Standard deviation	208	165	194	116	152
	No. animals	10	9	7	9	9
	% of control	100	98	**81**	**23**	**9**
12	Mean value	1180	1250	1080	**288****	**264****
	Standard deviation	131	168	248	127	142
	No. animals	10	10	10	10	10
	% of control	100	106	92	**24**	**22**
18	Mean value	1470	1307	**1246***	**348****	na
	Standard deviation	135	271	193	175	-
	No. animals	10	9	10	8	-
	% of control	100	89	**85**	**24**	-
24	Mean value	1662	1484	**1410***	**492****	na
	Standard deviation	371	74	158	159	-
	No. animals	10	10	10	10	-
	% of control	100	89	**85**	**30**	-
ChE Activity in Brain Tissue [I.U./g]						
Males						
12	Mean value	1.54	1.59	1.53	**1.23****	**1.01****
	Standard deviation	0.25	0.20	0.23	0.17	0.07
	No. animals	10	10	10	10	10
	% of control	100	103	99	**80**	**66**
24	Mean value	1.85	2.02**	1.87	**1.62****	na
	Standard deviation	0.11	0.11	0.09	0.11	-
	No. animals	10	10	10	10	-
	% of control	100	109	101	**88**	-
Females						
12	Mean value	1.36	1.41	1.37	**0.99****	**0.78****
	Standard deviation	0.13	0.19	0.17	0.12	0.08
	No. animals	10	10	10	10	10
	% of control	100	104	101	**73**	**57**
24	Mean value	1.77	1.86	1.74	**1.43****	na
	Standard deviation	0.12	0.13	0.11	0.12	-
	No. animals	10	10	10	10	-
	% of control	100	105	98	**81**	-

Sacrificed after 12 months
* Statistically different from control by Dunnett´s "t" test: $p<0.05$
** Significantly different from control by Dunnett´s "t" test: $p<0.01$
na Not applicable

Necropsy findings and organ body weights modifications: At 400 ppm in females, absolute and relative (to brain) kidney weights were statistically significantly reduced when compared to controls. Necropsy revealed no unusual macroscopic finding in male and female rats at any dose level.

Histopathology: There was evidence of changes in the liver associated with exposure to phosmet at 200 ppm dose level and higher. Liver fatty change was seen in both sexes but was not reflected in changes of serum cholesterol or triglycerides at this dose level. Males were more sensitive to the liver fatty change than females, as indicated by a significant increase in the incidence of fatty liver from the dose of 200 ppm in males removed as unscheduled deaths after 13–24 months of treatment and in all males (scheduled and un scheduled) during the study. Also, the severity of liver fatty change was notably increased in males which received 200 ppm. The histopathological evaluation of neoplastic lesions revealed no evidence of a treatment-related effect on the incidence of any tumour type in either sex.

Table 41: Incidences of relevant liver microscopic effects

(ppm):	**0**	**20**	**40**	**200**	**400**
Number of rats affected per group/number of rats examined per group					
Males:					
Fatty change 12 months interim sacrifice					
Grade 1&2	9/20	7/10	4/10	5/10	6/18
Grade 3	1/20	1/10	-/10	3/10	6/18
Grade 4&5	-/20	-/10	-/10	1/10	1/18
Fatty change 24 months terminal sacrifice					na
Grade 1&2	4/10	6/12	8/16	9/18	
Grade 3	1/10	4/12	1/16	2/18	
Grade 4&5	-/10	-/12	-/16	3/18	
Fatty change 0-12 months unscheduled deaths					
Grade 1&2	-/5	-/9	3/6	1/6	-/2
Grade 3	-/5	1/9	1/6	1/6	-/2
Grade 4&5	-/5	1/9	-/6	2/6	2/2
Fatty change 13-24 months unscheduled deaths					na
Grade 1&2	6/35	6/29	10/40	4/26	
Grade 3	3/35	9/29	3/40	5/26	
Grade 4&5	2/35	1/29	2/40	7/26	
Total	11/35	16/29	15/40	16/26*	
Fatty change for all males					
Grade 1&2	19/70	19/60	25/60	19/60	6/20
Grade 3	5/70	15/60	5/60	11/60	6/20
Grade 4&5	2/70	2/60	2/60	13/60	3/20
Total	26/70	36/60	32/60	43/60*	15/20*
Females:					
Fatty change 12 months interim sacrifice					
Grade 1&2	-/20	-/10	-/10	1/10	4/20
Fatty change 24 months terminal sacrifice					na
Grade 1&2	2/16	3/17	3/16	6/21	
Fatty change 0-12 months unscheduled deaths					
Grade 1&2	1/3	2/5	-/3	-/2	-/-
Fatty change 13-24 months unscheduled deaths					na
Grade 1&2	5/31	4/28	7/31	9/27	
Grade 3	2/31	1/28	2/31	3/27	
Grade 4&5	-/31	1/28	2/31	2/27	
Fatty change for all females					
Grade 1&2	8/70	9/60	10/60	16/60	4/20
Grade 3	2/70	1/60	2/60	3/60	-/20
Grade 4&5	-/70	1/60	2/60	2/60	-/20

* Statistically sdifferent from control by Fisher´s exact test with Bonferroni correction :p<0.05
na Not appliccable

Conclusion

Administration of phosmet to rats (Chang et al., 1991) resulted in toxicity at 200 ppm when fed for up to 2 years and at 400 ppm when fed for 1 year. Although there were changes in body weight gain, toxicity at these two dose levels was mainly manifested by statistically significant reduction in cholinesterase activities (brain and red blood cells). An increased incidence/severity of liver fatty changes was also observed from 200 ppm. At the dose level of 40 ppm toxicity consisted in statistically significant reduction in RBC cholinesterase activities in both sexes. There was no evidence of oncogenicity associated with phosmet administration.The incidence, type and severity of tumours observed in the treatment groups was comparable to the concurrent control group and in line with that expected for Sprague-Dawley rats.

In general, survival rates of both sexes of treated rats in the Chang study were comparable to or greater than controls. The median age of all unscheduled deaths was not adversely different from controls in all groups of treated animals of both sexes, i.e. the time span until death was comparable to or greater than controls. In addition, mortality rates for both sexes were in the typical ranges for Sprague-Dawley rat observed in contemporaneous studies performed with this strain in different testing facilities (Giknis and Clifford, 2001). Besides, poor survival is generally seen in long term studies with Sprague-Dawley rat. Notifier states the the shortened life-span generally seen in Sprague-Dawley rat at the time the study was conducted is associated with the “fat rat syndrome” of obesity.

The slightly higher death rate in control groups when compared to treated groups would only have biased the results such that there would be an increased likelihood of the observation of tumours in the test animals due to their greater longevity. In other words, the treated animals were at higher risk to develop tumours. However, no increase in the incidence, type or severity of neoplastic lesions was observed in any treated group.

At EPCO Expert Meeting 33 meeting 33 (12-16 September 2005) it was noted the limitations of this study, but experts concluded that it was adequate for the assessment of carcinogenicity in rats. Besides, this study was accepted by competent authorities such as EPA during the Interim Registration Eligibility Decision for Phosmet (IRED) in 2001 and WHO (JMPR, 1994, 1998), who concluded that phosmet was not carcinogenic in rat. Therefore, taking into account all the available data, the long-term/carcinogenicity study is valid and acceptable for assessing the carcinogenic potential of phosmet.

In the EFSA conclusion (EFSA, 2011) a chronic NOAEL of 20 ppm (1.1 mg/kg bw/day) was established based on the red blood cell cholinesterase inhibition at 40 ppm (about 1.8 mg/kgbw/day in males and about 2.1 mg/kg bw/day in females). Spanish CA in C&L agrees with this NOAEL.

Results of the study are supported by an earlier chronic/carcinogenicity study in rats with phosmet (Johnston, 1966; see section 4.7.1.4), where no oncogenicity were observable.

Carcinogenicity study in the mouse

Title Author (s) (year):	**Two-year dietary oncogenicity study in mice B6C3F$_1$-BR, with Imidan Technical. Katz, A.C.; Frank, D.W.; Zwicker, G.M.; Sprague G.L.; Turnier, J.C.; Freudenthal, R.I. 1984.** Additional information to this study: **2-year oncogenicity study in mice – Addendum I (Katz et al., 1986)** **2-year oncogenicity study in mice – Addendum II (Sprague, G.L., Turnier J.C. 1988)** **Neoplasm Inicidences in B6C3F1 mice: NTP Historical Data (Haseman et al., 1999)**
Administration	Two-year dietary oncogenicity study
Guideline	US EPA FIFRA 83-2 and OECD 451. Deviation with respect to guideline OECD 451: there should be done a haematology analysis at 18 months. There was a large difference between high and mid doses (100 and 25 ppm)
Species	B6C3F1 mice
GLP	Yes
Laboratory	Stauffer Chemical Company, Environmental Health Centre, Farmington,CT, USA
Purity:	94.7%
Groups	60 mice/sex/dose
Dose levels	0, 5, 25 and 100 ppm equivalent to: Males: 0, 1, 4 and 14 mg/kg bw/day Females: 0, 1.2, 5 and 18 mg/kg bw/day
	Acceptabe with some reservations

Two-year dietary oncogenicity study in mice B6C3F1-BR, with Imidan Technical. Katz, A.C.; Frank, D.W.; Zwicker, G.M.; Sprague G.L.; Turnier, J.C.; Freudenthal, R.I. 1984.

Executive Summary

In a 2-year oncogenicity study, phosmet (Imidan, technical, purity: 94.7%) was administered to groups of 60 B6C3F1 mice of each sex at concentrations of 0, 5, 25 or 100 ppm in the diet. An interim sacrifice (10 mice/sex/group) was performed at 12 months of treatment, and all survivors were sacrificed upon termination of the study at 24 months. The mean achieved daily doses were 1, 4 and 14 mg/kg bw/day in males, and 1.2, 5 and 18 mg/kg bw/day in females.

All animals were observed at least twice daily for clinical signs and mortality. Body weights and food consumption of each animal were determined weekly during the first 12 weeks of treatment and once every 2 weeks thereafter. Termination body weights were recorded on all animals. Palpation for the presence of tissue masses was performed each week. The first detection of palpable masses including size and location was noted.

Blood samples were taken from fasted anaesthetised animals (10 animals/sex/group) at 12 months and from additional animals (10 animals/sex/group) at termination. In these animals, the activity of cholinesterase was determined in red blood cells, plasma and brain tissue.

Necropsies were performed on all mice that died prematurely or were killed *in extremis* and on all surviving animals at the scheduled necropsies after 12 and 24 months of treatment. Selected organs

were weighed in mice sacrificed as scheduled after 12 and 24 months. All scheduled sacrificed mice were subjected to gross pathological assessment followed by histopathological examination.

Statistical Analysis: Quantitative continuous variables such as body weights, food consumption, clinical laboratory values, and absolute and relative organ weightes were analyzed by a one-way analysis of variance and Dunnett´s t-test to determine significance of differences. The critical level of significance in these tests was $p< 0.05$. Incidence data for gross and microscopic findings were analyzed using Fisher´s Exact Probability test. The incidence of hepatic tumors was analyzed using a level of significance of $p< 0.01$; this critical level is recommended when expected, background tumour incidence is $\geq$ 2%. Tumors were analysed by specific site, individual tumor tupe, organ system, and according to thie benign versus malignant classification.

Results

Mortality: Survival in males and females was unaffected by dietary test substance administration.

Clinical signs: The clinical observations for the most part consisted of occasional abrasions, alopecia and hair coat changes, mild lesions and scabs.

There was an incidence pattern of convulsions, among males, that appears approximately at the second half of the treatment. These convulsions were limited to occasions when animals were handled or within a short period of time after they were returned to their cages. The incidence among treated males compared to controls was statistically significant ($p<0.05$) only in the high dose group. Spontaneous, handling-induced convulsions in B6C3F1 and related strains of mice have been reported in the open literature and have been associated with extended individual housing of the test animals [Takemoto et al. (1975), King et al. (1955), Serota et al. (1986)]. B6C3F1 mice were caged individually in this study. Contrary, cholinergic convulsions are life-threatening events and they would be expected to be associated with mortality in at least some of the test animals. Convulsions observed in the study did not appear to be correlated with brain cholinesterase inhibition and were not associated with unscheduled (early) deaths. There was no evidence of any treatment-related effect on the other clinical signs of toxicity which typically precede or accompany cholinergic convulsions (*e.g.* diarrhea, salivation, lacrimation, miosis, coma).

Palpable masses were observed in the inguinal or pelvic area in approximately half of the males in this study. The incidence of this finding in each of the treated groups was comparable to that of the controls. Many of these masses observed in life were later diagnosed as preputial gland cysts, which commonly occur in male mice. No relevant difference was noted between treated females and the controls.

Body weights: The mean body weights of treated males for all doses were generally comparable to control values during the initial 3 months of treatment. At most of the biweekly determinations performed thereafter, the body weights of the high dose males were slightly but significantly greater ($p< 0.05$) than those of the controls. Body weights of the high dose females were also slightly elevated compared to controls from months 2 to 18 of treatment. At termination (week 104), high dose males weighed approximately 8 % more than controls, while high dose females weighed approximately 2 % less than controls. None of the differences between mean body weights for high dose vs. control animals were considered biologically significant. No trends in body weights were apparent for low or mid dose males or females.

Food consumption: Analysis of food consumption data revealed transient differences from control values for animals given the highest dietary concentration. Reduced food consumption and/or wastage occurred in high dose males intermittently during the first 9 months of the study, and in high dose females at most weekly determinations during the first 3 months. Since no concomitant body weight reduction was found, it is likely that the reduced food depletion noted for the high dose

groups during these periods reflects increased food efficiency and/or reduced spillage. No consistent or meaningful food consumption / wastage changes occurred in any other treated groups.

Haematology: Treatment was not related to haematological changes since all mean haematological values for males and females at termination were found to be within normal limits and no biologically significant changes were attributable to treatment.

Imidan produced cholinesterase inhibition in mice. Due to technical difficulties, measurements of RBC cholinesterase activity were not obtained at 12 months.

Plasma cholinesterase activity: Significantly reduced at 100 ppm (>20%) after 12 and 24 months for both sexes. At 25 ppm there was a slight (13%) statistically significant reduction in females after 12 months but not at termination.

Red blood cell cholinesterase activity: Not measured after 12 months. Not relevant changes after 24 months.

Brain cholinesterase activity: Mean brain cholinesterase values at 12 months for all doses were reduced with statistically significance by 22%-31% in phosmet treated males and by 28%-34% in phosmet treated females. At the termination of the study, no significant changes in brain cholinesterase were seen in males, while activity levels in mid and high dose females were reduced with statistically significance by 14 and 22%, respectively with a dose-relationship.

Table 42: Summary of cholinesterase activity as per cent of controls

		0 ppm	**5 ppm**	**25 ppm**	**100 ppm**
Males:					
Plasma I.U./l					
After 12 months	Mean value	7089	6316	6187	**3278***
	Standard deviation	633	1188	828	674
	No. animals	8	10	10	10
	% of control	100	89	87	**46**
After 24 months	Mean value	7264	6992	6597	**3828***
	Standard deviation	1898	1663	2371	1870
	No. animals	10	10	10	9
	% of control	100	96	91	**53**
RBC I.U./l					
After 12 months	Mean value	8861	nd	nd	nd
	Standard deviation	450	-	-	-
	No. animals	8	-	-	-
	% of control	100	-	-	-
After 24 months	Mean value	7892	6532*	8804	7704
	Standard deviation	1390	769	1176	1214
	No. animals	10	10	10	10
	% of control	100	83	112	98
Brain I.U./g protein					
After 12 months	Mean value	63.1	**44.9***	**49.1***	**43.6***
	Standard deviation	3.8	3.4	4.4	2.4
	No. animals	10	10	10	10
	% of control	100	**71**	**78**	**69**
After 24 months	Mean value	52.6	49.7	52.8	50.1
	Standard deviation	3.4	3.4	8.2	6.2
	No. animals	10	10	10	10
	% of control	100	94	100	95

		0 ppm	5 ppm	25 ppm	100 ppm
Females:					
Plasma I.U./l					
After 12 months	Mean value	8270	9133	**7175***	**3675***
	Standard deviation	820	1124	730	487
	No. animals	10	10	9	10
	% of control	100	110	**87**	**44**
After 24 months	Mean value	8158	8568	7613	**3950***
	Standard deviation	1119	1597	1386	781
	No. animals	10	10	10	9
	% of control	100	105	93	**48**
RBC I.U./l					
After 12 months	Mean value	9768	nd	nd	nd
	Standard deviation	765	-	-	-
	No. animals	10	-	-	-
	% of control	100	-	-	-
After 24 months	Mean value	7664	6606	8120	7962
	Standard deviation	767	928	1497	1035
	No. animals	10	10	10	10
	% of control	100	86	106	104
Brain I.U./g protein					
After 12 months	Mean value	68.4	**49.0***	**47.0***	**45.1***
	Standard deviation	5.4	3.1	4.9	3.5
	No. animals	10	10	10	10
	% of control	100	**72**	**69**	**66**
After 24 months	Mean value	55.3	53.2	**47.6***	**43.1***
	Standard deviation	5.4	5.3	4.1	6.7
	No. animals	10	10	10	10
	% of control	100	96	**86**	**78**

* statistically significant compared (Dunnett-t-test) to controls ($p<0.05$)
Nd: Not determined

Organ weight modifications: although a transient increase in mean relative liver weight at 100 ppm in males at the interim sacrifice was observed, the mean absolute and relative organ weights of male and female treated mice were comparable to those of their respective controls at termination.

Necropsy findings: At necropsy, there were no observations for scheduled or unscheduled deaths which suggest a relationship to dietary treatment. The number and nature of necropsy findings, including masses, were considered of normal incidence and degree in each group of this mouse strain. Necropsy observations indicated that liver tumours did not contribute to mortality. Treatment did not appear to affect the period of latency of liver tumour development. The mean ages at death of mice with hepatocellular tumours were comparable (*i.e.* 668 days for high dose males vs. 630 days for controls).

Histopathology

Non neoplastic findings

The incidence of hepatic foci of alteration was similar in all groups of mice regardless of the cytoplasmic straining whether eosinophilic, basophilic or mixed. Thus, there was no evidence of an increase in cellular changes considered putative of hepatocarcinogenicity in the male mice treated with 100 ppm.

The livers of 100 ppm male mice showed two patterns of mild vacuolative degenerative change which may have been related to treatment (individual randomly scattered hepatocytes contained either a single large cytoplasmic vacuole or a cluster of small vacuoles imparting a foamy appearance or randomly located clusters of foamy hepatocytes, some of which had poorly defined cytoplasmic vacuoles). Vacuolation of hepatocytes in female mice within dose groups was of random lobular orientation and number and therefore not considered compound-related. Other non-neoplastic findings did not have an incidence or severity pattern suggestive of a treatment effect.

Table 43: Selected incidences of microscopic effects at terminal sacrifice after 24 months – Non neoplastic findings

	0 ppm	**5 ppm**	**25 ppm**	**100 ppm**
	No. of mice affected/No. of mice examined			
Males:				
Liver				
Vacuolic degeneration (single cells)	3/49	1/50	2/50	16/50
Females:				
Liver				
Vacuolic degeneration (single cells)	-/49	-/50	-/48	-/50

Neoplastic findings

Evaluation of the distribution of all tumours shows that there was a significant increase in hepatic adenomas in 100 ppm males. Histologic evidence of adenomas included autonomous hepatocytic proliferation, lack of normal lobular architecture and compression of adjacent liver cells. Liver tumours were not found in females or control males that either died or were sacrificed during the first year of treatment. During the first year of treatment hepatic adenomas were found in one 5 ppm, two 25 ppm and two 100 ppm males. Hepatocellular carcinoma was also found in one 25 ppm and one 100 ppm male. Liver tumor malignancy was characterized by histologic evidence of anaplasia, vascular invasion and trabecular formation.

In male mice which died or were sacrificed during the second year, hepatocellular adenomas were found in 13 controls, 9 of the 5 ppm, 12 of the 25 ppm and 25 of the 100 ppm dose group animals. For the second year, the incidence of hepatic adenoma among female mice was 6, 4, 5 and 11 in the 0, 5, 25 and 100 ppm dose groups, respectively. Hepatocellular carcinoma was found in 13 controls, 11 of the 5 ppm, 10 of the 25 ppm and 13 of the 100 ppm male dose groups. There were 5, 4, 3 and 9 females with hepatocellular carcinoma in the 0, 5, 25 and 100 ppm dose groups, respectively. Pulmonary metastasis of hepatocellular carcinoma was similar in incidence in all male groups: 2 in controls, 4 in the 5 ppm, 2 in the 25 ppm and 3 in the 100 ppm group. Pulmonary metastases were found in the lungs of 2 female mice of the control group. No hepatic tumour metastasis was found in female mice after dietary treatment (Table 44).

Over the course of the entire study, combining the total number of animals with bening and /or malignant hepatocelullar tumors, there were 23, 21, 23 and 35 respectively in the 0, 5, 25 and 100 ppm male groups. Females had 10, 8, 8 and 18 tumors respectively in the 0, 5, 25 and 100 ppm groups. While the incidences were 3, 0, 2 and 6 for males and 1, 0, 0 and 2 for females, considering both adenoma and carcinoma in the 0, 5, 25 and 100 ppm groups (Table 46).

Hepatic adenomas were significantly increased in male mice given 100 ppm for two years. The authors stated that the biological significance of this observation is questionable based on the following findings:

- The distribution of hepatic adenomas has no overt biological implication since the distribution of hepatocellular carcinoma(s) was similar in all male dose groups. Likewise, the incidence of pulmonary metastasis of malignant liver tumours was similar

in all groups of male mice. These two observations suggest that phosmet treatment was not associated with malignancy in male mice.

- Evaluation of the individual data collected for two years reveal that most liver tumors were inicidental fingings at scheduled sacrifice. Necropsy observations unfrequently suggest that the liver tumor was the principal or contributory cause of death. Review of these data also indicate that mortality was not increased by treatment. Phosmet treatment don´t appear to affect the period of latency for the development of the liver tumors. A review of data for high dose and control males which died or were sacrificed during the second year, but prior to the scheduled termination, reveal that the mean ages at death of mice with hepatocellular tumors were comparable.
- Benign tumors were increased only in one dose group of one sex.
- Tumor incidence of hepatic tumors is considered within the normal background incidence for this strain of mice.

Harderian gland adenomas were increased in 5 and 100 ppm male mice but not in 25 ppm males. Two harderian gland tumours in 5 ppm males showed histological evidence of malignancy characterized by anaplasia, abortive tubule formation and stromal and/or capsular invasion. Because there was no clearly defined relationship to treatment, the distribution of these tumours is considered incidental. In females one adenocarcinoma was seen at 5 and 100 ppm (see Table 44).

The other types of tumours had either an incidence within the background expected in a study of this type or showed a statistical relationship to treatment.

Table 44: Selected incidences of microscopic effects after 12 months (interim sacrifice) + after 24 months (terminal sacrifice) – Neoplastic findings

	0 ppm	**5 ppm**	**25 ppm**	**100 ppm**
	No. of mice affected/No. of mice examined			
Males:				
Liver				
Hepatocellular adenoma	13/49	10/50	14/50	27/50
Adenocarcinoma	-/49	-/50	-/50	1/50
Hepatocellular carcinoma	13/49	11/50	11/50	14/50
Lymphoma	1/49	-/50	2/50	-/50
Hemangiosarcoma	-/49	-/50	3/50	1/50
Reticulum cell sarcoma	1/49	3/50	1/50	-/50
Sarcoma	-/49	1/50	-/50	1/50
Females:				
Liver				
Hepatocellular adenoma	6/49	4/50	5/48	11/50
Hepatocellular carcinoma	5/49	4/50	3/48	9/50
Lymphoma	6/49	6/50	7/48	6/50
Strom cell sarcoma	-/49	-/50	-/48	1/50
Hemangio sarcoma	-/49	-/50	2/48	-/50
Fibrosarcoma	-/49	1/50	-/48	-/50
Males				
Harderian gland				
Adenoma	3/49	7/50	4/49	9/50
Lymphoma	-/49	-/50	1/49	-/50
Adenocarcinoma	-/49	2/50	-/49	-/50
Females				
Harderian gland				
Adenoma	1//48	-/15	2/11	2/49

	0 ppm	5 ppm	25 ppm	100 ppm
	No. of mice affected/No. of mice examined			
Adenoma, papillary cyst	1/48	-/15	-/11	-/49
Adenocarcinoma	-/48	1/15	-/11	1/49
lymphoma	1/48	-/15	2/11	3/49

Statistics: According to study authors, hepatic tumors were analysed with a level of significance of $p < 0.01$ and stated that hepatic adenomas were significantly increased in males at 100 ppm.

Historical data (Katz et al. (1986); addendum I),

Historical control data on the incidence of hepatocelullar adenoma and hepatocelullar carcinoma in B6C3F1 mouse was generated in a 2-year study (Katz et al. (1986); addendum I), with the same strain of mice used for the study of Katz et al. (1984) purchased from the same supplier, housed in the same laboratory at approximately the same time and with the same principal pathologist. Sixty B6C3F1 mice of each sex were used for the study. Ten from each sex were sacrifice from each group at 12 months.

Results

Mortality: the total number of unscheduled deaths for males and females was 17 and 20 or 34% and 40%, respectively. Survival at 18 months (72 weeks) was 92% for males and 94% for females. Twenty-four months survival was 66% for males and 60% for females.

Liver Tumors in Historical Controls: the incidence of adenomas, carcinomas or either tumor was tabulated for male and female B6C3F1 mice in Table 45.

Table 45: Liver tumors in historical controls

	Incidence (affected/total, %)	
Tumor type	**Male**	**Female**
Hepatocellular adenoma(single or multiple)	25/60 (42%)	9/60 (15%)
Hepatocellular carcinoma (single or multiple)	10/60 (17%)	3/60 (5%)
Either hepatocellular adenoma or carcinoma.	31/60 (52%)	11/60 (18%)

Table 46 summarizes the liver tumor incidences for each group of the Katz et al. (1984) study and in the historical control group (0´) of Katz et al. (1986) study. Statistically significant trends and differences in incidences are also shown:

Table 46: Incidence of liver tumours in mice (12+24 months)

Tumor	Sex	0´	0	5	25	100	Trend [a]
Adenoma	**M**	25/60* (42%)	13/60 (22%)	10/60 (17%)	14/60 (23%)	27/60 (45%)	Increased
	F	9/60 (15%)	6/60 (10%)	4/60 (7%)	5/59 (8%)	11/60 (18%)	No Trend
Carcinoma	**M**	10/60 (17%)	13/60 (22%)	11/60 (18%)	11/60 (18%)	14/60 (23%)	No Trend
	F	3/60 (5%)	5/60 (8%)	4/60 (7%)	3/59 (5%)	9/60 (15%)	No Trend
Tumor	**Sex**	**0´**	**0**	**5**	**25**	**100**	**Trend [a]**
Adenoma or carcinoma	**M**	31/60 (52%)	23/60 (38%)	21/60 (35%)	23/60 (38%)	35/60 (58%)	Increased
	F	11/60 (18%)	10/60 (17%)	8/60 (13%)	8/59 (14%)	18/60 (30%)	No Trend

Adenomas and carcinoma	**M**		3/60 (5%)	-/60	2/60 (3.3%)	6/60 (10%)	
	F		1/60 (1.7%)	-/60	-/60	2/60 (3.3%)	

[a] Mantel-Haenszel trend analysis was used to compare tumor incidences in Katz, 1984 study using the concurrent control group (0 ppm). Incidences in the concurrent and historical (0´ppm) control groups were compared using Chi-squared analysis, and statistically significant differences are indicated by *. P< 0.05 was used in all comparisons.

In the Katz et al. (1984) study, there was a significant trend, the more increasing phosmet concentration, the more increased incidence of adenomas and either adenomas/carcinomas in the livers of male mice. In both cases, the incidence at 100 ppm was significantly greater than in the concurrent control group; however it was not significantly greater than the incidences in the historical control group, the incidence was only slight increased. In addition the incidence of adenomas in males of the historical control was significantly greater than in the concurrent control group of the Katz et al. (1984) study.

Report Addendum II: Two-year dietary oncogenicity study in mice with Imidan Technical. Sprague and Turnier (1988).

Objective: The purpose of this addendum is to correct an error that was discovered after the report was issued. The error was discovered for animal number 687 when data were reviewed.

Guidelines:US EPA Guideline: 83-2. OECD Guideline: 451. GLP: yes

This study is acceptable

Results

With respect to 100 ppm female 687, one adenocarcinoma detected does not involve the mammary gland. It was erroneously entered under mammary gland/skin.

Gross necropsy observation of mass:

"Soft subcutaneous bulge around left eye, 1 cm diameter. Skin removal reveals moderately firm, multilobulated tan mass envolving left side of face replacing frontal bones and tissues anterior to eye. Left eye caught up in caudal aspect of mass"

Microscopic description of mass:

"this expansive, invasive and highly cellular mass unilaterally involves the left periocular region and nasal cavity. The juxtaposed left eye is atrophied but uninvaded by neoplastic cells. Cells comprising the mass are arranged in haphazard sheets and island as well as glandular/tubular/duct-like structures. Finely reticulated round to oval basophilic nuclei containing variably prominent nucleoli are surrounded by variable abundant lightly eosinophilic foamy cytoplasm. Mitotic figures are numerous. The mass extend to a focally ulcerated cutaneous surface. Multiple metastases are present in the lung. The site of origin was most probably the Harderian Gland.

Conclusion

Morphologic diagnosis: Adenocarcinoma, Harderian Gland.

Neoplasm Incedences in B6C3F1 Mice: NTP Historical data. Haseman J.K., Elwell M.R, Hailey J.R. 1999.

Further historical control data (Haseman et al., 1999) were supplied. These data cover 25 years and 400 long term carcinogenicity studies with the same strain of mice (B6C3F1) conducted by NTP (National Toxicological Program) and the National Cancer Institute /NCI). These studies generally consist of two or three dose levels of the test material and an untreated or vehicle control group with 50 animals per group. NTP studies provide the single largest source of neoplasm incidence data for

B3C3F1 mice. Background neoplasm incidence is given for untreated and chamber control B6C3F1 mice from NTP two-year feeding and inhalation carcinogenicity studies. The procedures used to validate neoplasm incidences in these studies include a multistage quality assurance procedure to verify and assure that the pahotlogy data are consistent and accurate.

Table 47: Tumor incidences in control B6C3F1 mice from NTP carcinogenicity studies

	Male						**Female**					
	Untreated			**Chamber Controls**			**Untreated**			**Chamber Controls**		
	Mice with tumors	**Rate (%)**	**Range (%)**	**Mice with tumors**	**Rate (%)**	**Range (%)**	**Mice with tumors**	**Rate (%)**	**Range (%)**	**Mice with tumors**	**Rate (%)**	**Range (%)**
LIVER	(1350)			(1097)			(1350)			(1089)		
Adenoma	397	29.4	4-60	279	25.4	4-48	234	17.3	2-50	154	14.1	2-40
Carcinoma	241	17.9	6-29	244	22.2	11-46	113	8.4	0-20	150	13.8	0-38
Adenoma or Carcinoma	570	42.2	10-68	479	43.7	11-76	319	23.6	6-56	274	25.2	3-54

These historical controls showed that the incidence in hepatocellular adenoma in male mice was in the range from 4%-60%. So, the liver cell adenoma incidences in the high dosed males in the Katz et al. (1984) study was fully within the normal range of the historical NTP/NCI control data for the B6C3F1 strain of mice. These historical control, were ruled out by experts during the peer review, due to the high variability presented [17704/EPCO/BVL/05 rev. 2.1 (08.05.2006)].

Conclusion

In the 2-year mouse study, the liver was the target organ. Hepatic degenerative changes were observed as well as increased incidence of liver tumours. As it has been commented throughout this document, biologically relevant reductions of brain and RBC AChE are regarded as critical toxicological end point for human health risk assessments.

The EPCO 33 Experts' Meeting originally considered that a NOAEL could not be established since the brain AChE activity was decreased (>20%) in the low dose group at interim sacrifice (12 months). It was noted that the study report indicated that the incidence of convulsions increased at the end of the first year in males. Therefore, the agreed LOAEL was 5 ppm (1 mg/kg bw/day).

In March 2010, UK proposed the reevaluation of the IDA following the submission of additional Annex II data. The applicant provided the following new information and studies:

I. A comprehensive re-examination of raw data (clinical observation) from the chronic mouse study as well as other supporting published papers

   The raw clinical data on the convulsions were not available at the EPCO 33 Experts' Meeting and indicated that the convulsions were observed during the second half of the study. The pattern of convulsions does not indicate an effect due to cholinesterase inhibition since they were observed only in males in the second half of the study whereas brain cholinesterase inhibition was observed during the first one. Besides, a series of supporting published papers (Takemoto et al., 1975; King et al., 1955; Serota et al., 1986) that were not part of the original dossier, supportted the occurrence of spontaneous, handling-induced convulsions in B6C3F1 and related strains of mice, associated with extended individual housing of the test animals.

II. A relevant cholinesterase data from the chronic mouse study:

   A review of the study records indicated also that methodological deficiencies in the long-term study in mice may put the reliability of the brain cholinesterase measurements at interim

sacrifice after 12 months into question as concurrent cholinesterase controls were not employed (cholinestarase assays were performed on different days for control and treated groups in this study). When concurrent controls are not utilized, treated tissues cannot be compared with untreated tissues that have been assayed on the same day and under the same test conditions, using the same preparations of reagents and standards. The applicant states it is a well-kown occurrence to observe shifts in cholinesterase responses of similarly dosed animals or tissues when assayed at different times, and this was seen in the interim sacrifice in the two-year mouse study. It was the opinion of the applicant that the chronic mouse brain cholinesterase data should not be used for regulatory purposes. They considered that in addition to inconsistencies between the 1-month and 12-month mouse brain cholinesterase data noted by EPCO, the apparent 12-months brain ChE effects were not dose-responsive and comparable levels of inhibition were seen at all dose. Moreover, the 24-month data confirm the absence of a treatment related effect at 12-month interval. At study termination, no inhibition was seen in the male mouse brain at any treatment level.

III. Two new *in vitro* studies examining comparative inhibition of acethylcholinesterase (Barnett, 2007; Hobermann, 2008).

Higher sensitivity of RBC than of brain cholinesterase was shown in the mouse mechanistic study of Barnett (2007). The *in vitro* mechanistic study of Hoberman (2008) indicated that inhibition of plasma and RBC AChE was more pronounced in rats than that observed in humans. However, these *in vitro* studies were performed whitout metabolic activation system and they were not considered acceptable. The active metabolite of phosmet is an oxon derivative that needs the presence of metabolic activation system to be formed. Therefore, the *in vitro* studies express the AChE inhibition of the parent compound only and not of the main metabolite and they are not relevant.

During the re-discussion at the PRAPeR 86 Experts' Meeting in March 2011, it was agreed that "the brain cholinesterase inhibition at the low and mid doses, was only observed at the interim kill, without dose dependence and not associated to convulsions, was not an adverse effect". For convulsions it was suggested that the mid dose level of 25 ppm (4 mg/kg bw/day) could be considered as a NOAEL. For brain cholinesterase inhibition, a NOAEL of 25 ppm was identified in females after 24-months, using 20% inhibition as a cut-off criterion. After 24 months, no brain cholinesterase inhibition was observed in males. Therefore, according to EFSA Peer Review of Phosmet (2011), a NOAEL of 4 mg/kg bw/day was established based on an statistically increased incidence of convulsions in males, brain cholinesterase inhibition in females and histopathological findings in the liver of males (cytoplasmic hepatocellular vacuolic degeneration) at the highest dose group (14 mg/kg bw/day).

In our opinon, the apparent change of brain cholinesterase activity at the time of interim kill doesn´t seem to be attributed to phosmet administration. There are serious doubts on the validity of the one-year findings. The methodological deficiences in the long-term study in mice may put the reliability of the brain cholinesterase measurements at interim sacrificie after 12 months into question.

Brain cholinesterase activity was not different from the controls at the 5 ppm level at terminal sacrifice after 24 months in both sexes. However, the statistically significant and dose-related brain cholinesterase inhibition in female mice at the two upper dose levels at study termination after 24 months appears a valid finding, because controls were apparently examined concurrently. Therefore, giving the results obtained at study termination, the MSCA in C&L regards a relevant adverse effect the statistically significant dose dependent decrease of brain AChE (14%) at 25 ppm observed in females after 24 months (terminal sacrifice). Therefore, the MSCA considers a LOAEL of 5 mg/kg bw/day.

A statistically significant inhibition of brain, peripheral nerve or erythrocyte AChE ≥ 20% with respect to the concurrent control group or with respect to the 'pre-exposure' values in the treated group is considered toxicologically relevant ('adverse'). However, even statistically significant inhibition of less than 20% or statistically insignificant inhibitions above 20% indicates that a more detailed analysis of the data should be undertaken (JMPR, 1998; US EPA, 2000; Nielsen et al., 2008; EFSA, 2013).

The EFSA Panel on Plant Protection Products and their Residues (PPR) provided a scientific Opinion (EFSA, 2013) on the identificaction of pesticides to be included in the cumulative assessment groups (CAGs) based on their toxicological profile from the basis of datasets of oral toxicity studies evaluated in the Draft Assessment Reports (DARs) for pesticides having effects on thyroid or nervous system. It was decided that data collection needed to be re-evaluated with the aim of identifying adverse effects of pesticide active substances, among others, on the nervous system. The PPR panel regarded re-evaluated data provided from an external scientific report by the Danish Technical University (DTU) published by the EFSA in 2012 and a further revision of the DTU data published in 2013 (ANSES/ICPS/RIVM). The PPR panel recognized inhibition of brain and erythrocyte cholinesterase as a neurochemical effect which represents a level of grouping for neurotoxic substances based on mechanism of action rather that on phenomenological effects. Accordingly, phosmet was grouped in the acute and chronic CAGs for the nervous system based on neurochemical endpoints (inhibition of brain AChE). It has to be noted that a chronic LOAEL of 1 mg/kg bw/day for phosmet is established in this document (EFSA, 2013) based on brain AChE inhibition.

The treatment at 100 ppm was associated with a significant increase in liver cell adenomas in male mice and it was showed a significant trend, the more increasing phosmet concentration, but was not associated with earlier development of liver tumours. In addendum I to the report, data of historical controls indicated that the prevalence of liver adenomas in males of the highest dose group was only slightly higher than in the historical control. Besides, Haseman et al. (1999) reported neoplasm incidences in B6C3F1 mice originated from more than 400 NTP (U.S. National Toxicology Program) and NCI (U.S. National Cancer Institute) long-term carcinogenicity studies. According to this data compilation, the incidences of liver adenomas and carcinomas seen in the Katz et al. (1984) regulatory mouse study were fully in the range of the historical NTP/NCI control data for the B6C3F1 strain.

The carcinogenic potential of phosmet in mice was discussed by the Experts during the EFSA Peer Review (EFSA 2006a). In the Evaluation Table of phosmet [17704/EPCO/BVL/05 rev. 2.1 (08.05.2006)] it was commented that those values presented a high variability and they couldn't be used as historical control range.

However, it has been reported in the Guidance on the Application on the CLP Criteria (ECHA, November 2013) that liver tumours in B6C3F1 mice is one of the examples of animal tissues with a high spontaneous tumour incidence.

Therefore, based on the fact that the tumours occurred in the highly sensitive B6C3F1 strain of mice it can be considered that the significant increase in liver cell adenomas in the high dosed males is of uncertain biologic significance and of questionable relevance.

4.9.1.2 Carcinogenicity: inhalation

No inhalation carcinogenicity studies were conducted with phosmet.

4.9.1.3 Carcinogenicity: dermal

No dermal carcinogenicity studies were conducted with phosmet.

4.9.2 Human information

There are no human data available relevant for C&L. For further details on phosmet-related toxicity in humans, please refer to paragraph 4.11.1.4.

4.9.3 Other relevant information

No other relevant information is available.

4.9.4 Summary and discussion of carcinogenicity

In a two-year combined chronic toxicity/oncogenicity study, Sprague-Dawley rats were treated with 0, 20, 40, 200 and 400 ppm of phosmet equivalent to 0, 1.1, 1.8, 9.4 and 23 mg/kg bw/day for males and 0, 1.1, 2.1, 10.9 and 27 mg/kg bw/day for females (Chang et al., 1991). Administration of phosmet resulted in toxicity at 200 ppm when fed for up to 2 years and at 400 ppm when fed for 1 year. Although there were changes in body weight gain, toxicity at these two dose levels was mainly manifested by statistically significant reduction in cholinesterase activities (brain and red blood cells). An increased incidence / severity of liver fatty changes were also noted from 200 ppm. At the dose level of 40 ppm toxicity consisted in statistically significant reduction in RBC cholinesterase activities in both sexes. There was no evidence of oncogenicity associated with phosmet administration. The incidence, type and severity of tumours observed in the treatment groups was comparable to the concurrent control group and in line with that expected for Sprague-Dawley rats.

In a two-year dietary oncogenicity study, B6C3F1 mice were fed with a diet containing 0, 5, 25 or 100 ppm of phosmet equivalent to 0, 1, 4 and 14 mg/kg bw/day for males and 0, 1.2, 5 and 18 mg/kg bw/day for females (Katz et al, 1984). A statistically significant increase of convulsions was observed in males at 100 ppm during the second half of the study. Plasma cholinesterase was significantly reduced at 100 ppm (>20%) after 12 and 24 months for both sexes. Brain cholinesterase values at 12 months for all doses were statistically significant reduced by 22%-31% in phosmet-treated males, and by 28%-34% in the treated females. At the termination at 24 months there was statistically significant inhibition of brain cholinesterase in females at 25 ppm (14%) and at 100 ppm (22%) with dose-relationship. Target organ for systemic toxicity was the liver since cytoplasmic hepatocellular vacuolic degeneration was increased in high dose males at termination. The treatment at 100 ppm was associated with a statistically significant increase in liver cell adenomas in male mice not connected with earlier development of liver tumours and showed a significant trend (the more increasing of phosmet the more increased incidence). At this dose level the incidence of hepatocellular adenomas was higher (27/60 in males, 11/60 in females) than in the concurrent control group (13/60 in males, 6/60 in females). The incidence in the historical controls provided by the Testing Facility was 25/60 in males and 9/60 in females (Katz et al., 1986). Thus, the incidence of liver adenomas in males at the top dose level was only slightly higher than the historical controls (45% *vs*. 42%).

Haseman et al. (1999) reported neoplasm incidences in B6C3F1 mice originated from more than 400 NTP (U.S. National Toxicology Program) and NCI (U.S. National Cancer Institute) long-term carcinogenicity studies studies. According to this data compilation, the incidences of liver adenomas and carcinomas seen in the Katz et al. (1984) regulatory mouse study were fully in the range of the historical NTP/NCI control data for the B6C3F1 strain. Due to the high variability of the data (4%-60%), they were not regarded to be used as historical control range [Evaluation Table of Phosmet, 17704/EPCO/BVL/05 rev. 2.1 (08.05.2006)].

The significance of the increase in the incidence of hepatocellular adenoma in high dosed mice is questionable since the B6C3F1 strain of mouse has been well established as sensitive to liver tumour induction. The Guidance on the Application on the CLP Criteria (ECHA, November 2013)

has reported that liver tumours in B6C3F1 mice is one of the examples of animal tissues with a high spontaneous tumour incidence and a cautious view of these kind of tumours have to be done.

Additionally phosmet was tested for its potential to induce morphological transformation of cells. Phosmet did not induce morphological transformation of BALB/3T3 mouse cells (Dickey, 1986; see section 4.12.1.6).

In the ECB TC C&L it was agreed not to classify phosmet for carcinogenicity. The EFSA Peer Review of Phosmet (EFSA, 2011) did not include a proposal of classification regarding the carcinogenic potential of this substance but it mentioned that "the experts could not agree on the proposed classification as carcinogenic with the application of R40".

In our opinion, although the mode of action for induction of the tumours has not been clarified, the tumours occurred in the highly sensitive B6C3F1 strain of mouse. As phosmet is non-genotoxic, and no increases in liver tumours were seen in exposed rats, in our opinion the findings were most likely to have been specific to the strain and specie tested.

4.9.5 Comparison with criteria

The only tissue that showed evidence of increased tumour incidence was the liver in mice. The treatment at 100 ppm was associated with a statistically significant increase in the liver cell adenomas in male mice and there was a trend in the incidence of hepatocellular adenoma and hepatocellular adenoma carcinoma with increasing exposure in males.

As there is no epidemiological evidence regarding the carcinogenicity of phosmet to humans a classification in Category 1A is not appropriate.

Although evidence for carcinogenic response was found in mice, the tumour type found was largely sex-specific and there are significant doubts about the relevance to humans, and given that phosmet is not genotoxic, classification in Category 1B is also judged inappropriate.

Looking specifically at the criteria for deciding between category 2 and no classification, the Spanish MSCA conclude that phosmet doesn´t meet the criteria for classify phosmet into this category. After a detailed review of the available data (the B6C3F1 strain of mouse has been well stablished as sensitive to liver tumour induction, no other tumour type was detected in mice and no increases in liver tumours were seen in exposed rats; there is a robust genotoxicity/ mutagenicity database which confirms that phosmet is neither genotoxic nor mutagenic *in vivo*) the liver tumour findigs would not in themselves justify classification of phosmet.

4.9.6 Conclusions on classification and labelling

The classification criteria for carcinogenicity were not fully fulfilled. This is in agreement with the former discussion at ECB level and current classification and labelling in Annex VI, Table 3.1 of Regulation (EC) No 1272/2008.

CLP: A classification is not required.

4.10 Toxicity for reproduction

Table 48: Summary table of relevant reproductive toxicity studies

Method	Main results	Remarks	Reference
2-generation study EPA FIFRA Pesticide Guideline 83-4 and OECD 416 GLP: Yes **Study acceptable**	Parental toxicity 300 ppm: Statistically and biologically significant reduction (>20%) of RBC AChE, clinical signs (impaired general health status, dehydration in P0 females and chromorhinorrea in P1 females), significant absolute and relative organ weight reductions, liver impairment (mild to moderate centrilobular hepatocellular vacuolisation in P1 males) and significant decreasings in body weight, body weight gain and food consumption during growth, gestation and lactation periods. 80 ppm: Clinical signs (dehydration in P0 females), body weight gain reductions during growth, gestation and lactation periods that were statistically significant in P0 males on days 7 (14.9%), 21 (14.3%) and 112 (60%) of growth period and during lactation in P0 females (1st mating) on day 14 (105.4%), significant decreases of relative weight of liver and adrenals in P0 females and spleen in P1 females and statistically significant and biologically relevant reductions (>20%) in RBC cholinesterase activities. **NOAEL: 20 ppm (1 - 1.8 mg/kg bw/day)** Reproductive toxicity 300 ppm: Significant reduction in fertility indexes in P0 to generate F1b litters and P1 to generate F2a and F2b litters. Mating index decreased with statistical significance at 300 ppm in P1 for F2b litters. Significant changes in the weight of some reproductive organs ocurred in P0 and P1 males with reduction of absolute weight of testes and in P0 females with reduction of the relative weight of ovaries. Besides, significant decrease in the number of total born pups delivered was observed in F1b, F2a and F2b litters. 80 ppm: significant reduction of fertility indexes observed from 80 ppm in P0 to generate F1b litter. **NOAEL: 20 ppm (about 1 - 1.8 mg/kg bw/day)** Developmental toxicity 300 ppm: Significant reduction of numbers of total born pups delivered and live born pups per litter (postpartum day 0) in F1b, F2a and F2b litters. Significant decrease in live pups per litter (postpartum day 21) in F1a, F2a and F2b litters and in the pup bodyweight (day 21) in all generations. **NOAEL: 80 ppm (about 4.2-7.3 mg/kg bw/day)**	CD rats Purity: 95.2% (w/w) Dose range (diet): 0, 20, 80, 300 ppm equivalent to 0, 1-1.8, 4.2-7.3, 16.4-25.5 mg/kg bw/day male-female respectively	**Meyer, L.S. and Walberg, J.A. (1990)**
Prenatal developmental toxicity US EPA FIFRA Pesticide Guideline 83-3 GLP: Yes **Study acceptable**	Maternal toxicity: Statistically significant reduction on food consumprion at 15 mg/kg bw/day and on body weight gain from 10 mg kg/bw/day. **NOAEL: 5 mg/kg bw/day** Developmental toxicity: no indication of developmental toxicity or teratogenicity. **NOAEL: ≥ 15 mg/kg bw/day**	Alpk AP_fSD rat Purity: 96.4 % (w/w) Dose range (gavage): 0, 5, 10, 15 mg/kg bw/day	**Hodge, M.C.E. (1991)**
Prenatal developmental toxicity (range-finding study) No guideline GLP: No **Study acceptable as a range-finding study**	Main effects: At 15 mg/kg bw/day one female died and two animals, including the dead animal, showed clinical signs of organophosphorous compounds. Slight indications of impairment in foetal development were noted from 10 mg/kg bw/day onwards. Phosmet did not cause external malformations or variations in this range-finding study.	New Zealand White rabbit Purity: 96.4 % (w/w) Dose range (gavage): 0, 5, 10, 15 mg/kg bw/day	**Pinto, P.J. (1991)**

Method	Main results	Remarks	Reference
Prenatal developmental toxicity US EPA FIFRA 83-3 (virtually identical to OECD 414) GLP: Yes **Study acceptable**	Maternal toxicity: at 15 mg/kg bw/day two deaths than can be treatment related, clinical signs (unsteady gait, salivation, increased breathing rate and shaking) and significant maternal body weight decrease. NOAEL: 5 mg/kg bw/day Developmental toxicity: at 15 mg/kg bw/day slight foetotoxicity manifested primarily in form of few areas of reduced or lacking ossification by increased percentage of foetuses with unossified 5th sternebra and unossified 6th sternebra on a litter basis. The significant increase in the incidence of odontoid partially ossified and assymetrical development in 1st sacral vertebrae was statistically significant at 15 mg/kg bw/day without a clear dose-response relationship. **NOAEL: 5 mg/kg bw/day**	New Zealand White rabbit Purity: 96.4 % (w/w) Dose range (gavage): 0, 2, 5, 15 mg/kg bw/day	**Moxon, M.E. (1991)**
Prenatal developmental toxicity (scientific publication) No guideline GLP: No **Study acceptable as additional information**	Maternal toxicity (diet): significant reduction of body weight gain and food consumption at doses ≥ 22 mg/kg bw/day. **NOAEL: 10 mg/kg bw/day** Developmental toxicity (diet): no teratogenicity. **NOAEL: ≥ 29 mg/kg bw/day** Maternal toxicity (gavage): Survival was affected at 30 mg/kg bw/day (100% of mortality) and at 25 mg/kg bw/day (16% of mortality). Significant reduction in weight gain (>10%) at doses ≥ 20 mg/kg bw/day and in food intake at doses > 10 mg/kg bw/day (>10% from 20 mg/kg bw/day). **NOAEL: n.a.** Developmental toxicity (gavage): Foetuses weight was significantly reduced at all dose levels greater than 10% at doses ≥ 20 mg/kg bw/day. **NOAEL: n.a**	CD rats Purity: 95.8% w/w Dose range (diet): 0, 10, 22, 27, 29 mg/kg bw/day Dose range (gavage): 5, 10, 20, 25, 30 mg/kg bw/day. Oral gavage control group was not available. Instead, diet control group was used.	**Staples, R.E. et al. (1976)**
Study of the effect of Imidan (phosmet) on embryogenesis (scientific publication) No guideline GLP: No **Study acceptable as additional information**	Single dose of 30 mg/kg bw on day 9 of pregnancy resulted in significant decrease in the number of live foetuses (75.7% in comparison to 85.7 of controls). Single dose dose on day 13 of pregnancy of 30 mg/kg bw revealed hydrocephaly in 33 of the 55 embryos studied. Dose level of 1.5 mg/kg bw/day throughout pregnancy resulted in a statistically verifiable reduction in the number of live foetuses in the test group; 62.8% in comparison to 87% for the control group. No adverse effect was noted at a dose of 0.06 mg/kg bw/day.	Wistar rats Purity: unknown Dose range (gavage): single dose of 30 mg/kg bw on days 9 or 13 of pregnancy and doses of 0.06 and 1.5 mg/kg bw/day throughout pregnancy.	**Martson, L.V. and Voronina, V.M. (1976)**

4.10.1 Effects on fertility

4.10.1.1 Non-human information

Two-generation reproductive toxicity in the rats

Title	Two-generation reproduction study in rats with R-1504
Author (s) (year):	Meyer, L.S. and Walberg, J.A. (1990)
Administration	Diet
Guideline	EPA FIFRA Pesticide Guideline 83-4 and OECD 416
Species	CD Rats
GLP	Yes
Purity:	95.2%
Groups	25 animals/sex/dose level
Dose levels	0, 20, 80 and 300 ppm equivalent to 0, 1-1.8, 4.2-7.3, 16.4-25.5 mg/kg bw male-female respectively
Study acceptable	

Executive Summary

The test substance R-1504 Technical (phosmet, purity 95.2% w/w) was administered in the diet to groups of 25 rats/sex/dose levels of 0, 20, 80 or 300 ppm. The administration continued through two generations (P0, P1) with two litters (F1a, F1b; F2a, F2b) per generation. The b-litters were culled to eight pups leaving, approximately, four males and four females per litter.

The correctness of the dietary concentrations was analytically confirmed. The adequate homogenous distribution of phosmet in the diet was demonstrated. The concentration of the test substance in the diet was not reduced after storage for 30 days at 4 °C and slightly reduced by 7.3 % after 7 days when stored in the animal room.

Treatment of the rats of both sexes started at day 56 of age. After 56 days (at day 112 of age) the rats of both sexes (P0) were mated (ratio 1:1) to yield F1a litter. Pups from the F1a litter were weaned at 21 days and sacrificed. Shortly after weaning of the F1a litter, the P0 parents were mated again to yield the second litter (F1b). These litters were culled on post natal day 4 to eight pups (ideally to four males and four females). Culled pups were necropsied. The surviving pups from F1b litter were weaned at day 21 of age. From these F1b pups each 25 males and 25 females were selected and continued on treatment as P1 parental animals. All F1b weanlings not selected were necropsied. After delivery of the F1b pups, the P0 parental males were necropsied, while the P0 parental females were necropsied after weaning of the F1b pups. The selected F1b pups were treated for an additional 11 week period and the mating procedure was performed as described above to generate F2a and F2b pups. The P1 parental animals were necropsied after delivery of F2b pups (P1 parental males) or after weaning of F2b pups (P1 parental females).

All parental animals (P0, P1) were observed daily for signs of toxicity or poor health. During the mating phase, observations for the presence of a copulatory plug were conducted each morning and a vaginal smear was taken from each female in order to determine the stage of the oestrous cycle or the presence of sperm. During the perinatal phase, starting on gravid day 21, females were monitored frequently to observe parturition. On postpartum days 0, 4, 7, 14 and 21, total litter size, number of live pups, number of dead pups, external pup anomalies and, for F1b and F2b litters, the sex of each pup was determined.

Body weights were determined weekly during the growth phase and during the mating phase on males and cohabitated females without positive mating signs. During the gestational phase, body weights were determined on gravid days 0, 6, 13 and 20. During the lactational phase, dam and pup body weights were recorded on postpartum days 0, 4, 7, 14 and 21. For F1a and F2a litters, the total live litter weight was recorded on postpartum days 0, 4, 7 and 14 and the weight of each pup was recorded on postpartum day 21. Pups that weighed less than three-fourths of their litter mean were designated as runts. Food consumption was determined weekly during the growth phase. During the gestational phase, food consumption was determined for gravid day intervals 0-6, 6-13 and 13-20. During the lactational phase dam food consumption was recorded for postpartum day intervals 0-4, 4-7, 7-14 and 14-21. Blood samples were collected from all P0 and P1 parental animals at the time of sacrifice to determine serum and red blood cell cholinesterase activity.

The P0 and P1 parental animals from each group were subjected to gross pathology and selected organs were weighed. Histopathology was performed on control and high dose animals that died or were sacrificed as scheduled, special attention being paid to the organs of the reproductive system. In addition, the liver from all P1 parental males of the 20 and 80 ppm dose levels and the reproductive organs from the four P0 parental males of the 80 ppm level were examined.

Enumerated data for each dose group were evaluated using Fisher Test, using Bonferroni´s correction for multiple comparisons to a single control value. Enumerated data for each litter were analyzed by the non-parametric Mann-Whitney U two-sample rank test. Quantitative or continuous

data were analyzed by one-way analysis of variance and the Dunnett´s t-test. The Fisher probability test was one-tailed. All other tests were two-tailed. The level of significance selected for all statistical tests was $p < 0.05$, but values also significant at $p < 0.01$ were indicated.

Results

No relationship between premature death or sacrifice and administration of the test compound could be made. Signs of parental toxicity were dose-dependently confined to 80 and 300 ppm groups in both sexes of the P0 and P1. Impaired general health status including clinical signs indicative for an organophosphorous ester occurred at 300 ppm. Significative dehydration occurred from 80 and 300 ppm in the P0 parental females. Besides, the incidence of chromorhinorrhea was significantly increased in the P1 parental females at 300 ppm.

Significant reductions on mean body weight in both P0 and P1 parents to obtain the 1st mating occurred during growth phase at 300 ppm in both sexes.

Table 49: Mean body weight (grams) for parental rats P0 and P1 during growth period.

Study days	Males								Females							
	P0[a]				P1[b]				P0[a]				P1[b]			
	Doses (ppm)															
	0	20	80	300	0	20	80	300	0	20	80	300	0	20	80	300
0	311	311	309	312	177	175	180	**162* (-8.5%)**	203	200	199	202	145	148	155*	**134* (-7.6%)**
7	357	354	349	**345* (-3.4%)**	240	241	242	**220* (-8.3%)**	223	222	218	**207** (-7.2%)**	178	180	187	**165** (-7.3%)**
14	398	397	396	389	301	302	304	**279* (-7.3%)**	240	238	236	**219** (-8.8%)**	207	207	215	**193* (-6.8%)**
21	434	430	423	418	348	348	354	329	254	251	247	**229** (-9.8%)**	321	233	241	**216* (-32.7%)**
28	461	460	452	442	396	396	400	373	267	265	258	**243** (-9.0%)**	247	251	258	**232* (-6.1%)**
35	480	479	469	463	436	434	433	**409* (-6.2%)**	277	275	271	**260* (-6.1%)**	261	268	288	263
42	508	504	494	489	461	462	458	435	289	284	282	**273* (-5.5%)**	277	282	288	263
49	529	525	514	511	484	484	457[c]*	437[c]**	299	298	297	285	286	293	300	277
56	553	544	528	529	504	503	495	**470* (-6.7%)**	305	303	300	295	293	298	309	287
63	555	546	534	542	526	526	524	502					304	308	322	301
71	566	564	551	556	547	548	550	523					312	318	329	311
77	582	571	559	566	566	573	573	545					319	322	336	317
84	587	590	571	577	563	570	572	552								
91	602	598	581	590	585	588	590	568								
98	614	610	596	603	595	603	604	582								
105	622	616	606	613	604	612	615	592								
112	636	631	611	620	619	625	628	606								
119	649	637	620	637	632	640	647	618								
126	645	636	622	635	644	653	654	630								
133	657	652	638	644	659	664	666	638								
140	667	653	646	650	664	679	681	652								
147	665	659	653	660	661	676	681	655								
154	677	663	658	669	672	688	691	663								
161	684	671	662	673	681	696	699	678								
168	693	677	669	680	677	697	702	680	349	353	368	360	372	373	410	414

*significantly different from the 0 ppm dose level, $p<0.05$, two-tailed
**significantly different from the 0 ppm dose level, $p<0.01$, two-tailed

a the P0 generation was mated starting on study day 56 (1st mating) and study day 119 (2nd mating). Weekly body weights resumed on study day 168.
b the P1 generation was mated starting on study day 77 (1st mating) and study day 140 (2nd mating). Weekly body weights resumed on study day 168.
c malfunctioning automatic watering system. Most animals had no water

During gestation there was only a significant reduction in P0 (2nd mating) on day 20 at 300 ppm. During lactation, significant reductions of body weight occurred only at 300 ppm in P0 (1st mating) from day 4 to day 21, P0 (2nd mating) on day 21 and in P1 (1st mating) from day 7 to day 21.

Table 50: Mean body weights (grams) for gestation and lactation periods for P0 and P1 females.

	Females Gestation							
	P0 1st mating				**P1 1st mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
0	305	305	305	296	313	313	327	308
6	327	325	324	317	337	338	352	328
13	351	351	348	339	364	364	378	353
20	427	419	424	406	440	438	456	408
	P0 2nd mating				**P1 2nd mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
0	339	336	337	317	347	351	358	341
6	362	364	365	344	371	370	384	357
13	389	391	391	370	397	399	415	383
20	470	468	465	**129** ** **(-72.5%)**	483	484	497	445
	Females Lactation							
	P0 1st mating				**P1 1st mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
0	340	333	334	320	358	355	374	348
4	338	334	333	**317*** **(-6.2%)**	361	351	375	341
7	353	348	345	**318** ** **(-9.9%)**	366	358	375	**334*** **(-8.7%)**
14	364	355	345	**315** ** **(-13.5%)**	378	367	374	**332** ** **(-12.2%)**
21	339	335	322	**306** ** **(-9.7%)**	348	350	356	**311** ** **(-10.6%)**
	P0 2nd mating				**P1 2nd mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
0	373	370	386	357	389	388	410	391
4	379	372	380	358	397	392	408	380
7	378	373	380	359	401	393	410	376
14	381	379	375	356	411	402	412	379
21	372	367	370	**356*** **(-4.3%)**	385	376	388	374

*significantly different from the 0 ppm dose level, p<0.05, two-tailed
** significantly different from the 0 ppm dose level, p<0.01, two-tailed

There were body weight gain reductions from 80 ppm in P0 males during growth period on days 7 and 21 (statistically significant from 80 ppm) and on day 112 (statistically significant at 80 ppm but not at 300 ppm). Additionally, on day 141 there was also a relevant body weight gain reduction from 80 ppm not statistically significant. In P1 males a significant gain reduction occurred on day 7 at 300 ppm. There were also relevant reductions from 20 ppm, only statistically significant at 300 ppm, on day 133 prior to 2nd mating and on day 182. On day 154 the relevant reduction from 20 ppm was not significant at any dose level. In females only a statistically significant decrease in P0 on day 7 was observed correlated with a significant decrease in food consumption.

Table 51: Mean body weight change (grams) for parental rats P0 and P1 during growth period.

Study days	Males								Females							
	P0[a]				P1[b]				P0[a]				P1[b]			
	doses															
	0	**20**	**80**	**300**	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
7	47	43	**40* (-14.9%)**	**33** (-29.8%)**	63	65	62	**58* (-7.9%)**	21	21	20	**4.7** (-77.6%)**	32	33	32	31
14	43	43	46	44	60	61	62	59	16	16	18	14	29	26	28	28
21	35	33	**30* (-14.3%)**	**30* (-14.3%)**	47	49	50	50	15	13	11	12	24	26	26	23
28	29	30	29	28	48	47	46	44	13	14	12	15	16	19	17	16
35	19	19	17	22	40	40	34	37	10	9.9	14	17**	14	17	18	17
43	28	25	25	25	25	27	25	26	12	11	11	14	15	14	12	14
49	21	21	20	22	23	23	-1.0[c]**	2.0[c]**	9.7	14	15*	13	9.1	12	12	15*
56	22	19	**15* (-31.8%)**	18 (-18.2%)	20	19	38[c]**	33[c]**	6.1	5.2	3.0	10	8.6	4.9	9.2	10
63	4.3	2.0	5.0	14**	22	23	30**	30**					11	9.9	13	14
71	15	18	16	13	21	21	26	25					8.2	9.8	7.3	9.7
78	13	**6.6***	8.6	15	20	24	24	22					6.2	4.2	7.1	6.2
84	10	19**	12	10	-2.8	0.5	-0.6	9.0**								
91	14	10	9.9	16	21	18	17	15								
98	12	13	15	12	10	12	15	13								
105	8.4	8.9	9.4	11	8.7	8.9	11	12								
112	14	15	**5.6* (-60%)**	7.0 (-50%)	15	11	13	11								
119	4.9	6.2	8.4	17*	13	15	20*	16								
126	-3.8	-1.1	2.1	-2.4	12	14	7.2	11								
133	12	12	16	9.2	15	13 (-13.3%)	10 (-33.3%)	**8.6* (-42.7%)**								
141	9.4	9.0	8.5 (-9.6%)	6.0 (-36.2%)	11	14	17**	15								
147	-1.5	6.0*	7.0*	6.4*	-7.5	-2.5	0.6	0.9								
154	12	8.3	5.1	8.7	16	11 (-31.2%)	10 (-37.5%)	10 (-37.5%)								
161	7.1	5.2	3.2	4.4	4.9	8.6	7.4	15**								
168	8.5	5.6	7.3	7.0	-3.7	1.0	3.4**	2.0								
175					12	9.1	7.4	10	7.8	7.7	9.2	4.4				
182					13	9.8 (-24.6%)	10 (-23.1%)	**8.0* (-38.5%)**	7.5	11	9.7	9.0				
189					15	**9.1****	12	12	2.8	1.1	1.3	3.9				
196													7.0	9.5	11	3.5
231													0.4	4.8	8.2*	5.7

*significantly different from the 0 ppm dose level, p<0.05, two-tailed

** significantly different from the 0 ppm dose level, p<0.01, two-tailed

[a] The P0 generation was mated starting on study day 56 (1st mating) and study day 119 (2nd mating). Weekly body weights resumed on study day 168.

[b] The P1 generation was mated starting on study day 77 (1st mating) and study day 140 (2nd mating). Weekly body weights resumed on study day 189.

[c] malfunctioning automatic watering system. Most animals had no water

During gestation, relevant reduction occurred from 80 ppm on day 20 in P0 (2nd mating) only statistically significant at 300 ppm. Significant decrease was also observed in both matings of P1 at 300 ppm on day 20. Decreases of body weight gain during lactation were observed on P0 (1st mating) from 80 ppm on day 7 (statistically significant only at 300 ppm) and 14 (statistically significant from 80 ppm). There were also reductions from 20 ppm in P1 (1st mating) on days 7 and 14 and in P1 (2nd mating) on days 4, 7 and 14.

Table 52: Mean body weights change (grams) for gestation and lactation periods for F0 and F1 females.

	Females Gestation							
	P0 1st mating				**P1 1st mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
6	22	19	19	22	24	25	26	20 (-16.7%)
13	25	26	24	21	27	26	26	25
20	76	68	77	70	77	74	75	**55* (-28.6%)**
	P0 2nd mating				**P1 2nd mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
6	23	28	28	25	24	19	26	**16* (-33.3%)**
13	27	27	26	28	26	29	33	26
20	85	77	74 (-12.9%)	**64* (-24.7%)**	86	85	83	**63** (-26.7%)**
	Females Lactation							
	P0 1st mating				**P1 1st mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
4	-1.8	1.0	-0.8	-3.5	2.2	-2.5	0.7	-3.4
7	17	15	13 (-23.5%)	**1.1** (-93.5%)**	5.6	4.8 (-14.3%)	0.1 (-98.2%)	-2.0 (-135.7%)
14	11	6.3	**-0.6** (-105.4%)**	**3.0** (-72.7%)**	12	8.9 (-25.8%)	-0.3 (-102.5%)	-2.4 (-120%)
21	-25	-20	-23	-8.3**	-31	-17*	-18*	-20
	P0 2nd mating				**P1 2nd mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
4	5.7	2.1 (-63.2%)	**-5.4* (-194.7%)**	1.3 (-77.2%)	7.2	4.7 (-34.7%)	-1.2 (-116.7%)	-9.7 (-234.7%)
7	-0.9	0.7	0.7	0.7	4.3	0.3 (-93%)	1.7 (-60.5%)	-6.5 (-251.2%)
14	4.0	5.9	-5.6	-2.1	10	9.0 (-10%)	1.5 (-85%)	**-6.0* (-160%)**
21	-9.9	-12	-4.5	-11	-26	-25	-24	-5.2**

*significantly different from the 0 ppm dose level, p<0.05, two-tailed

** significantly different from the 0 ppm dose level, p<0.01, two-tailed

Transiently reduced values for food consumption were recorded at 300 ppm during the first week in P0 females and towards the end of the administration period in the P1 females at 300 ppm

Table 53: Mean Food consumption (grams/day) for parental rats P0 and P1.

Study days	Males								Females							
	P0				P1				P0				P1			
	doses															
	0	**20**	**80**	**300**	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
7	26	26	26	26	22	23	23	23	19	19	18	**17* (-10.5%)**	17	18	19**	20**
14	28	27	28	28	25	26	26	25	19	19	20	17 (-10.5%)	18	18	19	20*
21	29	28	28	29	27	28	28	28	20	21	20	22	19	198	20	20
28	29	28	28	28	27	28	28	29	20	20	20	20	19	19	20**	21**
35	29	29	28	29	27	27	27	27	19	20	20	22**	19	20	20*	20*
43	27	27	27	27	28	28	29	31**	19	19	20	21**	19	20	20	20
49	27	26	27	27	27	28	23[b]**	24[b]**	18	18	18	19	18	18	19	19
56	26	27	25	28	28	29	30	29	18[c]	18[c]	18[c]	19* [c]	19	19	20	19
63	[a]	[a]	[a]	[a]	28	28	30	29					19	19	20	19
71	27	26	26	26	28	29	30	29					19	19	20	20
78	27	26	27	27	30	30	31	30					19[d]	20 [d]	20 [d]	19 [d]
84	27	27	25	27	[a]	[a]	[a]	[a]								
91	27	26	25	27	29	29	29	30								
98	26	25	24	26	28	28	29	29								
105	25	26	25	25	29	28	29	29								
112	25	25	26	26	29	29	29	27								
119	26	26	26	26	29	29	29	29								
126	[a]	[a]	[a]	[a]	29	29	29	29								
133	26	27	26	26	29	29	29	28								
141	26	26	25	24	29	29	30	29								
147	26	27	26	25	[a]	[a]	[a]	[a]								
154	25	25	25	25	28	30	29	29								
161	26	26	25	26	27	29	28	29								
168	27	26	26	26	29	28	29	28								
175					29	30	29	29	23	23	23	21				
182					29	29	29	27	21	22	21	21				
189					29	30	28	28	19	20	20	19				
196									20	20	20	19	22	25	26*	22
203									20	20	19	19	24	25	25	**19** (-20.8%)**
210									19	19	19	19	21	22	24*	21

*significantly different from the 0 ppm dose level, p<0.05, two-tailed
** significantly different from the 0 ppm dose level, p<0.01, two-tailed
a valid food consumption not determined during mating phase
b malfunctioning automatic watering system. Most animals had no water
c The P0 generation was mated starting on study day 56 (1st mating) and study day 119 (2nd mating). Weekly body weights resumed on study day 168.
d The P1 generation was mated starting on study day 77 (1st mating) and study day 140 (2nd mating). Weekly body weights resumed on study day 189.

There were not significant reductions in the gestational period. During lactation reductions from 80 ppm occurred in P0 (2nd mating) on days 4 and 7 only significant on day 7 at 300 ppm. Significant reductions at 300 ppm were also observed in P0 (2nd mating) on days 14 and 21, P0 (1st mating) from day 7 to 21 and in both matings of P1 from day 4 to day 21.

Table 54: Mean food consumption (grams/day) for gestation and lactation periods for P0 and P1 females.

	Females Gestation							
	P0 1st mating				**P1 1st mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
6	20	19	19	21	21	21	22	20
13	20	20	20	21	21	21	23	22
20	21	20	20	20	24	22	23	23
	P0 2nd mating				**P1 2nd mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
6	22	23	24	25*	25	24	28	24
13	21	23	23	24*	23	23	27*	25
20	23	22	21	22	25	25	27	23
	Females Lactation							
	P0 1st mating				**P1 1st mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
4	28	26	28	26	28	27	28	**21** ** **(-25%)**
7	46	45	45	**33** ** **(-28.3%)**	45	44	45	**32** ** **(-28.9%)**
14	61	59	59	**43** ** **(-29.5%)**	62	61	60	**42** ** **(-32.3%)**
21	76	74	74	**55** ** **(-27.6%)**	72	73	73	**49** ** **(-31.9%)**
	P0 2nd mating				**P1 2nd mating**			
Day/dose	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
4	27	25	24 (-11.1%)	24 (-11.1%)	30	28	30	**21** ** **(-30%)**
7	40	37	36 (-10%)	**33** ** **(-17.5%)**	43	40	44	**28** ** **(-34.9%)**
14	50	48	48	**38** ** **(-24%)**	57	54	56	**37** ** **(-35.1%)**
21	62	63	60	**49** ** **(-20.9%)**	69	65	64	**41** ** **(-40.6%)**

*significantly different from the 0 ppm dose level, p<0.05, two-tailed

** significantly different from the 0 ppm dose level, p<0.01, two-tailed

Biologically relevant and statistically significant red blood cell cholinesterase (ChE) activity reductions (>20%) were noted at 300 ppm and 80 ppm in both sexes of P0 and P1. Plasma cholinesterase (ChE) activity only showed important reductions at 300 ppm and in P0 females at 80 ppm.

Table 55: Mean cholinesterase activity and variations at terminal sacrifice compared to controls

Location		Dose Level [ppm]			
		0	**20**	**80**	**300**
		Cholinesterase Activity [IU/L, IU/protein (%)]			
P0 Males					
Serum	Mean value	522	509	498	**365****
	Standard deviation	100	100	102	60
	No. animals	23	24	25	24
	% of control	0	-2	-5	**-30**
Red Blood Cells	Mean value	1587	**1460***	**997****	**411****
	Standard deviation	207	171	187	124
	No. animals	23	25	25	24
	% of control	0	**-8**	**-37**	**-74**
P1 Males					
Serum	Mean value	550	502	476	**326****
	Standard deviation	124	123	165	55
	No. animals	25	23	25	25
	% of control	0	-9	-13	**-41**
Red Blood Cells	Mean value	1360	1277	**710****	**203****
	Standard deviation	147	220	142	186
	No. animals	25	23	25	25
	% of control	0	-6	**-48**	**-85**
P0 Females					
Serum	Mean value	2611	2459	**1980****	**915****
	Standard deviation	691	667	628	229
	No. animals	25	24	23	24
	% of control	0	-6	**-24**	**-65**
Red Blood Cells	Mean value	1490	**1319****	**770****	**278****
	Standard deviation	208	169	187	66
	No. animals	25	23	22	23
	% of control	0	**-11**	**-48**	**-81**
P1 Females					
Serum	Mean value	2076	2042	1755	**771****
	Standard deviation	578	621	466	215
	No. animals	24	25	25	24
	% of control	0	-2	-15	**-64**
Red Blood Cells	Mean value	1427	**1204****	**589****	**291****
	Standard deviation	227	208	169	131
	No. animals	25	25	25	24
	% of control	0	**-16**	**-59**	**-80**

* Statistically significant difference from control group mean at the 5 % level (Dunnett's t-test)
** Statistically significant difference from control group mean at the 1 % level (Dunnett's t-test)

Significant reduction of the absolute mean testes weight in P0 and P1 males and spleen absolute weight in P1 males occurred at 300 ppm. At this same dose level absolute weight of spleen decreased in P0 and P1 females and also the absolute adrenals weight in P0 females. Significant reductions of relative weight of liver and adrenals in P0 females and spleen in P1 females from 80 ppm were observed. At 300 ppm there were also significant reductions in the relative weight in females of liver (P1), spleen (P0), kidneys (P0 and P1), ovaries (P0) and heart (P0 and P1). There were no treatment-related findings at necropsy.

Table 56: Summary table of relevant mean absolute and relative organ to body weight changes.

Males									
Parameter		**P0**				**P1**			
		0 ppm	**20 ppm**	**80 ppm**	**300 ppm**	**0 ppm**	**20 ppm**	**80 ppm**	**300 ppm**
Spleen	Absolute					1.017	0.980	1.052	**0.937* (-7.9%)**
Testes	Absolute	3.824	3.650	3.521	**3.436* (-10.1%)**	3.812	3.825	3.914	**3.549* (-6.9%)**
Females									
Parameter		**P0**				**P1**			
		0 ppm	**20 ppm**	**80 ppm**	**300 ppm**	**0 ppm**	**20 ppm**	**80 ppm**	**300 ppm**
Spleen	Absolute	0.682	0.667	0.656	**0.553** (-18.9%)**	0.756	0.720	0.726	**0.606** (-19.9%)**
	Relative	0.189	0.181	0.172	**0.145** (-23.3%)**	0.196	0.185	**0.168* (-14.3%)**	**0.146** (-25.5%)**
Liver	Relative	2.775	2.645	**2.611* (-5.9%)**	**2.451** (-11.7%)**	2.687	2.573	2.513	**2.377** (-11.5%)**
Kidneys	Relative	0.613	0.582	0.581	**0.564* (-8.0%)**	0.584	0.564	0.553	**0.514** (-12.0%)**
Adrenals	Absolute	0.073	0.069	0.066	**0.063* (-13.7%)**				
	Relative	0.020	0.019	**0.017** (-15.0%)**	**0.017** (-15.0%)**				
Heart	Relative	0.374	0.359	0.344	**0.328** (-12.3%)**	0.325	0.328	0.312	**0.286** (-12.0%)**
Ovaries	Relative	0.034	0.033	0.031	**0.027 (-20.6%)**				

* significantly different from the 0 ppm dose level, $p < 0.05$ two tailed
** significantly different from the 0 ppm dose level, $p < 0.01$ two tailed

The most relevant histopathological observations were mild to moderate centrilobular hepatocellular vacuolisation in 10/25 males of P1 at 300 ppm. Moderately decreased spermatogenesis was observed in a small number of P0 males from all dose groups, including controls, which was not considered treatment-related. Diminished spermatogenesis was also observed in P1 males in 3/25 animals (300 ppm), 2/25 animals (80 ppm) and 1/25 animal (20 ppm), but these changes were not statistically significant.

Indications of impaired fertility were observed at 80 and 300 ppm. Reduction in fertility indices was noted from 80 ppm in P0 to generate F1b litters and P1 to generate F2a and F2b litters. However this decrease was only statistically significant from 80 ppm in P0 to generate F1b litter and at 300 ppm in P1 to generate F2a and F2b litters. Mating index decreased from 80 ppm in P0 for F1b litters and P1 to generate both litters only reaching the level of statistical significance at 300 ppm in P1 for F2b litters. The mean length of gestation was not affected at any dose level. The gestation indices decreased without statistical significance from 80 ppm in P0 females for F1b litters and P1 females for F2a litters and at 300 ppm in P1 females for F2b litters.

Table 57: Summary of reproductive indices

Males																
	P0 (1st mating for F1a)				**P0 (2st mating for F1b)**				**P1 (1st mating for F2a)**				**P1 (2st mating for F2b)**			
	Doses (ppm)															
Males	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**	**0**	**20**	**80**	**300**
Number of males	24[e]	25	25	25	23	25	25	25	25	25	25	25	25	25	25	25
Cohabited	24	25	25	25	23	25	25	25	25	25	25	25	25	23	25	25
Positive Mating Sign	24	25	24	25	23	25	22	20	24	23	22	18	22	22	18	**14***
Mating index (%) [a]	100	100	96	100	100	100	88	80	96	92	88	72	88	96	72	**56***
Fertile	22	24	18	21	22	22	**17***	**15***	22	21	15	**12****	19	20	15	**9***
Fertility index (%) [b]	92	96	72	84	96	88	**68***	**60***	88	84	60	**48****	76	87	60	**36***
Females																
Number of females	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Cohabited	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Positive mating sign [c]	25	25	24	25	25	25	22	20	24	23	22	18	22	24	18	**14***
Mating index (%)	100	100	96	100	100	100	88	80	96	92	88	72	88	96	72	**56***
Delivered	23	24	18	21	24	22	**17***	**15***	22	21	15	**12****	19	21	15	**9***
Without positive mating signs	0	0	1	0	2	0	2	1	4	0	1	2	0	1	0	0
Fertility index (%)	92	96	72	84	96	88	**68***	**60***	88	84	60	**48****	76	84	60	**36***
Gestation index (%) [d]	92	96	75	84	96	88	77	75	92	91	68	67	86	88	83	64
Number of litters with <6 pups	0	1	0	1	1	1	3	1	1	1	1	0	0	1	1	2
Mean gestation length (days)	21.9	21.9	21.8	22.1	22	21.8	21.7	22	21.8	22	21.8	21.9	21.9	21.8	21.7	22
Pups																
No. of pups/litter (total born) [f]	14.1	14.6	15	14	15.1	14.8	13.4	**12.6***	14.1	14.2	14.3	**11.8***	15.3	14.6	14.9	**11.0***
No. live pups/litter (day 0)	13.2	14.5	14.7	13.6	14.7	14.2	12.9	**11.9***	13.8	13.6	13.9	**11.5***	14.8	14.1	13.9	**9.6****
No. live pups/litter (day 21)	13.6	14.2	14.4	**11.9***	7.9	7.7	7.2	6.9	13.5	12.8	13.5	**9.4****	7.8	7.7	7.7	**5.2***
Pup weight on day 0 (g)	6.4	6.3	6.1	6.2	6.4	6.2	6.4	6.3	6.4	6.2	6.3	6.0	6.4	6.4	6.4	6.2
Pup weight on day 21 (g)	44.5	42.4	41.3	**33.2****	49.4	49.1	50.8	**41.8****	42.2	44.3	41.8	**33.8****	56.4	54.5	53.1	**33.0****

a The percentage of animals in a dose group that had positive mating signs or whose females became pregnant.
b The percentage on animals in a dose group that had live born pups
c Females that delivered without positive mating signs are included
d The percentage of sperm-positive mating resulting in the birth of live pups: a female that delivered offspring was considered to have been sperm-positive even if sperm was not detected.
e Male No. 110 was cohabited on 6/13/88, however, he died on 6/16/88. It is not included in the summary of reproductive indices.
f Number of live pups on day 4 plus all pups either dead or missing from day 0 to day 4.
* Significantly different from the 0 ppm dose level, $p<0.05$, two-tailed
** Significantly different from the 0 ppm dose level, $p<0.01$, two-tailed

Reduced number of total born pups delivered, number of live born pups per litter during lactation and reduced pup body weight were observed at 300 ppm. No biologically relevant soft tissue anomalies or malformations were observed in incidental pup deaths or in the examined F1b and F2b pups (culled or weaned). No increased external pup anomalies were noted in the progeny of the P0 and P1 parental generation at any dose level.

Conclusions

The NOAEL for parental toxicity of the test substance was stated at 20 ppm (about 1 - 1.8 mg/kg bw) for the P0 and P1 parental rats taking into account the effects observed at 80 ppm which included statistically and biologically significant reduction (>20%) of red blood cell cholinesterase in P0 and P1 parental animals of both sexes, clinical signs as significative dehydration in P0 females, relative organ weight reductions in females of P0 (liver and adrenals) and P1 (spleen) and decrease in body weight gain during growth, gestation and lactation periods that was statistically significant in P0 males during growth (days 7, 21 and 112) and during lactation in P0 females (1st mating) on day 14.

The NOAEL for reproductive toxicity of the test substance was stated at 20 ppm (about 1 - 1.8 mg/kg bw) for rats considering the statistically significant reduction in fertility indexes observed from 80 ppm in P0 to generate F1b litters. Lower fertility indexes were also decreased from 80 ppm in P1 for F2a and F2b litters but only significant at 300 ppm. Lower mating indexes were observed from 80 ppm in P0 for F1b litters and P1 for F2a and F2b litters only reaching the level of statistical significance in P1 to generate F2b litters at 300 ppm. Besides, it has to be pointed out that these effects were observed under signs of maternal toxicity.

The NOAEL for developmental toxicity (growth and development of the offspring) can be fixed at 80 ppm (about 4.2-7.3 mg/kg bw) based on reduced number of total born pups delivered, impaired pup survival and reduced pup body weights at 300 ppm. Hence, indications for developmental toxicity occurred only at a dose level which was also toxic to the parental animals.

4.10.1.2 Human information

There are no human data available relevant for C&L.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

Teratogenicity test by the oral route in the rat

Title	**Phosmet: Teratogenicity study in the rat**
Author (s) (year):	Hodge, M.C.E. (1991)
Administration	Oral gavage
Guideline	US EPA FIFRA Pesticide Guideline 83-3
Species	Alpk APfSD rat
GLP	Yes, except that there is no documentation indicating whether the test substance was characterised in a GLP-accredited laboratory.
Purity:	96.4 % w/w
Groups	24 female rats/sex/dose level
Dose levels	0, 5, 10 and 15 mg/kg bw/day
	Study acceptable

Executive Summary

Groups of 24 female Alpk AP_fSD rats were dosed by gavage with 5, 10 or 15 mg/kg bw/day Imidan tech. (Phosmet, purity 96.4 % (w/w)) in corn oil from days 7-16 (inclusive) of gestation. A control group of animals received corn oil alone. The day of confirmation of mating by vaginal smear was designated day 1 of gestation. On day 22 of gestation, all females were killed and their uteri examined for live foetuses and intra-uterine deaths. The foetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination.

Dose formulations were analyzed for test item concentrations and stability. Appropriate concentration and stability of the test item in the formulation was shown analytically. The preparations were solutions, thus homogeneity was guaranteed.

Any changes in behaviour or clinical condition were recorded daily during the dosing period and on other days when the animals were weighed. On the other days, the animals were observed for signs of significant toxicity. Body weight and food consumption were recorded at regular intervals. On day 22 of gestation, all surviving animals were sacrificed for Caesarean section. Foetuses were

weighed, sexed and examined for external abnormalities including cleft palate, soft tissue anomalies. The stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed.

Analysis of variance was carried out using the GLM models in SAS (1985). Each treatment group mean was compared with the control group mean using Student's t-test, based on error mean square in the analysis. The following parameters were analysed by Fisher's Exact Test, comparing each treated group with the control group: proportion of females with pre-implantation loss, post implantation loss, the proportion of females with early and late intra-uterine deaths, proportion of male foetuses and the proportion of foetuses with major or minor external/visceral defects, skeletal defects, external/visceral variants, skeletal variants and each individual finding. All statistical analysis were one-sided with the following exceptions: Maternal bodyweight gain, maternal food consumption and the proportion of male fetuses, not ossified odontoid and not ossified centra of the 2^{nd}, 3^{rd} and 4^{th} cervical vertebrae.

Results

One control group female was killed *in extremis* on day 14; macroscopic examination showed gas-filled and distended stomach. All remaining animals survived until the day of scheduled necropsy. The highest dose of 15 mg/kg bw/day was toxic to the dams causing clinical symptoms indicative for an organophosphorous ester (including shaking, piloerection and salivation). It is important to point out that signs of urinary incontinence were produced at all group doses and showed an increase (8 animals) at 15 mg/kg bw/day.

Statistically significant retarded body weight gain was noted at 15 mg/kg bw/day during dosing (days 7-16) with respect to controls (47.5%). Smaller but statistically significant effects on body weight gain were also present during dosing (days 7-16) at 10 mg/kg bw/day (11.9%), while there were no effects at 5 mg/kg bw/day. Food consumption at 15 mg/kg bw/day was significantly reduced from the start of the dosing period for the remainder of the study (15.7% for days 7-16 and 14.1% for days 16-22). No effect on food consumption was noted at 10 and 5 mg/kg bw/day.

No compound-related macroscopic abnormalities were noted. There were no effects on intrauterine survival, sex ratio and post-implantation losses. Mean foetal weight at 15 mg/kg bw/day was reduced (3.7%) with statistical significance although the value (4.89 g) was within the historical control range (4.87-5.25 g), with no adverse effect on litter weight. No evidence of any compound-related effect on the incidence or type of major, minor or variant external/visceral or skeletal defects was observed. There were only differences in some skeletal variants (Table 58) not dose-related and/or only statistically significant at the intermediates dose level. Calcaneum not ossified was higher at 15 mg/kg bw/day but the number of foetus examined at this dose level was also higher than the control dose group (280 versus 241) and occurred in presence of maternal toxicity.

Table 58: No. of remarkable variants occurred at different doses (mg/kg bw/day) of phosmet

Variants	0	5	10	15
Odontoid not ossified	62	19**	56	69
Transverse processes of 4th lumbar fully ossified	26	34	22	40
5th sternebrae partially ossified	58	65	81**	79
Calcaneum not ossified	158	132	148	200

** $p<0.05$

Conclusion

No indications of embryo- or foetotoxicity were noted up to and including the high dose level. Especially, no signs of malformations or variations were recorded. Maternal toxicity at high dose, 15 mg/kg bw/day, was shown throughout the dosing period by reduced body weight gain (47.5%),

reduced food consumption (15.7% for days 7-16 and 14.1% for days 16-22) and clinical signs of toxicity. Reduced body weight gain (11.9%) was also noted at mid dose during dosing. Mean foetal weight and other litter parameters were not affected by the treatment with phosmet. There was no evidence of any compound-related effect on the incidence or type of major, minor or variant external/visceral or skeletal defects.

The NOAEL for maternal toxicity was achieved at 5 mg/kg bw/day. The NOAEL for prenatal developmental toxicity including teratogenicity was at least 15 mg/kg bw/day, the highest dose level investigated.

Teratogenicity tests by the oral route in the rabbit

Title	**Phosmet: Embryotoxicity study in the rabbit**
Author (s) (year):	Pinto, P.J. (1991)
Administration	Oral gavage
Guideline	Not applicable to range-finding study
Species	New Zealand White rabbits
GLP	No (range-finding study)
Purity:	96.4 % w/w
Groups	10 female rabbits/dose level
Dose levels	0, 5, 10 and 15 mg/kg bw/day
Study acceptable as a range finding study	

Executive Summary

Groups of each ten time-mated female New Zealand White rabbits were dosed by gavage with 0 (corn oil) and 5, 10 or 15 mg/kg bw/day Imidan tech. (phosmet, purity: 96.4%) at a dose volume of 1 ml/kg bw from days 7 to 19 (inclusive) of gestation. The concentration and stability of phosmet in corn oil was determined. Appropriate concentrations and stability of phosmet in the vehicle was shown analytically.

The animals were observed regularly for clinical symptoms, and body weight and food consumption were recorded at regular intervals. On the day of scheduled sacrifice (day 30), the uteri were examined for live foetuses and intra-uterine deaths. All foetuses were weighed, killed and examined for external abnormalities including cleft palate and then discarded.

Each treatment group mean was compared with the control group mean using Student's t-test. All statistical tests were two-sided.

Results

One female died on day 13 of gestation about 2 h post dosing with 15 mg/kg bw/day. Two animals given 15 mg/kg bw/day (including the one found dead on day 13) showed clinical signs of an organophosphorous compound (shaking, constricted/dilated pupils and salivation in both animals and high stepping gait in the one that survived) shortly after dosing, one on the day of its death (day 13), the other one during days 9 to 18 of gestation.

There were no adverse effects of treatment with phosmet on body weight gain and food consumption during the study. Macroscopic examination of the premature decedent at 15 mg/kg bw/day revealed stomach mucosa which sloughed off when washed. Overall, macroscopic findings in dams killed on day 30 were few and were considered not to be related to treatment with phosmet.

Pregnancy data only revealed a statistically significant reduction (22.8%) in mean litter weight at 15 mg/kg bw/day. Slight reductions of the mean litter weight at 10 mg/kg bw/day (19.9%) and

slightly reduced uterus weight at mid and high doses (11.1% and 18.9% respectively) were observed. These changes were only minor and did not achieve the level of statistical significance. Indications for substance-related external abnormalities were not obtained at any dose level.

Conclusion

Dose of 15 mg/kg bw/day produced maternal toxicity including one premature death while no substance-related effects were recorded at 10 mg/kg bw/day. Slight indications of impairment in foetal development were noted from 10 mg/kg bw/day onwards. Phosmet did not cause external malformations or variations in this range-finding study.

Title	**Phosmet: Teratogenicity study in the rabbit**
Author (s) (year):	Moxon, M.E. (1991)
Administration	Oral gavage
Guideline	US EPA FIFRA 83-3 (virtually identical to OECD 414)
Species	New Zealand White rabbits
GLP	Yes
Purity:	96.4 % w/w
Groups	20 female rabbits/dose level
Dose levels	0, 2, 5 and 15 mg/kg bw/day
	Study acceptable

Executive Summary

Groups of 20 female New Zealand White rabbits were dosed by gavage with 2, 5 or 15 mg/kg bw/day Imidan tech (phosmet, purity: 96.4 % (w/w)) in corn oil from days 7-19 (inclusive) of gestation. A control group of animals received corn oil alone. The day of insemination was designated day 1 of gestation. On day 30 of gestation, all females were killed and their uteri examined for live foetuses and intra-uterine deaths. The foetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination.

The appropriate concentration and stability of phosmet in corn oil were assessed analytically. The correctness of the nominal test substance concentrations in corn oil was analytically verified. The determined concentrations were within 6 % of the nominal levels. The preparations were solutions, thus homogeneity was guaranteed.

Details of changes in behaviour or clinical condition of the rabbits were recorded daily during the study. If no abnormalities occurred this was also recorded. Rabbits requiring euthanasia were killed. These animals together with the one animal found dead were given a macroscopic examination *post mortem* and pregnancy status was recorded. Body weight and food consumption were recorded at regular intervals. On day 30 of gestation, all surviving animals were sacrificed. The uteri were examined for live foetuses and intra-uterine deaths. All foetuses were weighed, killed and examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination.

Analysis of variance was carried out using the GLM models in SAS (1985). Each treatment group mean was compared with the control group mean using Student’s t-test, based on error mean square in the analysis. The following parameters were analysed by Fisher’s Exact Test, comparing each treated group with the control group: proportion of females with pre-implantation loss, post implantation loss, the proportion of females with early and late intra-uterine deaths, proportion of male foetuses and the proportion of foetuses with major or minor external/visceral defects, skeletal defects, external/visceral variants, skeletal variants and each individual finding.

Results

One animal was found dead on day 10 of the study one hour after dosing with 15 mg/kg bw/day without relevant clinical signs. Other dam had to be killed for humane reasons on day 14 at this same dose level. This last death was observed in presence of subdued, salivation, diarrhea and mucus on faeces. It cannot be dismissed that both deaths at the high dose level are treatment related. Three animals were killed for humane reasons at 5 mg/kg bw/day, two on day 26 and one on day 27 of gestation, following signs of abortion and one animal was also killed at 2 mg/kg bw/day. No evidences of any treatment-related changes are associated with these deaths at 5 and 2 mg/kg bw/day.

Clinical signs typical for an organophosphorous compound (unsteady gait, salivation, increased breathing rate and shaking) occurred in a low incidence at 15 mg/kg bw/day.

Maternal body weight gain at the highest dose level was clearly reduced statistically significant on days 10-13 during dosing. At 5 mg/kg bw/day reductions occurred on days 7-10 and 10-13 but during the whole dosing period (days 7-19) the body weight gain increased. Slight reduction was observed at this same dose level in post-dosing period. It has to be noted that no adverse effects of phosmet on maternal food consumption were observed at any dose level.

Table 59: Body weight gain (g) before, during and after phosmet administration (* p<0.05)

Period (days)	**Doses of Phosmet (mg/kg bw/day)**			
	0	**2**	**5**	**15**
Pre-dosing (1-7)	143.9	112.4	107.4	98.1
During dosing (7-19)	51.8	69.9	57.4	13.5 (-73.9%)
7-10	-42.4	-51.3	-53.8 (-26.9%)	-72.9 (-71.9%)
10-13	54.4	39.6	25.6 (-52.9%)	**-2.0* (-103.7%)**
13-16	71.1	61.4	81.6	87.4
16-19	-31.2	20.2	3.8	0.9
Post dosing (19-30)	277.3	273.7	246.8 (-11.0%)	249.6 (-10.0%)
Overall (1-30)	473	456	411.6 (-13.0%)	361.2 (-23.6%)

* p<0.05

None of the macroscopic findings noted in dams surviving to scheduled necropsy were considered to represent an adverse effect due to test substance administration. Macroscopic findings in intercurrent deaths were haemorraghic areas in the stomach in the animal found dead on day 10 at 15 mg/kg bw/day and stomach mucosa sloughed off when washed in the animal dead at 2 mg/kg bw/day.

No adverse effects on the number, growth and survival of the offspring *in utero* were noted at any dose level. Phosmet did not seem to affect litters. Although there were some findings, they were not considered relevant. These findings are the following: The mean % of post implantation losses were higher in females dosed with 2, 5 and 15 mg/kg bw/day of phosmet (17.3, 22.8 14.2 % compared to 11.6 % of control) without a clear relation dose-effect. The mean % of early intra-uterine deaths were also higher for those animals dosed with phosmet (12.6; 10.1 and 9 % at doses of 2, 5 and 15 mg/kg bw/day and 2.1% of the control) also without a dose response relationship.

The only increase in an external and major defect was observed in the extreme flexion of forepaw/s at 5 mg/kg/bw/day but it was not reproduced at 15 mg/kg bw/day and then not dose-related. No evidence of any compound-related effect on the incidence of visceral defects was observed at any dose level. The number of foetuses with minor skeletal defects (areas of reduced or lacking ossification) was statistically significantly increased at 5 and 15 mg/kg bw/day. Evaluation of the specific defects revealed only a minimal effect at 15 mg/kg bw/day indicated by an increased percentage of foetuses with unossified 5th sternebra and unossified 6th sternebra on a litter basis. These minor effects occurred in absence of reduction in foetal weight. The significant increase in the incidence of odontoid partially ossified and assymetrical development in 1st sacral vertebrae was statistically significant at 15 mg/kg bw/day but it is not clear a dose-response relationship. The foetal effects seen at 5 mg/kg bw/day were minimal and not clearly related to a single dose of phosmet. There was no evidence for teratogenicity.

Table 60: No. of foetal defects occurred at different doses (mg/kg bw/day) of phosmet (* p<0.05)

Skeletal variants	**0**	**2**	**5**	**15**	**Historical control data**
Odontoid partially ossified (foetus)	55	39	49	68*	0-58.2
Asymmetrical development (foetus) 1st sacral vertebrae	34	24	32	44*	-
Asymmetrical development (foetus) 2nd sacral vertebrae	25	22	22	29	-
No. of foetuses with minor skeletal defects (mean %)	50	42	62*	56*	35.2-55.8
Unossified fifth sternebrae. Foetus (%)	4	5	7	15*	1.1-25.8
Unossified sixth sternebrae. Litter (%)	4	5	6	9*	0-42.9
Seventh transverse process partially ossified. Foetus (%)	1	1	5	0	0-2.3
External defects	**0**	**2**	**5**	**15**	**Historical control data**
Extreme flexion of forepaw-foetus (%)	0	0	4*	1	0-4.2

* p<0.05

Conclusion

Phosmet is not teratogenic in the rabbit and slight foetotoxicity was seen only in presence of maternal toxicity at dose of 15 mg/kg bw/day.

NOAEL for maternal toxicity can be established at 5 mg/kg bw/day regarding mortality, clinical signs typical for an organophosphorous compound and decrease in body weight gain observed at 15 mg/kg bw/day.

NOAEL for development can be stated at 5 mg/kg bw/day taking into account the slight foetotoxicity manifested by areas of reduced or lacking ossification at 15 mg/kg bw/day.

Scientific publications on teratogenicity in the rat after oral administration

Title	**Developmental toxicity in the rat after ingestion or gavage of organophosphate pesticides (Dipterex, Imidan) during pregnancy**
Author (s) (year):	Staples, R.E., Kellam R.G. and Haseman J.K. (1976)
Administration	Diet and oral gavage
Guideline	No (scientific publication)
Species	CD rats
GLP	No
Purity:	95.8% w/w
Dose levels	0, 10, 22, 27 and 29 mg/kg bw/day (diet) and 5, 10, 20, 25 and 30 mg/kg bw/day (gavage)
Study acceptable as additional information	

Executive Summary

Staples et al. (1976) published an oral prenatal developmental toxicity study performed in CD rats with either dietary administration or application by gavage.

Phosmet (purity 95.8 % w/w) was administered to CD rats. Doses for administration by diet were 0, 10, 22, 27 or 29 mg/kg bw/day on days 6-15 of pregnancy. The size of the groups varied from 47 controls to 17 and 23 at the two higher doses. Phosmet was also administered by gavage at doses of 5, 10, 20, 25 or 30 mg/kg bw/day (with no separate control group; comparison of data was made with diet control group) to 9, 9, 18, 32 and 2 rats respectively on days 6-15 of pregnancy. In both methods of administration rats were killed on day 21.

Results

Diet: Food consumption and body weight gain were significantly reduced at doses ≥ 22 mg/kg bw/day through diet. No increase of malformation was observed. Phosmet was neither teratogenic nor foetotoxic after administration through diet at any dose level.

Gavage: Survival was affected at 30 mg/kg bw/day (100% of mortality) and at 25 mg/kg bw/day (16% of mortality). It has to be noted that diet control group was used to carry out the comparison at different dose levels after oral gavage. There was a significant reduction in weight gain (>10%) at doses ≥ 20 mg/kg bw/day and in food intake at doses ≥ 10 mg/kg bw/day (>10% from 20 mg/kg bw/day). Foetuses weight was significantly reduced at all dose levels but this decrease was greater than 10% at doses ≥ 20 mg/kg bw/day. No increase of malformation was observed.

Conclusions

After diet administration NOAEL maternal was established at 10 mg/kg bw/day and the NOAEL for development ≥ 29 mg/kg bw/day. No NOAEL was determined after gavage administration in the absence of a suitable control group.

Title	**Experimental study of the effect of a series of phosphoroorganic pesticides (Dipterex and Imidan) on embryogenesis**
Author (s) (year):	Martson, L.V. and Voronina V.M. (1976)
Administration	Gavage
Guideline	No (scientific publication)
Species	Wistar Rats
GLP	No
Purity:	Unknown
Dose levels	0.06, 1.5 mg/kg bw/day throughout gestation and 30 mg/kg bw on day 9 or 13 of pregnancy
Study acceptable as additional information	

Executive Summary

To study the embryotoxic effects of Imidan, the compound was given orally (gavage) in a single dose of 30 mg/kg bw (1/5 LD_{50}) to intact pregnant CD rats on day 9 or 13 of pregnancy; an additional group of rats were given phosmet daily throughout pregnancy at doses of 1.5 and 0.06 mg/kg bw/day (1/100 LD_{50}).

Results

Introduction of Imidan on day 9 of pregnancy at dose of 30 mg/kg bw resulted in a significant decrease ($p<0.05$) in the number of live foetuses (75.7% in comparison to 85.7 of controls) and in an insignificant increase in postimplantation mortality of embryos and in malformations such as hypognathia, general edema and dislocation of extremities. Introduction of Imidan on day 13 of

pregnancy did not affect embryo mortality. However, examination of serial sections revealed hydrocephaly in 33 of the 55 embryos studied.

Introduction of the compound at a level of 1.5 mg/kg bw/day throughout pregnancy resulted in a statistically verifiable reduction in the number of live foetuses in the test group; 62.8% in comparison to 85.7% for the control group. Hydrocephaly and subcutaneous hemorrhages were also seen but the significance is of these lesions is unknown.

No adverse effect was noted at a dose of 0.06 mg/kg bw/day.

Conclusion

Imidan administered orally (purity unknown) exhibited embryotoxicity and teratogenicity at the dose of 1.5 mg/kg bw/day, if the rats were exposed throughout the embryogenesis period. There are no data available about maternal toxicity.

Information from this scientific publication has to be taken with precaution.

4.10.2.2 Human information

There are no human data available relevant for C&L.

4.10.3 Other relevant information

No other relevant information is available.

4.10.4 Summary and discussion of reproductive toxicity

Fertility

In the 2-generation study in rats (Meyer and Walberg, 1990), phosmet caused reduction in fertility indexes from 80 ppm in P0 to generate F1b litters and P1 to generate F2a and F2b litters. However this decrease was only statistically significant from 80 ppm in P0 to generate F1b litters and at 300 ppm in P1 to generate F2a and F2b litters. Mating index decreased from 80 ppm in P0 for F1b litters and P1 to generate both litters only reaching the level of statistical significance at 300 ppm in P1 for F2b litters. The mean length of gestation was not affected at any dose level. The gestation indices decreased without statistical significance from 80 ppm in P0 females for F1b litters and P1 females for F2a litters and at 300 ppm in P1 females for F2b litters. Significant changes in the weight of some reproductive organs were observed at the top dose level of 300 ppm in P0 and P1 males with reduction of absolute weight of testes and in P0 females with reduction of the relative weight of ovaries. Besides, decrease in the number of total born pups delivered was observed in F1b, F2a and F2b litters at 300 ppm.

Hormonal changes associated with phosmet were not investigated. Only moderate decreased in spermatogenesis was observed in a small number of P0 males from all dose groups, including controls, which were not considered treatment-related. Diminished spermatogenesis was also observed in P1 males in 3/25 animals (300 ppm), 2/25 animals (80 ppm) and 1/25 animal (20 ppm), but these changes were not statistically significant.

Findings associated with impaired fertility observed mainly at 300 ppm and incidentally at 80 ppm happened at dose levels in which maternal toxicity was manifested. At 300 ppm toxicity in parental animals manifested by statistically and biologically significant reduction (>20%) of red blood cell cholinesterase, clinical signs, significant absolute and relative organ weight reductions, liver impairment (mild to moderate centrilobular hepatocellular vacuolisation in P1 males) and significant decreasings in body weight, body weight gain and food consumption during growth,

gestation and lactation periods. At 80 ppm it was observed statistically and biologically significant reduction (>20%) of red blood cell cholinesterase in P0 and P1 parental animals of both sexes, clinical signs as significative dehydration in P0 females, relative organ weight reductions P0 (liver and adrenals) and P1 (spleen) females and decrease in body weight gain during growth, gestation and lactation periods that was statistically significant in P0 males during growth (days 7, 21 and 112) and during lactation in P0 females (1^{st} mating) on day 14.

Development

Data from studies following accepted guidelines are the most valuable information:

In a teratogenicity study in the rat (Hodge, 1991) there were no indications affecting development up to and including the high dose level tested of 15 mg/kg bw/day in which signs of maternal toxicity were observed.

In a range finding prenatal development study in rabbits (Pinto, 1991), significant reduction of litter weight was observed at 15 mg/kg bw/day (22.8%). However, at this dose level, maternal toxicity was manifested by mortality in one dam and signs of intoxication of an organophosphorous compound. Slight reductions of the mean litter weight at 10 mg/kg bw/day (19.9%) and slightly reduced uterus weight at mid and high doses occurred (11.1% and 18.9% respectively). However, these changes were only minor and did not achieve the level of statistical significance. Indications for substance-related external abnormalities were not obtained at any dose level.

In a prenatal developmental study in rabbits (Moxon, 1991) there was evidence of slight foetotoxicity at 15 mg/kg bw/day manifested primarily in form of few areas of reduced or lacking ossification in foetuses (significant increase in the incidence of 5th sternebrae not ossified) and litters (6th sternebrae not ossified). The significant increase in the incidence of odontoid partially ossified and assymetrical development in 1st sacral vertebrae was statistically significant at this dose level but it is not clear a dose-response relationship. At 15 mg/kg bw/day there were signs of maternal toxicity such as mortality, clinical signs and decrease in the maternal body weight.

Effects indicating developmental toxicity were also observed in the offspring of the 2-generation study in rat (Meyer and Walberg, 1990). Significant decrease in the number of total born pups delivered and in the number of pups per litter (postpartum day 0) was observed in F1b, F2a and F2b generations at 300 ppm. Besides, significant reduction of live pups per litter (postpartum day 21) was observed in F1a, F2a and F2b litters at this same dose level. Pup weight on postpartum day 21 was significantly reduced at 300 ppm in all generations. It has to be pointed out that those effects were observed under maternal toxicity at this dose level.

The following data were collected from scientific publications considered acceptable as additional information and they must be considered with precaution.

In a rat prenatal development toxicity scientific publication (Staples et al., 1976) considered only acceptable as additional information, significant reduction after oral gavage of foetus weight at all dose levels (from 5 mg/kg bw/day) but greater tan 10% from 20 mg/kg bw/day were observed. No increase of malformation was observed. These effects were seen in presence of clear maternal toxicity (mortality at 25 mg/kg bw/day, reduction in weight gain from 20 mg/kg bw/day and reduction in food intake from 10 mg/kg bw/day). No effects on development were observed after diet administration at any dose level. Besides, it has to be pointed that a separated control group was not available for oral gavage administration.

In a scientific publication focused on the effect of phosmet (purity unknown) on rat embryogenesis (Martson and Voronina, 1976) considered only acceptable as additional information, after administration of 30 mg/kg bw on day 9 of gestation there was a significant decrease ($p<0.05$) in the number of live foetuses (75.7% in comparison to 85.7 of controls) and

insignificant increase in post-implantation mortality of embryos and in malformations such as hypognathia, general edema and dislocation of extremities. After a single oral dose of 30 mg/kg bw on day 13 of gestation hidrocefaly in 33 of the 55 embryos studied was observed. Treatment throughout gestation of 1.5 mg/kg bw/day resulted in a reduction in the number of live foetuses with respect to controls (62.8% in comparison to 85.7% for the control group). No adverse effect was noted at a dose of 0.06 mg/kg bw/day. No data about maternal toxicity are available.

4.10.5 Comparison with criteria

Comparison with classification criteria for reproductive toxicity (sexual function and fertility)

According to CLP Regulation (section 3.7.1.3 of Annex I), *any effect of substances that has the potential to interfere with sexual function and fertility has to be regarded for a classification for reproductive toxicity. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.*

Significant effects on fertility were observed in the 2-generation study in rats (Meyer and Walberg, 1990) at 300 ppm and also incidentally at 80 ppm:

- Reduction in fertility indexes from 80 ppm in P0 to generate F1b litter and in P1 to generate F2a and F2b litters at 300 ppm. Besides, significant decrease of the mating index occurred in P1 to generate F2b litter at 300 ppm.
- Reduction of absolute testes weight in P0 and P1 males and ovaries relative weight in P0 females at 300 ppm.
- Decrease in the total born pups delivered in F1b, F2a and F2b litters at 300 ppm

Reduction in the fertility index in F1b at 80 ppm can be regarded as incidental. Gestation length remained unaffected. Besides, other fertility parameters such as mating index, gestation index or total number of pups delivered were only slightly affected in all generations at this dose level. Moreover, at 80 ppm there were signs of maternal toxicity manifested by statistically and biologically significant reduction (>20%) of red blood cell cholinesterase in P0 and P1 parental animals of both sexes, clinical signs such as significative dehydration in P0 females, relative organ weight reductions P0 (liver and adrenals) and P1 (spleen) females and decrease in body weight gain during growth, gestation and lactation periods that was statistically significant in P0 males during growth (days 7, 21 and 112).

Remarkable effects imparing fertility at 300 ppm occurred in presence of maternal toxicity in parental animals manifested by statistically and biologically significant reduction (>20%) of red blood cell cholinesterase, clinical signs, significant absolute and relative organ weight reductions, liver impairment (mild to moderate centrilobular hepatocellular vacuolisation in P1 males) and decreasings in body weight, body weight gain and food consumption at this dose level during growth, gestation and lactation periods.

Phosmet induced a reduction in fertility and mating indexes, reduction in absolute testes weight and relative ovarian weight and decrease in total number of pups delivered at dose level also inducing parental toxicity. Therefore, the MSCA considers that these findings are likely to be a secondary non-specific consequence of general toxicity and not a consequence of administration of phosmet. Moreover, there were no gross lesions or histopatholigical changes. Hormonal changes associated with phosmet were not investigated. Only moderate decreased in spermatogenesis was observed in a small number of P0 males from all dose groups, including controls, which were not considered treatment-related. Diminished spermatogenesis was also observed in P1 males in 3/25 animals (300

ppm), 2/25 animals (80 ppm) and 1/25 animal (20 ppm), but these changes were not statistically significant. Overall, no effects providing sufficient evidence of impaired fertility were observed in the absence of parental toxicity. Besides, it has to be pointed out that the TC C&L agreed no to classifiy phosmet according to its fertility imparment considering the maternal toxicity observed, the lack of gross lesions or hispatological findings associated with phosmet dosing and the absence of relation between this reduction in fertility indices and spermatic changes.

Therefore, the MSCA concludes that based on data available, comparing these data with the relevant classification criteria, there is not sufficient and convincing evidence for classifying phosmet for its effects on fertility.

Comparison with classification criteria for reproductive toxicity (development)

Adverse relevant effects on development regarded as significant and biologically relevant from studies following accepted guidelines were the following:

Significant reduction of litter weight at 15 mg/kg bw/day (22.8%) in a range finding prenatal development study in rabbits (Pinto, 1991) in presence of maternal toxicity manifested by mortality (1 dam) and signs of intoxication of an organophosphorous compound.

Significant increase in the incidence of areas of reduced or lacking ossification in a prenatal developmental study in rabbits (Moxon, 1991) in foetuses (5th sternebrae not ossified) and litters (6th sternebrae not ossified) at 15 mg/kg bw/day. At this dose level, the significant increase in the incidence of odontoid partially ossified and assymetrical development in 1st sacral vertebrae was statistically significant but not dose related. These findings were observed at 15 mg/kg bw/day in presence of maternal toxicity (mortality, clinical signs and decrease in the maternal body weight).

In a 2-generation rat study (Meyer and Walberg, 1990), significant decrease in the number of total born pups delivered and in the number of pups per litter (postpartum day 0) was observed in P0 for F1b and P1 for F2a and F2b generations at 300 ppm. Besides, significant reduction of live pups per litter (postpartum day 21) was observed in P0 for F1a and F2a litters and in P1 for F2b litters at this same dose level. Pup weight on postpartum day 21 was significantly reduced at 300 ppm in all generations. It has to be pointed out that those effects were observed under maternal toxicity at this dose level.

The following data were collected from scientific publications considered acceptable as additional information and they must be considered with precaution:

Reduction of foetus weight at all dose levels (from 5 mg/kg bw/day) greater than 10% from 20 mg/kg bw/day were observed in presence of maternal toxicity (mortality at 25 mg/kg bw/day, reduction in weight gain from 20 mg/kg bw/day and reduction in food intake from 10 mg/kg bw/day) in a scientific publication (Staples et al., 1976). It has to be noted that a separated control group was not available for oral gavage administration.

Hidrocefaly in 33/55 embryos studied was observed in a scientific publication (Martson and Voronina, 1976) after a single oral dose of phosmet (purity unknown) of 30 mg/kg bw on day 13 of gestation. Significant decrease in the number of live foetuses after administration of 30 mg/kg bw of phosmet on day 9 of gestation occurred (75.7% in comparison to 85.7% for the control group) and insignificant increase in post-implantation mortality of embryos and in malformations such as hypognathia, general edema and dislocation of extremities. Treatment throughout gestation of 1.5 mg/kg bw/day resulted in a reduction in the number of live foetuses with respect to controls (62.8% in comparison to 85.7% for the control group). No data about maternal toxicity are available.

According to section 3.7.2.4 of CLP Regulation and the ECHA Guidance on the Application of the CLP Criteria, in the interpretation of the developmental outcome to decide classification for developmental effects it is important to consider the possible influence of maternal toxicity. Adverse developmental effects after phosmet treatment were observed at doses with marked maternal toxicity (i.e. mortality, clinical signs, body weight and body weight gain reductions, food consumption decrease, variations in organ weights. liver impairment and reduction of red blood cell cholinesterase levels). The MSCA is of the opinion that reproductive effects observed can be discounted and regarded as irrelevant for classification since they are a secondary consequence of maternal toxicity.

The severity of the effects observed is not sufficient to deem a classification of phosmet due to development impairment. Criteria in section 3.7.2.4.3 say that *"Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity"*. Section 3.7.2.4.2 of CLP Regulation says that *"Classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies"*.

Classification for phosmet due to developmental toxicity is not necessary since the effects were not sufficiently severe and occurred at doses in presence of maternal toxicity. Therefore, phosmet does not require classification for developmental toxicity

4.10.6 Conclusions on classification and labelling

The TC C&L agreed not to classifiy phosmet for reproductive toxicity. At the TC C&L meting 14-15 November 06, the Spanish Competent Authority was requested to present arguments why there was no classification and labelling proposal made for effects on fertility. During the meeting of the Technical Committee on Classification and Labelling in Arona, 15-16 May 2007, it was agreed not to classify phosmet for effects on fertility. There was no concern about developmental toxicity.

Fertility

CLP: A classification is not required

Development

CLP: A classification is not required

4.11 Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity/delayed neurotoxicty

Table 61: Summary table of relevant neurotoxicity/delayed neurotoxicity studies

Method	Results	Remarks	Reference
Range-finding study for acute neurotoxicity Guideline: US EPA OPPTS 870.6200 GLP: Yes **Study acceptable**	No mortality. Systemic toxicity manifested by related clinical signs as whole body tremors, gait alterations and salivation at 36 mg/kg bw. AChE inhibition (>20%): Plasma: at 36 mg/kg bw in both sexes and at 9 mg/kg bw in females. RBC: at doses ≥ 6 mg/kg bw Brain: at 36 mg/kg bw. **The NOAEL for AChE inhibitory potential was 3 mg/kg bw.**	Sprague-Dawley rats Purity: 94.4 % (correction factor of 1.059 used for dose calculation) Dose range (gavage): 0, 1.5, 3, 6, 9 and 36 mg/kg bw	**Cappon, G.D. (1998a)**
Acute neurotoxicity study Guideline: US EPA OPPTS 870.6200 GLP: Yes **Study acceptable**	No mortality. Plasma, RBC and brain AChE inibition (>20%) at 22.5 mg/kg bw. No observations in FOB and locomotor activity measurements at any dose level. No neuropathological changes. **NOAEL for acute neurotoxicity: 22.5 mg/kg bw** **NOAEL for AChE inhibition: 4.5 mg/kg bw**	Sprague-Dawley rats Purity: 94.4 % (correction factor of 1.059 used for dose calculation) Dose range (gavage): 0, 3, 4.5, 22.5 mg/kg bw	**Cappon, G.D. (1998b)**
Subchronic (13-weeks) neurotoxicity (feeding) Guideline: US EPA OPPTS 870.6200 GLP: Yes **Study acceptable**	No indications for neurotoxicity, including no neuro-pathological findings, were observed at any dose level. Activity of plasma, RBC, whole blood and brain AChE was significantly reduced troughout the study in a dose-related manner at all dose levels. Following reductions were seen at the lowest dose level of 25 ppm: - ↓ Plasma AChE for females statistically significant at week 13 (29%) and also inhibition greater than 20% in females at week 3 (21%) not statistically significant. - ↓Whole blood AChE was significantly inhibited from 25 ppm in both sexes at week 13. - ↓RBC AChE statistically significant in males at week 13 (19%) and also in females at week 7 (42%). - ↓brain regions AChE regions at week 13 in females: olfactory region (36%) and brainstem (21%) and also not statistically significant in hippocampus (33%). **NOAEL for clinical signs, functional effects and neuropathological findings: 150 ppm (corresponding to 9.4 mg/kg bw/day for males and 11.0 mg/kg bw/day for females)** **[a]LOAEL for AChE inhibition: 25 ppm (corresponding to 1.6 mg/kg bw/day for females)**	Sprague-Dawley rats Purity: 94.4 % (correction factor of 1.059 used for dose calculation) Dose range (diet): 0, 25, 50 and 150 ppm equivalent to: Males: 1.5, 2.7 and 9.4 mg/kg bw/day Females: 1.6, 3.1 and 11.0 mg/kg bw/day	**Cappon, G.D. (1999)**

Method	Results	Remarks	Reference
Acute delayed neurotoxicity Guideline: US EPA FIFRA 81-7, OECD 418 GLP: Yes **Study acceptable with reservations**	Phase A: LD_{50}: 2020 mg/kg bw. Pretreatment with atropine and 2-PAM reduced toxicity to all groups. Half of the hens at the high dose group were treated with atropine and 2-PAM after dosage. Phase B: Significant reductions of plasma and brain ChE at doses ≥ 65.6 mg/kg bw. **NOAEL for AChE inhibition: 16.4 mg/kg bw** Phase C: Alterations in the appearance and behaviour and motor coordination impairment was observed at 200 and 2050 mg/kg bw but after the observation period there were no signs of delayed neurotoxicity activity.	Domestic hen Purity: 94.7% Dose range (diet): Phase A – Acute toxicity: 1130 to 2400 mg/kg bw Phase B – ChE inhibition: 4.2, 16.4, 65.6, 131, 525, 1050 or 2100 mg/kg bw Phase C – Acute delayed neurotoxicity: 0, 20, 200 or 2050 mg/kg bw	**Sprague, G.L. (1982)**
Acute delayed neurotoxicity Guideline: US EPA FIFRA 81-7, OECD 418 GLP: Yes **Study acceptable**	Phase A LD_{50}: 577 mg/kg bw Unsteadiness, subdued behaviour, recumbency and salivation mainly during first 4 days in all groups. No clinical signs of toxicity were observed after day 8. Phase B Only inhibition of brain aceticholinesterase (63%) was observed in hens treated with phosmet after 48 h at 600 mg/kg. All adult hens treated showed clinical signs of toxicity for 4 days including unsteadiness, subdued behaviour, recumbency and weakness. Birds of the positive and negative control group remained healthy. No potential to induce acute delayed neutotoxicity by phosmet was observed.	Lohman Brown hens Purity: 94.7% Dose range (gavage): Phase A Acute oral toxicity: 0, 323, 420, 546, 710, 923, 1200 mg/kg bw Phase B Acute delayed neurotoxicity: 0, 600 mg/kg bw and positive group (TOCP)	**Johnson, A.J. (1997)**

[a] EFSA did not take into account these cholinesterase reductions at 25 ppm during the peer review of phosmet and established a NOAEL of 25 ppm. However, the Spanish Competent Authority in Classification&Labelling regards that AChE inhibition cannot be ruled out at this dose level. Therefore, this effect is considered relevant for repeated dose toxicity (RDT) classification.

Acute neurotoxicity – rat

Title	**A dose range-finding acute neurotoxicity study of phosmet in rats**
Author (s) (year):	Cappon, G. D. (1998a)
Administration	Dose range-finding acute neurotoxicity study (oral gavage)
Guideline	US EPA OPPTS 870.6200
Species	Sprague-Dawley rats
GLP	Yes
Purity:	94.4%
Groups	6 rats/sex/dose
Dose levels	0, 1.5, 3, 6, 9 and 36 mg/kg bw
	The study is acceptable

Executive Summary

In this dose range-finding study, groups of each 6 Sprague-Dawley rats/sex/group were dosed by gavage with 0 (corn oil), 1.5, 3 or 9 mg/kg bw phosmet technical (purity: 94.4 % (a correction factor of 1.059 was used for dose calculation, study phase I) and with 6 or 36 mg/kg bw phosmet (study phase II) at a dose volume of 5 mL/kg bw (single dose).

The animals were observed twice daily for mortality and moribundity. Animals received detailed clinical examinations on the day of dosing prior to administration and approximately 1, 2, 4, 6, 8, 24 and 48 hours after dosing. Body weights were determined on the day prior to administration and on days 0, 1 and 2. Blood was collected for plasma and red blood cell cholinesterase (ChE) level determinations on the day prior to dosing and at 2, 4, 6, 8, 12, 24 and 48 hours following dosing. The study was terminated 48 hours post dosing. Following euthanasia, the brains were excised, weighed and brain ChE levels determined.

Statistical analysis was not performed in this study.

Results

Mortality: All animals survived until scheduled termination on day 2.

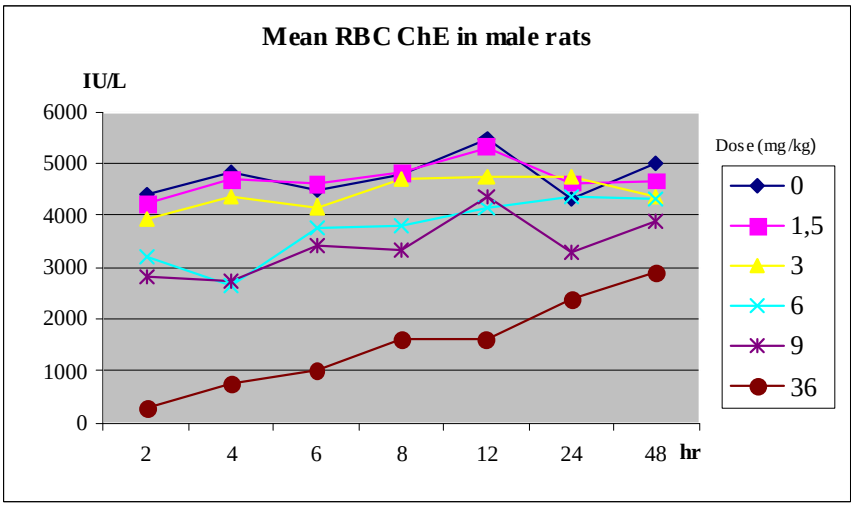
Body weights: No significant variations.

Clinical signs: Specific test substance-related clinical findings consisted of whole body tremors and gait alterations (animal rocks, lurches or sways as it walks and/or body drags, abdomen contacts surface and body sways) in the 36 mg/kg bw group. Gait alteration lasted about 4 hours, while whole body tremors were not observed after 6 hours post-dosing. Additionally, salivation occurred at 36 mg/kg bw lasting 1 - 2 hours after dosing.

Plasma cholinesterase levels: At 9 mg/kg bw was reduced by 14 - 36 % for females and at 36 mg/kg bw by 28 – 63 % for males and 41 – 59 % for females when compared to the control values from two hours onwards. The time of peak effect for reduced plasma ChE levels was between 2 - 4 hours post-dosing, while the inhibition persisted up to 48 hours post-dosing.

Red blood cell cholinesterase levels: Reduced by 13 – 45 %, 20 – 43 % and 42 – 94 % for males as well as by 14 – 41 %, 9 – 48 % and 53 – 94 % for females in the 6, 9 and 36 mg/kg bw groups, respectively, from two hours onwards. The time of peak effect for inhibited RBC ChE levels was between 2 – 6 hours after application and reduction persisted up to 12 hours after administration at 6 mg/kg bw and up to 48 hours at 9 and 36 mg/kg bw. At the high dose level 80%-90% of the RBC inhibition occurred up to 24 h post dosing.

Figure 2: Evolution of the RBC AChE activity after oral single dose of phosmet



Brain cholinesterase levels: determined 48 hours after application were reduced by 28 % for males and by 38 % for females at 36 mg/kg bw only.

Mean brain weights: Remained unaffected by treatment.

Conclusion

At 36 mg/kg bw systemic toxicity was manifested by related clinical signs as whole body tremors, gait alterations, salivation and depression (>20%) of plasma, RBC and brain cholinesterases. Besides, reductions of the cholinesterases activity greater than 20% was observed in plasma and RBC ChE at 9 mg/kg bw reduction and in RBC ChE at 6 mg/kg bw.

The NOAEL for ChE inhibitory potential was 3 mg/kg bw.

Based on the results of this study, dose levels of 3.0, 4.5 and 22.5 mg/kg bw and a time of peak effect of about 3 hours following dosing (*i.e.* day 0) were selected for the acute neurotoxicity study with phosmet in rats.

Title	**An acute neurotoxicity study of phosmet in rats**
Author (s) (year):	Cappon, G. D. (1998b)
Administration	Acute neurotoxicity study (oral gavage)
Guideline	US EPA OPPTS 870.6200
Species	Sprague-Dawley rats
GLP	Yes
Purity:	94.4%
Groups	30 rats/sex/dose
Dose levels	0, 3, 4.5 and 22.5 mg/kg bw
The study is acceptable	

Executive Summary

Phosmet technical with a purity of 94.4 % (correction factor of 1.059 used for dose calculation) was administered in corn oil as a single oral (gavage) dose to groups of 30 male and female Sprague-Dawley rats at 0, 3.0, 4.5 or 22.5 mg/kg bw. A dose volume of 5 mL/kg bw was used for all groups. 18 animals/sex/group were allocated to cholinesterase (ChE) activity evaluations. The remaining 12 animals/sex/group were assigned to functional observational battery and locomotor activity assessments.

Samples for concentration homogeneity and stability assessment were collected from formulations of each dose group. The animals were observed twice daily for mortality and moribundity for up to 15 days. Detailed clinical examinations were recorded daily for all animals with exception of animals assigned to the functional observational battery assessments on days 7 and 14. Body weights were determined pre-study and on days 0, 7, 14 and 15 (with few exceptions on days 7 and 14). Functional observational battery and locomotor activity evaluations were performed in selected 12 animals/sex/group at pre-treatment, at the time of peak effect (about 3 hours post-dosing on day 0 and on days 7 and 14).

Plasma, red blood cell and brain ChE activity determinations were conducted on 6 animals/sex/group at the time of peak effect (about 3 hours post-dosing on day 0), on day 7 and at termination on day 15.

After at least 15 days of observation, 12 animals/sex/group were euthanized and then perfused *in situ*. Fixed brain weights and dimensions were recorded. Qualitative neuropathological examinations were performed in 5 animals/sex/group of the control and the high dose groups (0 and 22.5 mg/kg bw, respectively).

All analysis were two-tailed (except as noted) for significance levels of 5% and 1%. Body weights, body weight changes, cholinesterase values, brain weights and brain dimensions were analysed by a one-way analysis of variance (ANOVA) and continuous Functional Observational Battery (FOB) data were analysed using a one-way ANOVA. If significant differences were indicated by the ANOVA, Dunnett's test was used to compare the control and treated groups. FOB data was analysed using Fisher's Exact Test.

Locomotor activity data were analysed using a two-way repeated measures ANOVA. If significant treatment or treatment-time interactions occurred, a one-way ANOVA was conducted at each time point. If significant treatment effects were observed at a time point, Dunnett's multiple T-test was conducted to determine significant treatment differences from the control group ($p<0.05$).

Results

Mortality: All animals survived until scheduled termination.

Body weights: No effects on body weight were observed.

Clinical signs: No test substance-related clinical signs were observed.

FOB parameters: No test substance-related effects were apparent between treatment and control group animals at the time of peak effect (3 hours post-dosing), and on days 0, 7 and 14. These observations covered home cage, open field, sensorimotor, neuromuscular and physiological observations.

Locomotor activity: On study day 0 (time of peak effect 3 h post-dosing) slight decreases in mean ambulatory and mean motor activity counts were observed in the 22.5 mg/kg bw group. However, the overall mean ambulatory and motor activity in this point of the study was not significantly different from controls. Evaluations at this dose level on days 7 and 14 were comparable to controls.

Mean ambulatory and total motor activity counts were unaffected in the 3.0 and 4.5 mg/kg bw groups on study days 0, 7 and 14.

Plasma ChE activity: Statistically significant reduced in the 22.5 mg/kg bw group males (57%) and females (46%) at the time of peak effect (3 hours post-dosing), when compared to the control group. Thereafter, no deviations were observed.

Red blood cell (RBC) ChE activity: At 22.5 mg/kg bw statistically significant inhibition in males (75%) and females (88%) at peak effect (3 hours post-dosing) and on day 15 in females (40%), when compared to the control group. Reduction in RBC ChE activity was also noted at study day 7 in females (25%) at this dose level, but it was not statistically significant.

Brain ChE activity: Statistically significant reduced in the 22.5 mg/kg bw group males (61%) and females (70%) at the study day 0 evaluations, when compared to the control group. These significant reductions persisted to study days 7 (15% for males and 20% for females) and 15 (9% for males and 17% for females).

Table 62: Cholinesterase (ChE) variations as per cent of controls

Time Point		**Males**				**Females**			
[day]		**Dose Level [mg/kg bw]**							
		0	**3.0**	**4.5**	**22.5**	**0**	**3.0**	**4.5**	**22.5**
Plasma [I.U./L (%)]									
0	Mean value	621	587	549	**266****	1125	1290	1037	**610***
	(% of control)	(100)	(-5)	(-12)	**(-57)**	(100)	(+15)	(-8)	**(-46)**
	Standard deviation	40.0	87.5	124.7	37.4	271.6	268.1	370.6	206.3
7	Mean value	488	602*	487	474	1314	1413	1415	1561
	(% of control)	(100)	(+23)	(0)	(-3)	(100)	(+8)	(+8)	(+19)
	Standard deviation	40.8	79.7	91.3	52.9	243.1	392.9	362.2	541.0
15	Mean value	533	603	527	502	1906	1761	1675	2239
	(% of control)	(100)	(+13)	(-1)	(-6)	(100)	(-8)	(-12)	(+17)
	Standard deviation	87.6	127.6	73.5	65.2	481.3	698.7	477.9	774.6
RBC [I.U./L (%)]									
0	Mean value	3696	3779	3162	**928****	3848	3259	3339	**443****
	(% of control)	(100)	(+2)	(-14)	**(-75)**	(100)	(-15)	(-13)	**(-88)**
	Standard deviation	417.7	208.1	553.4	239.4	431.6	563.2	294.3	318.7
7	Mean value	3560	3418	3791	3405	3101	3276	2673	2332
	(% of control)	(100)	(-4)	(+6)	(-4)	(100)	(+6)	(-14)	(-25)
	Standard deviation	745.9	361.1	482.2	435.7	544.4	1279.9	845.2	720.6
15	Mean value	3110	3114	3185	3056	2128	2146	2197	**1274***
	(% of control)	(100)	(0)	(+2)	(-2)	(100)	(+1)	(+3)	**(-40)**
	Standard deviation	591.7	746.4	278.0	648.6	406.7	528.0	883.8	234.4
Brain [I.U./g (%)]									
0	Mean value	22.83	22.57	22.59	**8.88****	23.27	22.85	22.39	**6.89****
	(% of control)	(100)	(-1)	(-1)	**(-61)**	(100)	(-2)	(-4)	**(-70)**
	Standard deviation	0.892	0.499	1.084	0.870	0.734	0.858	0.680	1.311
7	Mean value	22.88	22.82	22.65	**19.54****	23.80	23.30	22.91	**19.11****
	(% of control)	(100)	(0)	(-1)	**(-15)**	(100)	(-2)	(-4)	**(-20)**
	Standard deviation	0.535	0.372	0.439	0.828	0.827	0.588	1.086	0.736
15	Mean value	19.61	20.21	19.79	**17.87****	21.15	20.29	20.08	**17.59****
	(% of control)	(100)	(+3)	(+1)	**(-9)**	(100)	(-4)	(-5)	**(-17)**
	Standard deviation	1.173	0.842	0.825	0.651	1.130	1.317	1.086	0.892

* statistically significant difference from control group mean at the 5 % level (Dunnett's test)
** statistically significant difference from control group mean at the 1 % level (Dunnett's test)
No. of animals was 6 in all cases

Organ weights: No test substance-related changes were apparent in mean absolute brain weights or mean brain weights relative to final body weights for non-perfused animals as well as for fixed brain weights or brain dimensions of perfused animals.

Neurohistopathology: No treatment-related neuropathological lesions were noted.

Conclusion

The single oral administration of phosmet up to the high dose level of 22.5 mg/kg bw caused no specific treatment-related changes within the functional observational battery or locomotor activity measurements. Moreover, virtually no neuropathological changes due to treatment were observed.

The ChE inhibitory potential was confirmed since plasma, red blood cell and brain ChE activities were depressed at 22.5 mg/kg bw. No cholinesterase inhibition was observed at 4.5 mg/kg bw in rats after a single oral application. Consequently, the NOAEL for acute neurotoxicity was 22.5 mg/kg bw, while the NOAEL for ChE inhibition was 4.5 mg/kg bw in rats after a single oral application.

Subchronic neurotoxicity – rat – 90 day

Title	**Subchronic (13-weeks) neurotoxicity**
Author (s) (year):	Cappon, G.D. (1999)
Administration	Dietary subchronic 90-day neurotoxicity study of phosmet in rats (feeding)
Guideline	US EPA OPPTS 870.6200. Deviations: homogeneity did not meet the acceptance criteria.
Species	Sprague-Dawley rats
GLP	Yes
Purity:	94.4%
Groups	32 rats/sex/dose
Dose levels	0, 25, 50 and 150 ppm equivalent to: Males: 1.5, 2.7 and 9.4 mg/kg bw/day Females: 1.6, 3.1 and 11.0 mg/kg bw/day
The study is acceptable with reservations	

Executive Summary

In this subchronic neurotoxicity study, phosmet with a purity of 94.4 % (correction factor of 1.059 used for dose calculation) was administered in the diet to groups of 32 male and female Sprague-Dawley rats for 13 weeks at constant dietary levels of 0, 25, 50 or 150 ppm.

Samples of the test diet were analysed at regular intervals for concentration, homogeneity and stability. The animals were observed twice daily for mortality and moribundity. Detailed clinical examinations were recorded daily with few exceptions. Body weights were recorded weekly. Body weights were also recorded on treatment days when the functional observational battery and locomotor activity evaluations were conducted and prior to the scheduled termination. Food consumption was recorded weekly. Food left values were inadvertently not recorded for week 7 for animals scheduled for cholinesterase evaluation. The study was terminated at week 13 by sacrifice of all surviving animals. Blood cholinesterase activity levels (plasma, red blood cell and whole blood) were evaluated on 2 animals/sex/group during pretest and on 6 animals/sex/group for each week of the study (3, 7 and 13), involving a total of 20 animals/sex/group. From this same group of 20 animals/sex/group, for the pretest and study weeks 3 and 7, brain cholinesterase activity levels

were determined, and on week 13 brain regions cholinesterase activity levels were evaluated. The remaining 12 animals/sex/group from the total number of 32 animals/sex/group were assigned to Functional Observational Battery and Locomotor Activity assessments during pretest and study weeks 3, 7 and 12. *In situ* tissue perfusion was performed on these 12 animals/sex/group at study termination. Subsequent neuropathological evaluations were performed on 5 animals/sex in the control and high dose groups. The brains were excised, weighed and brain ChE activities were determined. Fixed brain weights and dimensions were recorded. Qualitative neuropathological examinations were performed.

Chemical analyses revealed that the diet preparations did not meet the acceptance criteria for homogeneity. The 25 ppm formulated diet did not meet the acceptance criteria for stability. When expressed as a percentage of targets, the overall diet concentrations averaged 75.7 %, 58.9 % and 71.5 % of 25, 50 and 150 ppm target concentrations. Since most of these analyses were not in the acceptable range, the nominal concentrations were adjusted for the analysed ones for the test item intake calculations in order to guarantee the validity of the study results. The average amount of test item consumed is listed in Table 63.

Table 63: Test item intake

Dietary Dose Level [ppm]	Test Item Intake [mg/kg bw]	
	Males	Females
25	1.7 / 1.5[#]	1.9 / 1.6[#]
50	3.4 / 2.7[#]	3.9 / 3.1[#]
150	10.4 / 9.4[#]	12.1 / 11.0[#]

[#]adjusted values based on the analytical results of the dietary preparations

All analysis were two-tailed (except as noted) for significance levels of 5% and 1%. Body weights, body weight changes, food consumption, cholinesterase determinations, brain weight data and brain dimensions were analysed by a one-way analysis of variance (ANOVA) and continuous Functional Observational Battery (FOB) data were analysed using a one-way ANOVA. If significant differences were indicated by the ANOVA, Dunnett's test was used to compare the control and treated groups. FOB data was analysed using Fisher's Exact Test.

Locomotor activity data were analysed using a two-way repeated measures ANOVA. If significant treatment or treatment-time interactions occurred, a one-way ANOVA was conducted at each time point. If significant treatment effects were observed at a time point, Dunnett's multiple T-test was conducted to determine significant treatment differences from the control group ($p<0.05$).

Results

Mortality: All animals survived until scheduled termination.

Mean body weight and food consumption: No test substance-related effects on body weight and food consumption were observed.

Clinical signs: No test-article related clinical signs were observed at any concentration.

FOB (Functional Observation Battery): No test article-related effects were apparent between treated and control group animals when functional observational battery evaluations were performed during study weeks 3, 7 and 12. These observations covered home cage, handling, open field, sensorimotor and neuromuscular and physiological observations.

Mean ambulatory and total motor activity counts: Unaffected at all phosmet concentrations.

Plasma cholinesterase (ChE) activity: Statistically significant reduction at 25 ppm for females at week 13 (29%). At this dose level there was also an inhibition greater than 20% in females at week 3 not statistically significant. At 50 ppm there was a significant reduction of the ChE activity in

males at week 3 (21%) and in females at weeks 3 (46%) and 13 (27%). In females there was also an inhibition of 27% at week 7 regarded as not statistically significant. At 150 ppm statistically and biologically significant reduction of ChE activity was observed for both sexes at all time evaluation points.

Red blood cell cholinesterase (ChE) activity: Mean RBC cholinesterase values in the 25 ppm group were statistically significant reduced by 19% (dose-dependent) in the males at week 13 and by 42% in the females at week 7. In the 50 ppm group, mean RBC cholinesterase levels were reduced statistically significant by 26%-38% in males during the study and by 38% for females at week 7. A not statistically significant inhibition of 54% in females was observed at week 13. In the 150 ppm group, mean RBC ChE levels were reduced by 65%-70% in the males and by 66%-89% in the females during the study. At this dose level all of the differences were statistically significant except for females at week 13 where the low n (n=1) precluded statistical analysis.

Whole blood cholinesterase activity: Reduced in a dose related manner at all doses for both sexes throughout the study. These reductions were statistically significant at all evaluation periods for the 50 and 150 ppm group males and females and at week 13 for the 25 ppm group in both sexes. Values were significantly reduced compared to the control group by 19% in the males and 16% in the females in the 25 ppm group at week 13, and during the study by 24%-36% in the males and 23%-32% in the females in the 50 ppm group and by 59%-64% in the males and 64%-74% in the females in the 150 ppm group.

Brain cholinesterase activity: Activity was reduced in a dose-related manner at weeks 3 and 7 in both sexes. There were no statistically significant reductions at weeks 3 or 7 in mean whole brain cholinesterase activity in the 25 ppm males or females. Brain ChE was reduced by 11%-17% in males and by 11%-19% in females of the 50 ppm group and by 43%-49% in the males and 61%-68% in the females in the 150 ppm group when compared to controls. These differences were statistically significant, with the exception of the value at week 3 in the 50 ppm group females.

At week 13 results for the brain ChE activities were divided by different brain regions. At 25 ppm, statistically significant reduction in olfactory region (36%) and in brainstem (21%) was observed in females. A reduction of 33% not statistically significant in hippocampus was also observed in females. At 50 ppm a statistically significant inhibition (27%) was observed in females in the olfactory region. Reductions greater than 20% not statistically significant were observed in females in hippocampus (34%) and in midbrain (21%). At 150 ppm there were reductions in the six brain regions (hippocampus, olfactory region, midbrain, brainstem, cerebellum and cortex) greater than 20% for both sexes, statistically significant in all cases with the exception of the olfactory region in males.

Table 64: Cholinesterase (ChE) variations as per cent of controls

Time Point		**Males**				**Females**			
[week]		**Dose level [ppm]**							
		[mg kg/bw/day]							
		0	**25**	**50**	**150**	**0**	**25**	**50**	**150**
			1.5	**2.7**	**9.4**		**1.6**	**3.1**	**11.0**
Plasma [I.U./L (%)]									
-1	Mean value (% of control)	754 (100)	576 (-24)	541 (-28)	624 (-17)	698 (100)	661 (-5)	761 (+13)	860 (+23)
	Standard deviation	204.4	26.9	57.3	91.2	51.6	54.4	184.6	509.8
	No. animals	2	2	2	2	2	2	2	2
3	Mean value (% of control)	555 (100)	505 (-9)	**437* (-21)**	**327** (-41)**	2609 (100)	2065 (-21)	**1398* (-46)**	**756** (-71)**
	Standard deviation	65.5	106.3	64.4	27.3	1022.5	1009.5	156.0	254.8

Time Point		Males				Females			
[week]		Dose level [ppm]							
		[mg kg/bw/day]							
		0	25	50	150	0	25	50	150
			1.5	2.7	9.4		1.6	3.1	11.0
	No. animals	6	6	6	6	6	6	6	6
7	Mean value (% of control)	586 (100)	488 (-17)	479 (-18)	**450* (-23)**	2657 (100)	2790 (+5)	1936 (-27)	**1066** (-60)**
	Standard deviation	48.8	88.6	50.5	115.0	682.6	676.3	375.0	298.2
	No. animals	6	6	6	6	6	6	6	6
13	Mean value (% of control)	589 (100)	514 (-13)	527 (-11)	**373** (-37)**	3250 (100)	**2312* (-29)**	**2363* (-27)**	**1698** (-48)**
	Standard deviation	121.7	67.0	85.5	113.0	569.3	825.7	355.4	548.5
	No. animals	6	6	6	6	6	6	6	6
RBC [I.U./L (%)]									
-1	Mean value (% of control)	4018 (100)	4961 (+23)	3902 (-3)	4692 (+17)	4377 (100)	4153 (-5)	3959 (-10)	4999 (+14)
	Standard deviation	699.3	137.2	159.1	305.5	291.3	34	533.2	666.1
	No. animals	2	2	2	2	2	2	2	2
3	Mean value (% of control)	3619 (100)	3145 (-13)	**2690** (-26)**	**1258** (-65)**	2065 (100)	2196 (+6)	2035 (-1)	**710** (-66)**
	Standard deviation	399.0	680.2	465.7	119.1	511.6	526.9	411.6	316.4
	No. animals	6	6	6	6	6	6	6	6
7	Mean value (% of control)	3321 (100)	3240 (-2)	**2043** (-38)**	**1130** (-66)**	2312 (100)	**1336** (-42)**	**1431** (-38)**	**252** (-89)**
	Standard deviation	347.1	439.3	783.2	359.5	320.3	538.7	505.6	223.7
	No. animals	6	6	6	6	6	6	6	4
13	Mean value (% of control)	3582 (100)	**2887** (-19)**	**2601** (-27)**	**1091** (-70)**	1295 (100)	1626 (+26)	594 (-54)	176# (-86)
	Standard deviation	466.3	227.1	326.8	358.2	353.3	806.1	487.0	0.0
	No. animals	6	6	6	6	6	5	6	1
Whole Blood [I.U./L (%)]									
-1	Mean value (% of control)	211 (100)	232 (+10)	180 (-15)	223 (+6)	220 (100)	208 (-5)	204 (-7)	249 (+13)
	Standard deviation	9.2	8.5	0.0	4.9	24.0	2.1	23.3	61.5
	No. animals	2	2	2	2	2	2	2	2
3	Mean value (% of control)	194 (100)	170 (-12)	**146** (-25)**	**74** (-62)**	234 (100)	212 (-9)	**168** (-28)**	**68** (-71)**
	Standard deviation	18.0	32.1	21.8	5.4	33.8	37.1	26.0	29.5
	No. animals	6	6	6	6	6	6	6	6
7	Mean value (% of control)	184 (100)	170 (-8)	**117** (-36)**	**76** (-59)**	250 (100)	214 (-14)	**171** (-32)**	**66** (-74)**
	Standard deviation	13.9	15.2	32.0	21.0	29.1	42.6	21.3	15.9
	No. animals	6	6	6	6	6	6	6	6
13	Mean value (% of control)	196 (100)	**158** (-19)**	**149** (-24)**	**70** (-64)**	239 (100)	**201* (-16)**	**159** (-23)**	**86** (-64)**
	Standard deviation	16.4	14.0	11.5	21.4	23.8	21.0	13.0	32.8
	No. animals	6	6	6	6	6	6	6	6
Whole Brain [I.U./g (%)]									
-1	Mean value (% of control)	22.28 (100)	22.02 (-1)	23.22 (+4)	22.05 (-1)	21.95 (100)	22.53 (+3)	22.87 (+4)	23.90 (+9)
	Standard deviation	2.150	0.884	0.170	0.057	0.014	0.219	0.594	0.446

Time Point		Males				Females			
[week]		Dose level [ppm]							
		[mg kg/bw/day]							
		0	25	50	150	0	25	50	150
			1.5	2.7	9.4		1.6	3.1	11.0
	No. animals	2	2	2	2	2	2	2	2
3	Mean value (% of control)	22.93 (100)	22.58 (-2)	**20.45* (-11)**	**11.61** (-49)**	23.42 (100)	21.88 (-7)	20.89 (-11)	**9.06** (-61)**
	Standard deviation	0.760	1.277	1.978	0.884	1.301	0.505	1.645	2.871
	No. animals	6	6	6	6	6	6	6	6
7	Mean value (% of control)	20.59 (100)	20.17 (-2)	**17.09** (-17)**	**11.72** (-43)**	21.44 (100)	19.30 (-10)	**17.26** (-19)**	**6.90** (-68)**
	Standard deviation	1.028	0.540	2.516	2.197	1.113	2.984	2.107	1.244
	No. animals	6	6	6	6	6	6	6	6

* statistically significant difference from control group mean at the 5 % level (Dunnett's test)
** statistically significant difference from control group mean at the 1 % level (Dunnett's test)
# no mean value, n=1

Table 65: Cholinesterase (ChE) activity in different brain regions

Brain Region		Males				Females			
		Dose level [ppm]							
		[mg kg/bw/day]							
		0	25	50	150	0	25	50	150
			1.5	2.7	9.4		1.6	3.1	11.0
		ChE Activity [I.U./g (%)]							
Hippocampus	Mean value (% of control)	15.16 (100)	15.73 (+4)	14.27 (-6)	**8.22** (-46)**	20.79 (100)	14.19 (-33)	13.77 (-34)	**7.04** (-66)**
	Standard deviation	2.251	3.094	1.807	4.436	11.983	2.796	3.365	3.834
	No. animals	6	6	6	6	6	6	6	6
Olfactory	Mean value (% of control)	29.80 (100)	29.61 (-1)	34.38 (+15)	17.56 (-41)	35.82 (100)	**22.92** (-36)**	**26.10* (-27)**	**12.00** (-66)**
	Standard deviation	11.635	6.687	5.444	5.413	7.787	5.880	4.286	7.422
	No. animals	6	6	6	4	6	6	6	6
Midbrain	Mean value (% of control)	23.70 (100)	22.18 (-6)	22.55 (-5)	**12.42** (-48)**	23.49 (100)	24.08 (+3)	18.60 (-21)	**12.72** (-46)**
	Standard deviation	2.021	1.658	3.028	2.489	1.532	5.464	1.747	5.669
	No. animals	6	6	6	6	6	6	6	6
Brainstem	Mean value (% of control)	20.25 (100)	19.41 (-4)	20.09 (-1)	**12.77** (-37)**	22.03 (100)	**17.44* (-21)**	18.57 (-16)	**12.13** (-45)**
	Standard deviation	0.987	1.778	1.090	1.874	1.411	3.175	1.886	3.134
	No. animals	6	6	6	6	6	6	6	6
Cerebellum	Mean value (% of control)	6.90 (100	7.16 (+4)	7.15 (+4)	**5.01** (-27)**	7.55 (100)	7.00 (-7)	6.62 (-12)	**4.83** (-36)**
	Standard deviation	0.264	0.681	0.407	0.432	0.458	0.792	0.926	0.992
	No. animals	6	6	6	6	6	6	6	6
Cortex	Mean value (% of control)	24.86 (100)	23.58 (-5)	23.06 (-7)	**11.87** (-52)**	24.09 (100)	22.38 (-7)	20.16 (-16)	**9.12** (-62)**
	Standard deviation	2.968	2.271	1.666	1.398	4.049	2.552	2.614	2.063
	No. animals	6	6	6	6	6	6	6	6

* statistically significant difference from control group mean at the 5 % level (Dunnett's test)
** statistically significant difference from control group mean at the 1 % level (Dunnett's test)

Organ weights: No effects were observed at any concentration on absolute brain and brain region weights, brain and brain region weights relative to final body weights or brain region weights relative to brain weights in non-perfused animals. Furthermore, remarkable differences were not observed in brain weights or dimensions for animals perfused at the scheduled terminal euthanasia.

Histopathology: No treatment-related neuropathological lesions were observed at the microscopic examination of perfused tissues.

Conclusion

No test article-related clinical signs, effects on body weight, effects on food consumption, on Functional Battery evaluations or total motor activity were apparent at any concentration. No effects were observed in organ variations and neuropathological findings.

Reduction of AChE values throughout the study were observed in a dose-related manner at all dose levels. At 50 and 150 ppm these reductions were significant and biologically relevant. At the lowest dose level of 25 ppm decrease of AChE plasma statistically significant for females at week 13 (29%) and also inhibition greater than 20% in females at week 3 not statistically significant (21%) were seen. Whole blood cholinesterase was clearly and significantly inhibited from 25 ppm in both sexes at week 13. Statistically significant inhibition of RBC cholinesterase values were observed at 25 ppm reduced at week 13 by 19% in males and by 42% in the females at week 7. Besides statistically significant reduction of cholinesterase in different brain regions were observed at week 13. Significant reductions in females were seen in the olfactory region (36%) and in the brainstem (21%) and also a reduction of 33% not statistically significant in hippocampus.

EFSA did not take into account these cholinesterase reductions at 25 ppm during the peer review of phosmet and established a NOAEL of 25 ppm. However, the Spanish Competent Authority in Classification&Labelling regards that AChE inhibition cannot be ruled out at this dose level. Therefore, this effect is considered relevant for repeated dose toxicity (RDT) classification.

Delayed neurotoxicity following acute exposure

Title	**Acute delayed neurotoxicity study with Imidan technical in adult hens**
Author (s) (year):	Sprague, G.L. (1982)
Administration	Acute delayed neurotoxicity study (diet administration)
Guideline	US EPA FIFRA 81-7, OECD 418 Deviations: Neuropathy target esterase (NTE) activity was not performed and histopathology examination was considered not valid.
Species	White Leghorn hens
GLP	Yes
Purity:	94.7%
Groups	Part A (acute toxicity): 5 hens/dose at all doses except top dose level (10 hens) Part B (AChE inhibition): no data available about the groups Part C (acute delayed neurotoxicity): 10-14 hens/dose level
Dose levels	Part A (acute toxicity): 1130-2250 mg/kg bw Part B (AChE inhibition): 4.2, 16.4, 65.6, 131, 525, 1050 and 2100 mg/kg bw Part C (acute delayed neurotoxicity): 0, 20, 200 and 2050 mg/kg bw and one positive control with 500 mg/kg bw of TOCP
The study is acceptable with reservations	

Executive Summary

In this delayed neurotoxicity study following acute exposure, Imidan technical (phosmet, purity: 94.7%) was administered neat in gelatine capsules by the oral route to domestic adult White Leghorn hens. The study comprised acute oral toxicity, cholinesterase inhibition and potential acute delayed neurotoxicity phases.

Phase A – Acute oral toxicity

In the acute oral toxicity phase, phosmet was given to five hens per dose level (1130-2250 mg/kg bw) for LD_{50} assessment. After treatment, the hens were observed daily for up to two weeks and body weights, food consumption and behavioural neurotoxicity scores were recorded. Atropine and pyridinealdoxime methochlorine (2-PAM) were administered subcutaneously as antidotes before phosmet administration and also in half of the hens after dosage at 2250 mg/kg bw.

Phase B – Cholinesterase inhibition

In the cholinesterase inhibition phase, the effect of phosmet on plasma cholinesterase and brain acetylcholinesterase was determined after single application of 4.2, 16.4, 65.6, 131, 525, 1050 or 2100 mg/kg bw. Heparinised blood samples were collected 4 and 24 hours after treatment and the activity of ChE was determined.

Phase C – Acute delayed neurotoxicity

In the acute delayed neurotoxicity phase, the birds were treated twice with phosmet at 20, 200 or 2050 mg/kg bw (10 hens for the low and mid doses and 14 heins for the high dose) within an interval of three weeks and were observed for further 3 weeks after the second treatment. Positive controls (10 hens) were dosed with tri-o-cresylphosphate (TOCP) at 500 mg/kg bw and negative controls (10 heins) received empty capsules. Body weights, food consumption, behavioural neurotoxicity scores and egg production were measured at regular intervals. Three weeks after the second treatment the study was terminated and specimens of the central and peripheral nervous system were examined histopathologically. Atropine and 2-pyridinealdoxime methochlorine (2-PAM) were also administered subcutaneously as antidotes at least on days 1 and 22 to all groups. Hens showing severe cholinergic signs were also given atropine and 2-PAM up to 2 days after Imidan treatment.

Statistics: Mean values for body weights, 24-hour food consumption and cholinesterase activities were compared by Dunnett's test. Group scores for behavioural neurotoxicity and weekly egg production were compared statistically using the Mann-Whitney U-test.

Results

Phase A – Acute oral toxicity

The oral LD_{50} value of phosmet in birds was calculated to be 2020 mg/kg bw, with 95 % confidence limits of 1700 to 2400 mg/kg bw. No deaths occurred up to 14 days after administration of the lowest dose of 1130 mg/kg bw, and only mild motor incoordination was observed. Deaths occurred between days 1-7 after treatment in the additional groups dosed up to 2250 mg/kg bw. The severity of motor incoordination and diarrhoea increased with increasing dose levels. Pre-treatment with atropine and 2-PAM reduced the toxicity. Ten birds were treated with 2250 mg/kg bw, half of which were also given three daily treatments of atropine and 2-PAM. All 5 birds given phosmet alone died 1 to 2 days after application, while the "protected" hens survived. When these hens were killed after a two week observation period most appeared normal.

Phase B – Cholinesterase inhibition

Both, plasma cholinesterase and brain acetylcholinesterase activities were inhibited by phosmet. Statistically significant reductions of both enzymes occurred 24 hours after application of a single

dose equal to or greater than 65.6 mg/kg bw with 55% of reduction in plasma ChE and 45% in brain ChE.

Phase C – Acute delayed neurotoxicity

Mortality: All hens of the control and mid dose group (200 mg/kg bw) survived the entire study period. One hen of the low dose group died on day 2 and two hens of the high dose group died on day 3. Only six positive control hens treated with TOCP survived until day 43. Advanced paralysis developed in 3 hens of the TOCP group killed on days 31 (two hens) or 38 (one hen) and one died unexpectedly on the last day of study.

Body weight and food consumption: There were some significant reductions of both body weight and food consumption at the high dose level from week 1 after treatment. Transient signs of recovery in this group were observed during treatment. In TOCP group impaired food comsumption and resulting reduction conspicuous late in the study.

Clinical signs and other findings: The main alterations of appearance and behaviour were found at high doses of 200 and 2050 mg/kg bw. Effects observed at the lower dose of 20 mg/kg bw were similar than those observed in controls and corresponded with the presence of diarrhea (attributed to the atropine and 2-PAM treatment) and feather loss. Animals dosed with 200 mg/kg bw also presented diarrhea and feather loss and noticeably non-vocal 1-2 days after each treatment and transient motor incoordination for up to 4 days after treatment. The diarrhea, non-vocal and motor incoordination occurred with greater frequency and severity when hens were dosed at 2050 mg/kg bw and in the most severe cases hens were unable to stand in their cages. In addition, at this dose level, the behavioural depression was evident in most hens up to 6 days after treatment and these hens were described as “listless”. In survivor animals at 2050 mg/kg bw, these adverse signs were transient and the severity decreased with time after each treatment, except for the feather loss. Significant cessation of the egg production was observed at the high dose level during the study.

No clinical signs indicative for delayed neurotoxicity in form of weakness or incoordination having a delayed onset occurred in any of the phosmet treated hens.

Observation of animals treated with TOCP showed the presence of diarrhea after the first treatment, motor incoordination by day 14, that increased to paresis and by day 16 several animals were sitting on their hocks rather than standing, and finally, at day 26, hens were laying and unable to stand. The motor incoordination showed impairment increased through the course of the study.

It has to be taken into consideration that hens dosed with phosmet showed a pattern of transient impairment (or reversible), since the effects disappeared 3 week after the second treatment, not occurring the same in hens dosed with TOCP, that showed a pattern of impairment growing (score) with time.

Neurohistopathology: TOCP treated hens showed neurohistopathologic changes characteristic of delayed neurotoxicity. These changes include axonal degeneration in located, myelinated fiber tracts of the brain and the spinal cord as well as bilateral axonal degeneration in peripheral nerves with severe appearance. More neurohistopathological changes were observed in the negative control group than the group treated with TOCP. The author explained that the changes observed in control animals were due to field and vaccine viruses, very common to commercila hens.

Conclusion

Under the conditions of this study, phosmet revealed acute oral toxicity in adult domestic hens after a single treatment with a LD_{50} of 2020 mg/kg bw. Pre-treatment with atropine or 2-PAM reduced markedly the acute lethality of phosmet. Phosmet inhibited plasma cholinesterase and brain

acetylcholinesterase in hens at dose levels from 65.6 mg/kg bw onwards. The NOAEL for brain AChE inhibition was 16.4 mg/kg,

Only doses of 200 and 2050 mg/kg bw produced neurotoxicity, manifested by alterations in the appearance, behaviour and motor coordination impairment. These alterations cannot be considered delayed neurotoxicity, because the onset of the signs was not delayed, most adverse signs were transient and the severity decreased with time after each treatment.

Title	**Acute delayed neurotoxicity study in the domestic hen**
Author (s) (year):	Johnson, A.J. (1997)
Administration	Acute delayed neurotoxicity study (gavage)
Guideline	US EPA FIFRA 81-7, OECD 418
Species	Lohmann Brown hens
GLP	Yes
Purity:	97.4%
Groups	Preliminary study: 2 hens/dose Acute oral toxicity: 10 hens/dose Acute delayed neurotoxicity: 12 hens/dose for TOCP and control groups 14 hens/dose for the test group
Dose levels	Preliminary study: 500, 1000 and 2000 mg/kg bw Acute oral toxicity: 0, 323, 420, 546, 710, 923 and 1200 mg/kg bw Acute delayed neurotoxicity: 0, 600 mg/kg bw and positive control with 1000 mg/kg bw of TOCP.
	The study is acceptable

Executive Summary

In this study, domestic adult hens were used to investigate acute oral toxicity and any acute delayed neurotoxic effects of dosing with phosmet by oral gavage route. The study comprised two phases to determine acute oral toxicity and potential acute delayed neurotoxicity including assessment of cholinesterase inhibition. No statistical analysis was performed in this study.

Phase A – Acute oral toxicity

In the acute oral toxicity phase, a dose range-finding test was performed using 3 groups of 2 birds each at oral dose levels of 500, 1000 and 2000 mg/kg bw. Thereafter, single doses of 0, 323, 420, 546, 710, 923 or 1200 mg/kg bw, selected on the basis of the dose range-finding test were orally administered by gavage to 10 hens/group at a dosing volume of 10 mL/kg bw. After treatment, the birds were observed for 14 consecutive days for clinical signs and mortality. At the end of this observation period all animals were sacrificed.

Phase B – Acute delayed neurotoxicity

The acute delayed neurotoxic potential of phosmet was assessed after oral administration (gavage) in comparison with a negative (corn oil) and a positive control group. Positive control birds were dosed with TOCP and negative control birds were dosed with vehicle (corn oil). Phosmet at 600 mg/kg bw was administered orally (gavage) as a 6.2 % solution in corn oil. All birds were given a subcutaneous injection of atropine sulphate prior to dosing. Birds with severe clinical symptoms following dosing were given a further atropine sulphate injection. After treatment, animals were observed daily for a period of up to 21/22 days for clinical signs,

mortalities and delayed locomotor ataxia. The degree of ataxia was scored according to a scale from 0 – 8. Body weights were recorded once weekly. Brain acetylcholinesterase (AchE), brain neuropathy target esterase (NTE) and lumbar spinal cord NTE in 3 pre-determined animals per group were determined after 48 hours of treatment. At the end of the study all birds were sacrificed and 6 birds/group were gross necropsied and examined histopathologically.

Results

Phase A – Acute oral toxicity

In the dose range-finding study both birds of the two highest dose levels died. On this basis, dose levels were selected to determine acute oral toxicity.

In the main study mortality occurred at all dose levels. All birds treated with phosmet showed dose-dependent body weight losses during the week following treatment. In the subsequent week, all surviving birds gained body weight but mean body weights at 710, 923 and 1200 mg/kg bw remained lower than control. Clinical signs of toxicity were recorded mainly during the first 4 days in all groups dosed with phosmet. These signs included unsteadiness, subdued behaviour, recumbency and salivation. No clinical signs of toxicity were observed after day 8. The oral LD_{50} value of phosmet in birds was calculated using a logistic model for which 95 % confidence limits were estimated by the likelihood ratio method.

LD_{50} oral (gavage) value: 577 mg/kg bw (411 to 767 mg/kg bw)

Phase B – Acute delayed neurotoxicity

Mortality: Five birds dosed with phosmet were either found dead (4 animals) or were sacrificed for humane reasons (1 animal) within the first 5 days after treatment at 600 mg/kg.

Mean body weight: During the first week after treatment there was a decrease in mean body weight in the phosmet and TOCP-treated animals. Subsequently, the phosmet-dosed birds with 600 mg/kg gained weight, while the TOCP group showed a decrease in mean body weight.

Clinical signs: All adult hens treated with phosmet at 600 mg/kg showed clinical signs of toxicity for 4 days including unsteadiness, subdued behaviour, recumbency and weakness. Birds of the positive and negative control group remained healthy.

Delayed locomotor ataxia: There were no clinical signs of delayed locomotor ataxia in any birds treated with phosmet or the control group. The susceptibility was demonstrated since eight birds of the positive control group developed clinical ataxia commencing between days 11 and 18. At study termination, the ataxia grades ranged from 2 to 5 (max. grade: 8) in the positive control group.

Brain acetilcholinesterase: Treatment with 600 mg/kg of phosmet led to a reduction of brain acetylcholinesterase activity by 63 % at 48 h relative to the vehicle control group, while birds of the positive TOCP control group remained unaffected.

Neuropathy esterase: brain and spinal cord NTE activities were similar in the phosmet group compared to the vehicle control group and thus, in agreement with the findings of the ataxia scoring, where no signs of delayed neurotoxicity were observed in any bird. Birds treated with TOCP showed a marked reduction in NTE activities (90% reduction in brain and 80 % reduction in spinal cord NTE).

Table 66: Brain AChE and NTE determination 48 hours after administration as per cent of controls

Group	Dose Level		Brain AChE	NTE Brain	NTE Spinal Cord
[#]	[mg/kg bw]		[µmol/g/min (% of control)]	[nmol/g/min (% of control)]	[nmol/g/min (% of control)]
1	0 (Control)	Mean	13.49	2417	504
		S.D.	2.9	96.5	92.5
2	1000 (TOCP)	Mean (% of control)	13.73 (+2%)	231 (-90%)	100 (-80%)
		S.D.	1.880	61.4	43.3
3	600 (phosmet)	Mean (% of control	4.94 (-63%)	2122 (-12%)	490 (-3%)
		S.D.	0.867	302.6	2.0

No. of animals was 3 in all cases

Macroscopic post mortem examination: Only oedermatous areas in the lumbo-sacral region of the spinal cord were found in two negative control and one positive control birds examined at the termination of the study and therefore, were considered unrelated to treatment.

Histopathology: In the negative control group no significant axonal degeneration was recorded in any of the six birds examined in this group. In four birds, trace axonal degeneration was recorded in at least one level of the spinal cord. One of these birds also showed trace swollen axons in one level of the spinal cord. In four birds, trace axonal degeneration was recorded in at least one level of peripheral nerve. No axonal degeneration was seen in the birds of this group. These findings are consistent with the normal background.

All examined birds treated with phosmet showed trace axonal degeneration in at least one level of spinal cord, and three hens showed trace axonal degeneration in at least two levels of peripheral nerve. One bird showed trace swollen axons in the medulla/pons and in one level of spinal cord, and a trace focus of gliosis in the cerebellum. No significant axonal degeneration was recorded in any of the birds of this group. These findings were considered to be normal background incidence and not related to treatment with phosmet.

All animals of the positive control showed minimal axonal degeneration in the cerebellum, and minimal or moderate axonal degeneration in at least one of the four levels of spinal cord examined and in at least one of the six regions of peripheral nerve examined. As expected, these findings revealed significant axonal degeneration related to treatment with TOCP.

Conclusions

The oral LD_{50} value of phosmet in birds was 577 mg/kg bw (95 % confidence limits: 411 to 767 mg/kg bw). Clinical signs of toxicity were recorded in this acute oral phase mainly during the first 4 days in all groups dosed with phosmet. These signs included unsteadiness, subdued behaviour, recumbency and salivation. No clinical signs of toxicity were observed after day 8.

Oral administration by gavage of a single dose of 600 mg/kg bw resulted in clinical signs such as unsteadiness, subdued behaviour, recumbency and weakness for 4 days after dosing and inhibition of brain AChE (63%) was also seen at 48 h. Treatment with phosmet did not elucidate any clinical signs of delayed neurotoxicity when assessed as clinical locomotor ataxia seen 10-20 days later. There were no biologically relevant or significant reductions in NTE in brain or spinal cord within 24-48 hours after dosing. Histopathology revealed no evidence of specific acute delayed neurotoxicity. Thus, phosmet showed no potential to induce delayed neuropathy in adult domestic hens at a dose level in excess of an unprotected LD_{50}.

Further investigations

In an old, acute neurotoxicity study (Hendricks, 1983) prior to implementation of valid testing guidelines phosmet (batch: 4921-31-31, purity: 94.7 %, white granular) was orally administered to male and female CD rats (Charles River Laboratories, Portage, MI, USA). The aim was to evaluate the effect on plasma, RBC and brain cholinesterase (ChE) activity as a measure for potential acute neurotoxicity. Phosmet diluted with 20 % Tween 80 in distilled water was orally administered by gavage to each 5 male and 5 female rats (fasted for 17 – 24 hours) at dose levels of 10 or 100 mg/kg bw. Rats treated with 20 % aqueous Tween 80 only were used as controls. The animals were killed after 4 and 24 hours and ChE activities in plasma, RBC and brain were measured 4 or 24 hours post dosing.

Treatment caused death in one out of five males and two out of five females at 100 mg/kg bw 4 hours post dosing and additionally one after 24 hours. No mortality was recorded at 10 mg/kg bw, either 4 hours or 24 hours after treatment. The high dose led to prolonged ChE inhibition in brain and RBC (> 34 %) 24 hours after dosing. At 10 mg/kg bw only brain ChE was inhibited by 14 % in males and 21 % in females at the sampling point 4 hours post dosing. Plasma ChE was not as consistently inhibited as RBC ChE. The authors concluded that either plasma or RBC ChE might be suitable for monitoring Phosmet exposure but RBC ChE is preferred due to consistent inhibition.

4.11.1.2 Immunotoxicity

No immunotoxicity studies were conducted with phosmet.

4.11.1.3 Specific investigations: other studies

Table 67: Summary table of relevant specific investigations

Method	Results	Remarks	Reference
Morphological transformation assay Guideline: Not mentioned, but based on the procedure described by Kakunaga (1973) GLP: Yes **Study acceptable**	No transformation activity	BALB/3T3 cells Purity: 95.7% (w/w) Dose range: First experiment: 0.0005, 0.0010, 0.0020, 0.0040, 0.0080, 0.0120 mg/mL Second experiment: 0.004, 0.006, 0.008, 0.010, 0.012, 0.014 mg/mL	**Dickey, J.K. (1986)**
Comparative *in vitro* inhibition of acetylcholinesterase (AChE) in mouse brain homogenate and mouse red blood cells Guideline: Not applicable GLP: Yes **Study acceptable as additional information**	Mouse RBC-AChE inhibition more pronounced than brain-AChE inhibition.	Mouse brain tissue (Crl:CD I (ICR)) and mouse red blood cells (RBCs) Purity: 98% Dose range: 0.52, 1.737, 5.202, 17.37, 52.02, 173.7 to 520.2 µM	**Barnett jr., J.F. (2007)**
Comparative inhibition of AChE in rat plasma and rat red blood cells versus human plasma and human red blood cells Guideline: Not applicable GLP: No **Study acceptable as additional information**	AChE inhibition observed at the highest tested dose level (0.421 mM: – Humans: 17% in RBC AChE and 19.2% in plasma AChE. – Rats: 31% in RBC AChE and 26.5% plasma AChE. Inhibition of plasma and RBC AChE was more pronounced in rats than that observed in humans.	Plasma and RBC from three rats [Crl:CD(SD)] and three humans. Purity: 98% Tissue exposure levels: 0, 0.0421, 0.140, 0.421 mM	**Hoberman, A.M. (2008)**

Title	Morphological transformation of BALB/3T3 cells
Author (s) (year):	Dickey, J.K. (1986)
Guideline	Not mentioned, but based on the procedure described by Kakunaga (1973)
GLP	Yes
Purity:	95.7%
The study is considered acceptable	

Executive Summary

Phosmet (purity: 95.7 % (w/w)) was tested for its potential to induce morphological changes of BALB/3T3 cells *in vitro* by determination of the number of foci/flask in exposed and control cultures.

Cells from the C-14 subclone of a clone I-13 of BALB/3T3 mouse cells were plated at a density of $1x10^4$ cells/flask approximately 24 h before treatment. Fifteen flasks were treated for each of the six concentrations of the test substance, the positive control (3-methylcholanthrene; 1.0 µg/mL) and the negative (culture medium) and solvent (DMSO) controls. Culture medium was Eagles minimum essential medium. After a 3-day exposure period, the cells were washed and fresh culture medium was added. Medium was changed twice weekly for 4 weeks. After termination, the number of foci/flask was determined to evaluate the transformation potential of the chemical. Two independent experiments were conducted. A concurrent toxicity assay for the solvent control and treatment concentrations was included with each transformation experiment.

The selected concentrations, 0.0005, 0.0010, 0.0020, 0.0040, 0.0080 and 0.0120 mg/mL in the first experiment and 0.004, 0.006, 0.008, 0.010, 0.012 and 0.014 mg/mL in the second experiment were based on the results of an initial cytotoxicity experiment. The test substance was initially dissolved in DMSO before being applied to the culture medium.

In the cytotoxicity study, an excessive reduction in clonal survival was seen at concentrations ≥ 0.015 mg/mL.

Significance was determined by the method of Kastenbaum and Bowman. A positive test is determined on the basis of a dose related increase in foci, and a statistical significance of $p<0.01$ at the highest responding dose.

Results

In the initial transformation assay, in flasks treated with 0.0040 mg/mL, with relative clonal survival of 100%, there was a significant increase in foci number (0.53 foci / flask) as compared to solvent controls (0.07 foci / flask) but not as compared to negative controls (0.33 foci / flask). In addition, no dose response was obtained and the cloning efficiency for this assay was considered unacceptable (only 9%). Therefore, this slight increase was not regarded as biologically relevant.

In the second independent experiment, no significant or biologically relevant increase in transformed foci was seen in any of the treated flasks at any of the test substance concentrations (0.004 - 0.014 mg/mL). The relative clonal survival ranged from 52% at the lowest concentration to 10 % at the highest concentration. The cloning efficiency was 29%.

The positive control (3-methylcholanthrene) induced an average of 1.4 and 1.13 foci / flask in the first and second experiment, respectively.

Conclusion

Phosmet had no transformation activity under the conditions of the BALB/3T3 morphological transformation assay.

Title	**Comparative *in vitro* inhibition of acetylcholinesterase in mouse brain homogenate and mouse red blood cells following exposure to Phosmet**
Author (s) (year):	Barnett jr., J.F. (2007)
Guideline	Non-guideline study
GLP	Yes
Purity:	98%
	This study was provided for the re-evaluation of the ADI of phosmet in March 2010 by the EFSA and it is considered acceptable only as additional information since no metabolic activation system was used. The active metabolite of phosmet is an oxon derivative that needs the presence of a metabolic activation system to be formed (Fukuto, 1990). Therefore, this *in vitro* study expresses the AChE inhibition of the parent compound only and not of the main metabolite.

Executive Summary

In this comparative *in vitro* acetylcholinesterase inhibition study, mouse brain tissue (Crl:CD I (ICR)) and mouse red blood cells (RBCs) were exposed to tissue concentrations of phosmet (purity: 98%, vehicle: 0.1 M sodium phosphate buffer with 0.1 % Tween 80, pH 6.5, with 1.67 % dimethyl sulfoxide (DMSO)) ranging from 0.52, 1.737, 5.202, 17.37, 52.02, 173.7 to 520.2 µM. Following incubation with phosmet, acetylcholinesterase activity was measured in each tissue using the modified Ellman's method. The concentration of the dosing solution (578.0 µM) used for the highest final concentration of treated tissue (520.2 µM) was verified.

The brain and RBC tissues were separately treated with the same dosing solutions. The whole blood samples were processed by centrifugation and the plasma (and the interface) was removed from the packed RBC samples. Phosphate buffer (pH 8) was added to each packed RBC sample resulting in a dilution of 1:11.25. After sonication for a period of 5 seconds, each RBC sample was further diluted with phosphate buffer. This resulted in a total dilution of 1:112.5 for each of the RBC samples.

Each mouse brain was excised, weighed and placed into a tube containing chilled saline. The brain weights ranged between 0.487 and 0.520 g. The mouse brains were then transferred into a tube containing phosphate buffer (pH 8) homogenized and further diluted with phosphate buffer. This resulted in a total dilution of 1:110 for each of the brain samples.

A sufficient volume of each mouse RBC sample or mouse brain sample was placed into duplicate columns on a single plate (sample #1 was placed into all eight wells of columns 1 and 2, sample #2 was placed into all eight wells of columns 3 and 4, etc.). An additional plate was then used to combine 50 µL of the RBC sample or 10 µL of the brain sample with 90 µL of the appropriate dosing solution. Once the combination occurred, this plate was then allowed to incubate for ten minutes at 37°C. After the incubation period, 50 µL of each of the dosing solution/RBC sample mixtures or 10 µL of each of the dosing solution/brain sample mixtures were removed from the plate and added to the final RBC sample plate and the final brain sample plate, respectively. Once the 50 µL (RBC sample) or 10 µL (brain sample) of the appropriate mixtures was placed into the wells, 200 µL of 0.65 mM 5,5'-dithio-bis (2-nitrobenzoic acid) was also added to each well. The samples were then allowed to incubate at 37°C for 10 minutes. After the incubation period, 100 µL of 3.5 mM acetylthiocholine iodide was then added to each well and the samples were analyzed via absorption.

The brain and RBC sample analysis plates were analyzed for cholinesterase activity using the modified Ellman's reaction technique, *i.e.* by means of a colour change which is directly proportional to the amount of cholinesterase enzyme present within each sample. Each plate was analyzed at 435 nm for 60 minutes at 37°C. At the beginning of each analysis, there was a 10 minute lag-time used to reduce the variability within the samples. The remaining 50 minutes of the analysis was integrated to generate a linear rate.

The result of the concentration analysis was found to be +1.7 % of the targeted concentration.

No statistical analysis for comparison between groups was performed in this study.

Results

Acetylcholinesterase in mouse was inhibited at a substantially greater rate and extent in RBCs than in the brain tissue. At the highest concentration tested (520.2 µM), RBC acetylcholinesterase was 36.4 % of the control value (63.6 % inhibition, compared to the control). In comparison, the brain acetylcholinesterase response was 85.4 % of control (14.6 % inhibition) at the highest concentration tested. Substantial cholinesterase inhibition continued to be observed in the RBCs at 173.7 µM; however, no inhibition was seen in brain tissue at this 3-fold lower dose. The mean RBC response was 75.6% of controls at 173.7 µM. In comparison, the brain acetylcholinesterase response was 100.7 % of controls at this concentration.

Table 68: Acetylcholinesterase responses to phosmet treatment

Test Group	Concentration	Response [% of Control]			
		RBC		Brain	
[#]	[µM][a]	mean[b]	S.D.	mean[b]	S.D.
I	0 (Vehicle)	na	na	na	na
II	0.520	101.0	3.8	102.2	8.5
III	1.737	101.7	5.0	110.4	12.2
IV	5.202	99.6	2.8	108.9	15.1
V	17.37	97.1	5.5	109.3	13.0
VI	152.02	86.9	6.8	112.0	12.4
VII	173.7	75.6	9.0	100.7	11.2
VIII	520.2	36.4	10.5	85.4	9.3

a Values are the concentrations of phosmet in the analysis plate wells
b Mean values of each 5 samples per test group
RBC Red blood cells
S.D. Standard deviation
na Not applicable

Conclusion

The observed comparative inhibition of cholinesterase in RBCs versus brain in this *in vitro* assay closely matches that seen in a variety of rodent and non-rodent *in vivo* studies with phosmet. The mouse *in vitro* data fully support the conclusion that inhibition of RBC acetylcholinesterase is a highly protective biomarker for possible brain cholinesterase effects of phosmet.

Title	**Comparative inhibition of acetylcholinesterase in rat plasma and rat red blood cells versus human plasma and human red blood cells following *in vitro* exposure to Phosmet**
Author (s) (year):	Hoberman, A.M. (2008)
Guideline	Not applicable
GLP	No
Purity:	98%
	This study was provided for the re-evaluation of the ADI of phosmet in March 2010 by the EFSA and it is considered acceptable only as additional information since no metabolic activation system was used. The active metabolite of phosmet is an oxon derivative that needs the presence of a metabolic activation system to be formed (Fukuto, 1990). Therefore, this *in vitro* study expresses the AChE inhibition of the parent compound only and not of the main metabolite.

Executive Summary

In this comparative *in vitro* acetylcholinesterase (AChE) inhibition study, blood samples were drawn from three rats [Crl:CD(SD)] and three humans at approximately the same times (within 30 minutes). All handling and processing of the human and rat blood was analogous. Using a plate reader with 96 wells per plate, diluted rat and human plasma or red blood cell (RBC) samples were placed into the wells on the plate. Each sample was then dosed with 0 (vehicle), 0.0468, 0.156 or 0.468 mM of phosmet (purity: 98%) for 10 minutes at 37º C, resulting in tissue exposure levels of 0 (vehicle), 0.0421, 0.140 or 0.421 mM. There were five replicates of each sample on the plate. Following incubation, a portion (50 µL) of each exposed sample was then transferred to a second 96 wells plate and analyzed for cholinesterase levels.

Since all samples were exposed and analyzed together, a direct comparison of response values was used to determine the percent of inhibition or percent of control. Averages and percentages were calculated based on the response values.

Evaluation of the dosing solution indicated that the concentration of this dosing solution was 19 % (0.468 mM) below the target value of 0.578 mM. Therefore, the result of 0.468 mM was used to calculate the actual concentrations of the dosing solutions (0.0, 0.0468, 0.156 and 0.468 mM) used in this study and the resultant tissue exposure (0.0, 0.0421, 0.140 and 0.421 mM).

No statistical analysis for comparison between groups was performed in this study.

Results

At the highest tissue concentration tested (0.421 mM), human RBC AChE was 83 % of the control value (17 % inhibition). In comparison, rat RBC AChE was 69% of control (31 % inhibition) at this dose level. Human plasma AChE response was 80.8 % of control (19.2 % inhibition) at the highest concentration tested. In comparison, rat plasma AChE response at the high dose group was 73.5 % of control (26.5 % inhibition).

Table 69: Acetylcholinesterase responses to phosmet treatment and comparison as per cent of controls

Test Group	Concentration	Response [% of Control]			
		Rat		Human	
[#]	[mM][a]	Mean ± S.D.[b]	Inhibition (% of control)	Mean ± S.D.[b]	Inhibition (% of control)
RBC					
I	0 (Vehicle)	22.82±0.71	na	163.88±7.23	na
II	0.0421	23.41±1.88	+2.6	156.48±7.52	-4.5
III	0.140	20.75±0.40	-9.1	152.75±4.16	-6.8
IV	0.421	15.74±0.34	-31.0	136.03±6.33	-17.0
Plasma					
I	0 (Vehicle)	61.44±9.26	na	202.55±7.46	na
II	0.0421	60.69±0.72	-1.2	210.23±13.99	+3.8
III	0.140	56.04±1.65	-8.8	200.25±14.12	-1.1
IV	0.421	45.13±2.53	-26.5	163.67±19.94	-19.2

a Values are the final concentrations of Phosmet in the analysis plate wells
b Mean values of each 5 samples per test group
RBC Red blood cells
na Not applicable

Human RBC responses of 93.2 and 95.5 % of control were found at the middle (0.140 mM) and low doses (0.0421 mM), respectively. Rat RBC responses of 90.9 and 102.6 % of control were observed at the mid and low doses, respectively.

Human plasma responses were 98.9 and 103.8 % of control at the middle and low doses, respectively. The comparative rat plasma responses at the respective dose levels were 91.2 and 98.8 % of control.

Conclusion

This *in vitro* study indicated that inhibition of plasma and RBC AChE was more pronounced in rats than that observed in humans.

Further investigations

Implications use of phosmet-ocurrence of bovine spongiform encephalopathy (BSE)

Publications deduced from the open literature revealed the possible implication of phosmet in the mechanism of increased susceptibility for the induction of spongiform encephalopathy.

In his first publication on BSE and phosmet (Purdey, 1996), Purdey expresses the opinion that organophosphorus chemicals such as phosmet were likely to target the prion protein in the same manner as they attack the catalytic site on organophosphorus ester sensitive esterases and proteases. In a second paper (Purdey, 1998) Purdey presumes that phosmet applied to the bovine's spinal column would penetrate and concentrate in phospholipids of the CNS membranes.

In addition, Gordon et al. (1998) reported an increase in the externally accessible fraction of PrP of cultured human SKNSH neuroblastoma cells after phosmet treatment, what may be relevant, but further research is needed to establish whether this represents a common effect of the class of organophsophate persticides as well as the relevance of these phenomena to the UK BSE epidemic.

Dr. David Ray (1998), an independent member of the Scientific and Medical Panel reporting to the MAFF Sub-committee on Pesticides, carried out an experiment in order to verify Mr. Purdey hypothesis that phosmet is likely to target the prion protein in the same manner as it attack the catalytic site on esterases and proteases. Data showed conclusively that prion protein was not a target for organophosphorylation in the way that Mr. Purdey had proposed in his publication.

In this context, Shaw et al. (2002) revealed that it is crucial that the concentration of phosmet in the tissues was sufficiently high to facilitate the conformational change from PrPc to PrPsc. The second pre-requisite is that phosmet is present in cattle tissue for a sufficiently long period to allow its reaction with PrPc. Several studies in animals showed that phosmet is not persistent in tissues and that 10 days after exposure phosmet residues are very low, even in adipose tissue. In addtion, the experiment carried by Shaw et al. (2002), suggests that interactions between phosmet and/or its metabolites and r[mouse]PrPc do not result in the conformational change necessary to result in the generation of PrPsc. These arguments were supported by Dr. Colin Leonard Berry, from the Department of Morbid Anatomy and Histopathology of the Royal London School of Medicine (Berry et al., 1999).

Roger Cook, Director of the National Organisation of Animal Health (NOAH), alleged that from the epidemiological point of view, there is no link between the use of OP insecticides and the occurrence of BSE by several reasons (Cook, 1998):

- Warble treatment was required for all cattle, beef and dairy, however BSE predominantly occurs in dairy herds.
- OPs have been used to treat cattle for warbles, at the same rate as in the UK, in a number of other EU countries
- In Ireland in the late 1970s to early 1980s an average of 2 million doses per year were used of one product alone, however only 128 cases of BSE have occurred in Ireland
- In Switzerland the authorities have investigated the allegation. Although OPs were used in warble campaigns in some cantons the Swiss Authorities could find no correlation between those areas and the areas where BSE has occurred.
- OPs have been widely used throughout the world to protect cattle from ticks and other external parasites. BSE is found only in Europe
- In the Channel Islands warble treatments using OPs took place in Jersey but not in Guernsey. There are 5 times more cases of BSE in Guernsey than in Jersey.

The Scientific Steering Committee meeting of 25-26 June 1998, concluded that there was no relationship between the use of and exposure to organophosphates and the occurrence of BSE: “There was no reference or studies that can prove the presence of a receptor site on the surface of the prion protein. In addition a possible affinity and covalent binding of Ops fro PrP protein should be shown”. Also concluded that Purdey work is not a true epidemiological study and it was not scientifically valid.

A.H. Andrews, an independent veterinary consultant from the UK, reported that the peak levels of organophosphate use was around the introduction of the respective law i.e. 1978 –1982 (Andrews, 1998). However, most cases of BSE occurred in animals born after 1986, which would be at a time when levels of all warble fly compounds would have reduced. In three consecutive years (1978 – 1980) about 2 million doses per year were used, millions of animals were treated. Nonetheless there were proportionally few cases of BSE in Ireland and a lot more in the UK (1998: 0.000989 % (IRL) and 1.458 % (UK) of the total cattle population).

Experimental studies showed that neither phosmet nor its metabolites interacted with the prion protein. Considering its pharmacokinetic properties, it is highly unlikely that phosmet reaches the

CNS after dermal application in an appreciable amount as proposed by Purdey. Furthermore, epidemiological data from different countries clearly showed that there was no correlation between phosmet use and BSE occurrence, neither temporally nor geographically as it was stated in the Evaluation table 17704/EPCO/BVL/05 rev. 2-1; 08.05. 2006 (EFSA, 2006a).

This issue was also discussed in the EPCO 33 (12.–16.09.05) (EFSA, 2006a). Experts agreed that there was no apparent link between phosmet application and BSE occurrence. They considered Purdeys´ arguments as a theory, since it cannot be supported by scientific studies and the epidemiological data did not show a direct relationship between phosmet application and BSE occurrence.

4.11.2 Human information

Report on medical surveillance on manufacturing plant personnel

Observations concerning to phosmet exposure of several manufacturing companies
Author: Staff, 1966. Not a publication. OECD and GLP not applicable.
Study acceptable with reservations

> There have been no illnesses which might have been related to Imidan (phosmet) exposure. No side effects and no deaths were observed related to phosmet. Periodic blood cholinesterase checks were performed on involved persons. In general, the results showed normal average pattern of plasma and red blood cell cholinesterase values .

Chromosome studies in workers producing organophosphate insecticides
Author: Kiraly, J. et al., 1979. Publication: *Arch. Environm. Contam. Toxicol. 8, 309-319 (1979).* OECD and GLP not applicable.
Study acceptable with reservations

Chromosome mutations were investigated in:

- Normal control group (group 1) which consisted of persons (mainly males), which did not suffer from any manifest disease, had not been exposed to any mutagenic effect (therapeutic and/or repeated diagnostic radiography, handling of chemicals, intoxication, etc.). Their ages ranged between 26 and 52 years.
- Factory employees control group consisted of engineers, technicians, office staff workers working with the company for 10 to 30 years. They were never directly exposed to pesticides. Their ages ranged between 28 and 47 years.
- Positive control groups consisted of workers engaged in the production of TECB (1,2,4,5-tetrachlorobenzene) the basic ingredient of several pesticides.
- Ditrifon producing workers.
- Basudin E producing workers .
- Safidon 40 WP (assumed to contain 40% phosmet).

The workers had been engaged in the production of insecticides for at least six months, working 8 hours per day, using “Tucan”-type face mask during work hours and using air ventilation (minimal 15 times per hour). The indoor concentration of Safidon 40 WP was determined to be 0.26 mg/m^3.

Lymphocytes of the peripheral blood samples were examined by a slightly modified method of Moorhead *et al*. (1960). After 48 hours of cultivation, 50 metaphase chromosomes were evaluated according to the Edinburgh classification (Buckton *et al*., (1962), Court-Brown (1967), Buckton and Evans (1973)) by one and the same person. At least 50 metaphases were evaluated in each person.

In addition, randomly chosen five metaphases and every cell showing chromosome aberrations were photographed and karyotyped.

Only gaps and isogaps (achromatic lesions) Chromatid-type aberration were moderately increased in the group of workers producing Safidon 40 WP. This was attributable to the increase in gaps and isogaps. There were no obvious differences between this group and the control group concerning all other chromosome mutations, *e.g.* chromatid-type aberrations, chromosome-type aberrations (labile and stable) and numerical aberrations as polyploidy, endoreduplications etc.

However, due to the following reasons the study is of limited value:

- It is assumed that Safidon 40 WP contains about 40 % of the active ingredient Phosmet. However, no information regarding the remaining co-formulants is available.
- No reliable information concerning confounding factors like previous disease and therapeutic measures, previous scope of activity and working conditions (*e.g.* mixed exposure with other pesticides/chemicals during manufacturing) and smoking behaviour is available.
- No smoking status was included as a covariate in any of the groups assumed.
- The average age of the groups is different or not specified at all.
- The number of persons examined in the different groups varies.
- Only 50 metaphases/person were examined, which is not sufficient for this kind of study.
- No statistical evaluation was performed on any parameter investigated.
- The author concluded that chromatid-type aberrations were moderately increased for the Safidon 40 WP group. This was attributable to the increase in gaps and isogaps. However, according to recent guidelines (OECD 473) gaps and isogaps - which are regarded as achromatic lesions according to recent definitions - should be recorded separately and reported but generally not included in the total aberration frequency. No increase was observed for breaks, iso breaks and all other investigated types of chromosome mutations. Hence, no increase in chromatid-type or chromosome-type aberration relevant for the total aberration frequency was noted in this study.

Conclusion:

Due to the reasons mentioned above the study is of limited value, but indicated that chromatide-type aberrations were moderately increased, attributable to the increase in gaps and isogaps. No increase for breaks, iso breaks, and the other chromosome mutations were observed in workers manufacturing Safidon 40 WP.

Report on clinical cases and poisoning incidents

It has to be noted that in the following reports, cholinesterase activity was only measured in Good (1993) and Rosenberg and Quenon (1998).

Blondell and Spann (1998) compiled information from the U.S. EPA Office of Pesticide Programs' incident data system (IDS), the Poison Control Centers (PCCs) and the Californian Department of Food and Agriculture about poisoning incidents with phosmet.

- IDS: Five cases of poisoning incidences with phosmet were reported between 1986 and 1996 in the US. The primary symptoms mainly included headaches, ocular irritation, respiratory symptoms, weakness, disorientation, lethargy, nausea, vomiting and diarrhoea. No further information on the disposition was reported.
- There were a total of 2548 phosmet cases in the PCC data base from 1985-1992. Of these, 136 cases were occupational exposure, 101 (74.3 %) involved exposure to phosmet alone and 35 (25.7 %) involved exposure to multiple chemicals, including phosmet. There were a

total of 1432 adult non-occupational exposures, 1350 (94.3 %) involved in this chemical alone and 82 (5.7 %) were attributed to multiple chemicals. For non-occupational exposure, one life-threatening case was reported for exposure to phosmet alone and two life-threatening cases which involved exposure to phosmet and other products. Phosmet cases were less likely to be seen in a health care facility and slightly less likely to be hospitalized than the other pesticides investigated. Among the non-occupational cases, phosmet was less likely to result in symptoms.

A separate analysis of the number of exposures in children five years of age and under from 1985 – 1992 was conducted. For phosmet, there were 980 incidents, 960 involved exposure to phosmet alone and 20 involved other pesticides as well. There was one life-threatening case reported in children under age six.

More recent data from the nation's Poison Control Centres (PCCs) in the US has been analysed to determine the risk associated with residential use of 13 organophosphates including phosmet. The current review is based on 424,469 records of pesticide-related exposures (excluding cases exposed to multiple products, attempts suicides, malicious intent and confirmed non exposures) reported to PCC. Of the 424,469 exposures, 392,188 occurred in a residential setting and 62,915 (16 %) of these were due to organophosphate pesticides and 1,242 (0.3 %) were due to phosmet.

- Detail descriptions of 57 cases submitted to US California Pesticide Illness Surveillance Program (1982-1995) were reviewed. In 36 of these cases, phosmet alone was judged to be responsible for the health effects. One individual was hospitalised within the reviewed time period 1982 and 1995. A total of 25 persons (69 %) had systemic illnesses. The symptoms noted included light-headedness, shortness of breath, difficulty of breathing, slow heart beat, dizziness, rash on face and hands, numb hands, weight loss and blurred vision.

Ames et al. (1989) reported on a telephone survey conducted in California, March through August 1987, to determine whether health symptoms were associated with occupational exposure to flea control products among pet care facility workers. The survey was conducted of workers from veterinary clinics, pet stores, pet boarding kennels, pet grooming shops, and animal control facilities. Information was gathered on exposure to flea control products, industrial hygiene practices, protective clothing use, and symptoms that occurred three months prior to being interviewed. Six hundred and ninety-six employees (496 exposed and 200 unexposed) were interviewed and asked to report symptoms experienced, from a list of 45 symptoms, within the past three months. Among exposed employees, only 295 reported wearing rubber gloves and only 21 % wore a protective apron. After applying flea control products, the following symptoms experiences were found to be statistically significant when comparing applicators to non applicators: skin rash, increased tearing, unusual tiredness, burning eyes, and flushing of skin after controlling for gender, current smoking status, and years of school completed. However, phosmet alone did not exhibit any statistically significant increase in symptoms frequency when compared to non exposed workers at the same facility. It should be noted that 23 % of the employers contacted for this study refused to cooperate which may have biased the results.

Good at al. (1993) reported on a fifty-one year old man who got phosmet on his face and hands and experienced diplopia, light-headedness, a progressive staggering gait, dysphagia, change in voice tone, excessive diaphoresis, facial, jaw, and neck weakness, mild distal and proximal muscle weakness, and droopy eyelids. He experienced these symptoms after he was exposed to phosmet for the past five weeks and was admitted to the hospital five days later. On the nineth day of hospitalisation, he experienced paralysis around the neck and shoulders, visual hallucinations, disorientation, and myoclonic jerks in his extremities which persisted for 10 days. The paralysis continued for 30 days, with some weakness persisting for about four months. His red blood cell

cholinesterase level was normal upon admission and two and three weeks later. He was administered a nerve conduction and repetitive stimulation neuromuscular drug while in the hospital. His motor responses were somewhat reduced in amplitude and prolonged in latency for the median, ulnar, and peroneal nerves. His neuromuscular exam was normal about five months later. The authors concluded that subacute neuromuscular syndrome can be experienced without marked symptoms of acute toxicity. The concentration of phosmet in this report was not known.

Rosenberg and Quenon, 1988, reported two incidents on exposure to flea-dip products in September 1986 in California. In one incident, a thirty-three years old woman, who was a pet groomer, experienced periodic headaches, nausea, dizziness, tiredness, blurred vision, sweating, confusion, and feeling spaced out. The woman treated dogs for 18 months (about 10 dogs a day in the summer) with products containing 11.6 % phosmet and frequently spilled the concentrated chemical on her skin while diluting the product. Her red blood cell cholinesterase level was normal, however, symptoms diminished after atropine treatment. A telephone survey was conducted in September 1986 of twenty four pet groomers by the California Health Department. The groomers were randomly selected from telephone directory lists in the San Francisco Bay and Los Angeles area. Twelve groomers reported symptoms, most commonly headache, dizziness, nausea, fatigue, and dermatitis. Flea-control products containing phosmet were most often reported as being related to the symptoms. Most groomers reported they did not wear aprons or gloves and often worked with undiluted concentrate with bare hands.

Tracey and Gallagher (1990), reported a 21-year old male ingested 500 mL of phosmet (Porect). On admission to the casualty unit gastric lavage was performed. Initial treatment was atropine 1.2 mg i.v. and diazepam 10 mg. He was then transferred to the intensive care unit. On arrival he was conscious but drowsy. He had pin point pupils and marked muscle fasciculations with a pulse rate of 120 bpm and blood pressure of 120/70 mm Hg. Blood gases showed moderate hypoxia, metabolic acidosis and compensatory alkalosis. His respiratory status deteriorated over the first 12 hours, and he was intubated and ventilated. Initial pharmacological treatment was atropine, glycopyrrolate and pralidoxime. He continued on this combination for 3 d. Then the glycopyrrolate was discontinued. The atropine was increased and continued for a total of 13 d. Pralidoxime was continued for 5 d. He had two convulsions within the first 24 h of admission to the unit. CNS manifestations were controlled initially with a midazolam infusion and thereafter by boluses as required.

Midazolam was continued until his discharge from the intensive care unit. Plasma cholinesterase levels were measured on the first 3 d of admission and were consistently low, 227, 309 and 318 U 1^{-1}, respectively (normal range: 3500-8500 U 1^{-1}).

He was ventilated for 11 d and extubated after 13 d. He was discharged to a psychiatric unit after 15 d with no neurological sequelae.

Clinical tests

Title	**A randomised double blind ascending single oral dose study with Phosmet to determine the no effect level on plasma and RBC cholinesterase activity**
Author (s) (year):	Cameron, B.D. (1999)
Administration	Single oral dose
Guideline	Not applicable
Species	Human volunteers
GLP	Yes
Purity:	96.0%
Groups	36 healthy male and (27 males and 9 females)
Dose levels	Males: 1.0, 2.0 and 4.0 mg/kg bw. Females: 2.0 mg/kg bw
	The protocol, volunteer information/consent form and toxicology report were submitted to the Independent Ethics Committee of Inveresk Research for consideration. The committee met on 17 December 1998 and agreed it was ethically acceptable for the study to proceed. It was also conducted under the provision of Helsinki Declaration and later revisions.

Executive summary

The study was performed to investigate the highest no observed effect level (NOEL) in human volunteers investigating the most sensitive endpoint, *i.e.* inhibition of cholinesterase activity. None of the volunteers suffered on adverse effects.

Subjects were healthy males and females aged 18 to 50 years, selected from a panel of volunteers recruited by Inveresk Clinical Research (ICR). The study was a double-blind randomised, placebo-controlled, ascending leading oral dose study in 36 healthy male and female volunteers. Twenty seven male subjects were randomised to receive a single oral dose of 1.0, 2.0 or 4.0 mg/kg bw phosmet (purity: 96.0%) or placebo (Lactose BP). Nine female subjects were then added to be studied at 2.0 mg/kg bw. The planned dosing schedule is presented in the following table.

Table 70: Dosing schedule

	Placebo	**1.0 mg/kg bw (males)**	**2.0 mg/kg bw (males)**	**4.0 mg/kg bw (males)**	**2.0 mg/kg bw (females)**
Cohort 1 Subjects 001-002	1	1	-	-	-
Cohort 2 Subjects 003-010	2	5	1	-	-
Cohort 3 Subjects 011-019	3	-	5	1	-
Cohort 4 Subjects 020-027	3	-	-	5	-
Cohort 5 (females) Subjects 028-036	3	-	-	-	6

Females were not dosed higher for the following reasons: (i) the testing laboratory generally limited testing for females and had few subjects available for testing; (ii) the addition of the 4.0 mg/kg bw dose for males was done to be able to show comparability between species (humans and rats). Since no sex differences in responses have been seen in former animal testing, the testing of females at 4.0 mg/kg bw was not warranted.

The test substance was administered as an oral capsule with 150 mL of water approximately 5 minutes after breakfast. Subjects were dosed in the sitting position and remained sitting until 4 h after dosing.

Subjects received the test substance or placebo on one occasion (day 1). Subjects remained in the clinic for 48 hours post dose and returned for follow up visits 96 hours post dose and on day 8. Physical examination included vital signs, electrocardiogram (ECG) (12-lead), continuous single channel ECG, urinalysis, haematology, clinical chemistry, oral temperature, adverse events and plasma and red blood cell cholinesterase activity. Samples for cholinesterase activity determination were taken at the following time points: day –10, –8, -4, -2, -1 and –30 minutes pre-dose, 1, 2, 4, 8, 12, 24, 48 and 96 hours post-dose and on day 8 (168 hours). All samples before dosing and those at 24, 48 and 96 hours post-dose were taken in the morning, at the same time of day if possible.

RBC and plasma AChE activity levels were summarised (*i.e.* mean, standard deviation, minimum, maximum and n) at each timepoint including changes from baseline, by gender and dose level. Additionally, the percentage change from baseline at each timepoint was tabulated by gender and dose level and illustrated graphically. Baseline is defined as the mean of all available predose values, except screening (*i.e.* -10, -8, -4, -2, -1 and 30 min). For the male and female data, percentage change from baseline for RBC and plasma cholinesterase were analysed separately using a repeated measures analysis of variance (ANOVA) including terms for dose level, timepoint (*i.e.* 1, 2, 4, 8, 12, 24, 48, 96 and 168 h) and dose level by timepoint interation. In addition, using the error variance from the ANOVA, pairwise comparisons between placebo and each dose level were carried out, using Student's t-distribution. For the male subjects, at each timepoint separately, a test for a linear trend with dose was performed using a linear contrast. At each timepoint, if the test for linear trend was found not to be significant at the 5% level, a Bonferroni adjustment was applied to the pairwise comparisons at that timepoint.

Results

Plasma Cholinesterase data:

For male subjects at all time points, the 2 mg/kg bw dose group has slightly larger percentage decreases from baseline compared with the remaining dose groups. The p-value using error variance from ANOVA Student‘s t-distribution was <0.05 for the reduction observed at 2 mg/kg bw (8 h). The test for a linear trend with dose was not found to be significant at any of the time points. A Bonferroni adjustment was therefore applied at all time points to the pairwise comparisons between each of the dose levels and placebo. None of these pairwise comparisons at any time points were found to be statistically significant after Bonferroni adjustment.

For female subjects, placebo has a higher percentage decrease from baseline values than the 2 mg/kg bw dose group at all time points.

Table 71: Plasma cholinesterase change from baseline (%) statistical analysis: all male subjects.

Time point [h]		Dose of phosmet				Linear trend[3]
		Placebo	1 mg/kg bw	2 mg/kg bw	4 mg/kg bw	
1	Adjusted means[1]	-6.68	-6.60	-8.25	-5.10	-
	p-value[2]	-	0.97	0.47	0.47	0.52
2	Adjusted means[1]	-5.79	-4.68	-7.36	-4.82	-
	p-value[2]	-	0.61	0.48	0.66	0.84
4	Adjusted means[1]	-4.70	-1.79	-5.99	-3.90	-
	p-value[2]	-	0.19	0.56	0.72	0.88
8	Adjusted means[1]	-2.49	-4.70	-7.17	-0.26	-
	p-value[2]	-	0.32	0.035 #	0.31	0.25
12	Adjusted means[1]	-3.47	-3.26	-6.49	-1.81	-
	p-value[2]	-	0.92	0.12	0.45	0.57
24	Adjusted means[1]	1.48	1.22	0.39	3.22	-
	p-value[2]	-	0.91	0.62	0.43	0.42
48	Adjusted means[1]	-1.68	3.29	-4.45	0.87	-
	p-value[2]	-	0.028 #	0.22	0.26	0.78
96	Adjusted means[1]	0.95	1.67	-0.49	4.74	-
	p-value[2]	-	0.75	0.52	0.092	0.12
168	Adjusted means[1]	1.26	0.85	-0.18	2.53	-
	p-value[2]	-	0.86	0.52	0.57	0.56

[1]represented adjusted means from repeated measure ANOVA
[2]p-value for comparison of dose group with placebo using error variance from ANOVA student´s t distribution
[3]if p>0.05 for the linear trend, the Bonferroni adjustment was used for pairwise comparisons with placebo
Note: pairwise comparison with placebo not significant after Bonferroni adjustment

Table 72: Plasma cholinesterase change from baseline (%): statistical analysis: all female subjects

Time point [h]		Dose of phosmet	
		Placebo	2 mg/kg bw
1	Adjusted means[1]	-9.34	-6.24
	p-value[2]	-	0.36
2	Adjusted means[1]	-7.85	-4.24
	p-value[2]	-	0.28
4	Adjusted means[1]	-5.63	-1.18
	p-value[2]	-	0.19
8	Adjusted means[1]	-8.59	-5.94
	p-value[2]	-	0.43
12	Adjusted means[1]	-13.39	-4.55
	p-value[2]	-	0.010
24	Adjusted means[1]	-7.96	-0.70
	p-value[2]	-	0.040
48	Adjusted means[1]	-7.49	-0.70
	p-value[2]	-	0.055
96	Adjusted means[1]	-0.97	-0.058
	p-value[2]	-	0.91
168	Adjusted means[1]	-11.57	-9.10
	p-value[2]	-	0.46

[1]represented adjusted means from repeated measure ANOVA
[2]p-value for comparison of dose group with placebo using error variance from ANOVA student´s t distribution

RBC Cholinesterase data:

For the male subjects, the p-value using error variance from ANOVA Student‘s t-distribution was <0.05 for the decreases observed at 1 mg/kg bw (12 and 24 h), at 2 mg/kg bw (8 h) and at 4 mg/kg bw (1 h). The test for a linear trend with dose was not found to be significant at any of the time points. Therefore, a Bonferroni adjustment was applied at all time points to the pairwise comparisons between each of the dose levels and placebo. Significant pairwise comparisons after Bonferroni adjustment were observed at 1 mg/kg bw (24 h) and at 2 mg/kg bw (8 h).

For female subjects at the 2 mg/kg bw dose level there were not significant pairwise comparisons using error variance from ANOVA Student‘s t-distribution between placebo and tested dose levels.

Table 73: RBC cholinesterase change from baseline (%): statistical analysis: all male subjects

Time point [h]		**Dose of phosmet**				**Linear trend[3]**
		Placebo	**1 mg/kg bw**	**2 mg/kg bw**	**4 mg/kg bw**	
1	Adjusted means[1]	4.39	0.37	3.04	-4.44	-
	p-value[2]	-	0.35	0.76	0.042#	0.068
2	Adjusted means[1]	2.51	3.25	-2.47	4.52	-
	p-value[2]	-	0.86	0.25	0.64	0.77
4	Adjusted means[1]	-0.65	1.53	-1.33	-6.87	-
	p-value[2]	-	0.61	0.87	0.15	0.093
8	Adjusted means[1]	10.51	2.33	-0.67	12.70	-
	p-value[2]	-	0.060	0.010[a]	0.61	0.38
12	Adjusted means[1]	7.57	-4.57	2.24	9.10	-
	p-value[2]	-	0.005	0.22	0.72	0.23
24	Adjusted means[1]	7.50	-4.51	1.14	0.24	-
	p-value[2]	-	0.006[a]	0.14	0.095	0.34
48	Adjusted means[1]	6.47	0.29	1.40	2.69	-
	p-value[2]	-	0.16	0.25	0.39	0.59
96	Adjusted means[1]	-2.93	-6.98	-4.41	-5.50	-
	p-value[2]	-	0.36	0.74	0.56	0.74
168	Adjusted means[1]	1.97	-5.11	5.00	0.44	-
	p-value[2]	-	0.11	0.49	0.73	0.78

[1]represented adjusted means from repeated measure ANOVA
[2]p-value for comparison of dose group with placebo using error variance from ANOVA student´s t distribution
[3]if p>0.05 for the linear trend, the Bonferroni adjustment was used for pairwise comparisons with placebo
Note: pairwise comparison with placebo not significant after Bonferroni adjustment
[a] pairwise comparison with placebo significant at a 5% significance level after Bonferroni adjustment

Table 74: RBC cholinesterase change from baseline (%): statistical analysis: all female subjects

Time point [h]		Dose of phosmet	
		Placebo	**2 mg/kg bw**
1	Adjusted means[1]	-6.85	-6.36
	p-value[2]	-	0.93
2	Adjusted means[1]	-7.02	-4.06
	p-value[2]	-	0.61
4	Adjusted means[1]	3.65	5.99
	p-value[2]	-	0.69
8	Adjusted means[1]	5.05	8.65
	p-value[2]	-	0.54
12	Adjusted means[1]	3.60	2.43
	p-value[2]	-	0.84
24	Adjusted means[1]	2.04	4.24
	p-value[2]	-	0.71
48	Adjusted means[1]	10.95	4.72
	p-value[2]	-	0.30
96	Adjusted means[1]	0.40	-1.41
	p-value[2]	-	0.76
168	Adjusted means[1]	-1.30	-2.38
	p-value[2]	-	0.85

[1]represented adjusted means from repeated measure ANOVA
[2]p-value for comparison of dose group with placebo using error variance from ANOVA student´s t distribution

Adverse events

Safety results: There were no clinically significant changes in either mean or individual safety measurements, *i.e.* vital signs, ECGs, haematology, clinical chemistry, urinalysis, or physical examination observed for subjects receiving phosmet or placebo.

There were no adverse events considered to be related to the test compound.

Conclusion

Phosmet administered as a single oral doses of 1.0, 2.0 or 4.0 mg/kg bw to healthy male subjects resulted in p-values <0.05 using error variance from ANOVA Student‘s t-distribution in RBC AChE at 1 mg/kg bw (12 and 24 h), 2 mg/kg bw (8 h) and 4 mg/kg bw (1 h) and in plasma AChE at 2 mg/kg bw (8 h). The test for a linear trend with dose was not found to be significant at any of the time points. RBC AChE significant pairwise comparisons after Bonferroni adjustment were observed at 1 mg/kg bw (24 h) and at 2 mg/kg bw (8 h).

4.11.3 Summary and discussion

Neurotoxicity

A range-finding study in Sprague-Dawley rats (Cappon, 1998a) was performed to elucidate the time of peak effect and to select dose levels for the subsequent main acute neurotoxicity study. Dose levels of 0, 1.5, 3, 6, 9, and 36 mg/kg bw were investigated. Clinical signs of whole body tremors, gait alterations and salivation were observed at 36 mg/kg bw. Plasma cholinesterase (ChE) was inhibited at ≥ 9 mg/kg bw, while red blood cell (RBC) ChE inhibition occurred at ≥ 6 mg/kg bw. Brain ChE depression was limited to the 36 mg/kg bw group. All these inhibitions were greater than 20% (no statistics available). The NOAEL for ChE inhibitory potential was 3 mg/kg bw.

Based on the results of the dose-range finding study the subsequent acute neurotoxicity study (Cappon, 1998b) investigated dose levels of 3.0, 4.5 and 22.5 mg/kg bw and considered a time of peak effect of about 3 hours following dosing of phosmet in Sprague-Dawley rats. The single oral (gavage) administration of phosmet up to the high dose level of 22.5 mg/kg bw caused no specific treatment-related changes within the functional observational battery or locomotor activity measurements. Virtually no neuropathological changes due to treatment were observed. The AChE inhibitory potential was confirmed since at the time of peak effect (3 hours post-dosing) at 22.5 mg/kg bw plasma AChE was depressed by 57%-46% and RBC AChE by 75%-88% in males and females respectively. Thereafter no variations were observed in plasma ChE and only on day 15 in RBC ChE in females (40%). At this same dose level brain ChE was significantly reduced in males (61%) and females (70%) at the study day 0 evaluations, when compared to the control group. These significant reductions persisted to study days 7 (15% for males and 20% for females) and 15 (9% for males and 17 % for females). Consequently, the NOAEL for acute neurotoxicity was 22.5 mg/kg bw, while the NOAEL for ChE inhibition was 4.5 mg/kg bw in rats after a single oral application.

The potential of phosmet to induce neurotoxicity after repeated oral administration was tested in a subchronic neurotoxicity study (Cappon, 1999) using a neurotoxicity screening battery including specific neuropathology and evaluation of AChE activities in plasma, RBC, whole blood and brain/brain regions. Each 32 male and 32 female Sprague-Dawley rats were administered constant dietary levels of 0, 25, 50 and 150 ppm for 13 weeks. This treatment led to no clinical evidence of systemic toxicity. Virtually no indication for neurotoxicity was observed in the FOB, locomotor activity and neuropathology assessments up to 150 ppm. Significant and biologically reduction of AChE values throughout the study were observed in a dose-related manner at all dose levels. Regading plasma ChE activity, it was significantly reduced at 25 ppm for females at week 13 (29%). At 50 ppm there was a significant reduction of the plasma ChE activity in males at week 3 (21%) and in females at weeks 3 (46%) and 13 (27%). In females there was also an inhibition of plasma AChE activity of 27% at week 7 regarded as not statistically significant. At 150 ppm statistically and biologically significant reduction of plasma ChE activity was observed for both sexes at all evaluation points. About mean RBC cholinesterase, values in the 25 ppm group were statistically significantly reduced by 19% in the males at week 13 and by 42% in the females at week 7. In the 50 ppm group, mean RBC cholinesterase levels were reduced statistically significant by 26%-38% in males during the study and by 38% for females at week 7. Inhibition of 54% in females was observed at week 13 not statistically significant. In the 150 ppm group, mean RBC ChE levels were reduced by 65%-70% in the males and by 66%-89% in the females during the study. At this dose level all the differences were statistically significant except for females at week 13 where the low n (n=1) precluded statistical analysis. Whole blood AChE was reduced in a dose related manner at all doses for both sexes throughout the study. These reductions were statistically significant at all evaluation periods for the 50 and 150 ppm group males and females and at week 13 for the 25 ppm group in both sexes. Whole brain AChE was also inhibited at 150 and 50 ppm in both sexes at weeks 3 and 7. The ChE inhibitory potential of phosmet was confirmed for the different brain compartments examined (hippocampus, olfactory, midbrain, bainsteam, cerebellum and cortex). Significant inhibition (≥20%) of brain ChE in the six brain regions was observed at 150 ppm in animals of both sexes. Inhibitory effect was also noted at 50 ppm in females with statistically significant or biologically relevant decreases. Significant reductions in females at 25 ppm were seen in the olfactory region (36%) and in the brainstem (21%) and also not statistically significant in the hippocampus (33%). EFSA did not take into account these cholinesterase reductions at 25 ppm during the peer review of phosmet and established a NOAEL of 25 ppm. However, the Spanish Competent Authority in Classification&Labelling regards that AChE inhibition cannot be ruled out at this dose level. Therefore, this effect is considered relevant for repeated dose toxicity (RDT) classification.

The potential of phosmet to induce delayed neurotoxicity was investigated in two acute delayed neurotoxicity studies in adult domestic hens. In the former study (Sprague, 1982) phosmet was orally administered in gelatine capsules to fasted hens. The acute oral toxicity in hens revealed oral LD_{50} value for phosmet of 2020 mg/kg bw. Pre-treatment with atropine or 2-PAM reduced markedly the acute lethality of phosmet. Statistically significant reductions of cholinesterase inhibition ChE tests after oral administration in gelatine capsules of phosmet inhibited plasma and brain ChE levels (>20%) at dose levels ≥ 65.6 mg/kg bw. In the delayed acute neurotoxicity test from the three levels of dosing (20, 200 and 2050 mg/kg bw), the two higher dose levels produced alterations in the appearance, behaviour and motor coordination impairment, not considered delayed neurotoxicity, because the onset of the signs was not delayed and most adverse signs were transient and the severity decreased with time after each treatment. NOAEL for AChE inhibition was 16.4 mg/kg.

In a more recent acute delayed neurotoxicity study (Johnson, 1997) gavage was chosen as the oral administration mode. The acute toxicity in hens revealed an oral LD_{50} value of 577 mg/kg bw after treatment by gavage as oily preparation. Unsteadiness, subdued behaviour, recumbency and salivation were observed mainly during first 4 days in all groups in this acute oral phase test. No clinical signs of toxicity were observed after day 8. In the acute delayed neurotoxicuty phase, inhibition of brain ChE activity after 48 h was noted at the only tested dose level of 600 mg/kg bw by 63%. At this dose level all adult hens treated showed clinical signs of toxicity for 4 days including unsteadiness, subdued behaviour, recumbency and weakness. Birds of the positive and negative control group remained healthy. There were no biologically relevant or significant reductions in NTE levels at two locations (brain and spinal cord). Moreover, neurohistopathology of specimen from the central and peripheral nervous system revealed no evidence of specific acute delayed neurotoxicity. Phosmet showed no potential to induce acute delayed neuropathy in adult domestic hens at dose levels including or exceeding the unprotected oral LD_{50}.

In a randomised double blind single oral dose study with phosmet in human volunteers (Cameron, 1999), phosmet administered as a single oral doses of 1.0, 2.0 or 4.0 mg/kg bw and 2.0 mg/kg bw to healthy male and female subjects resulted in p-values <0.05 using error variance from ANOVA Student‘s t-distribution only in males at 1 mg/kg bw (12 and 24 h), 2 mg/kg bw (8 h) and 4 mg/kg bw (1 h) and in plasma ChE at 2 mg/kg bw (8 h). The test for a linear trend with dose was not found to be significant at any of the time points for both plasma and RBC AChE. RBC AChE significant pairwise comparisons after Bonferroni adjustment were observed for males at 1 mg/kg bw (24 h) and at 2 mg/kg bw (8 h).

Effects associated with neurotoxicy (cholinesterase inhibition) were observed in other toxicity studies. These signs are compiled in Tables 30, 31, 32 and 33.

4.11.4 Comparison with criteria

Neurotoxicity

Phosmet is a neurotoxic compound with a well-known toxicity attributed to their ability to inhibit acetylcholinesterase (AChE), which is a class of the enzymes that catalyses the hydrolysis of the neurotransmitting agent acetylcholine (ACh). Inhibition of AChE has been widely observed in neurotoxicity studies but also in other acute subacute, subchronic, chronic and reprotoxicity studies through this report. No more signs of neurotoxicity apart from cholinesterase inhibition and some cholinergic effects have been observed after phosmet dosing. The MSCA is of the opinion that these neurotoxic effects are covered by the proposed classification of phosmet for acute oral toxicity (Acute Tox. 3 – H301) and repeated dose toxicity after oral and dermal exposition (STOT RE 1).

Phosmet showed no potential to induce delayed neurotoxicity in two studies with adult domestic hens. The alterations in appearance, behaviour and motor coordination observed in treated hens (Sprague, 1982 and Johnson, 1997) were already observed on the day of dosing and declined after dosing. Therefore, according to the definition of delayed neurotoxicity in the OECD 418 guideline: "Delayed neurotoxicity is a syndrome associated with prolonged delayed onset of ataxia, distal axonopathies in spinal cord and peripheral nerve, and inhibition and ageing of neurotoxic esterase in neural tissue", these alterations cannot be considered delayed neurotoxicity. This was also concluded by the Joint Meeting on Pesticide Residues (1994).

4.11.5 Conclusions on classification and labelling

Neurotoxic effects of phosmet are covered by acute and repeated dose toxicity (sections 4.2 and 4.7 respectively) classification of phosmet according to CLP (Acute Tox. 3 - H301 and STOT RE 1-H372).

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate and ecotoxicological properties of Phosmet were assessed in the Draft Assessment Report, in 2005. The summaries included in this proposal are partly copied from the revised DAR, its addenda and the EFSA Conclusion of this active substance, finalised on 12th May 2006.

The classification regarding environmental hazards was already discussed and finally agreed at ECB level at the January 2007 meeting (N;R50 – very toxic to aquatic organisms, according to the Dangerous Substance Directive (DSD).

Regarding the classification according to CLP Regulation, no changes are needed, only M factor for aquatic chronic toxicity should be included.

5.1 Degradation

Table 75: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aerobic and anaerobic transformation in soil: OECD guideline 307	DT_{50} and DT_{90} in three soils, under aerobic conditions. Loamy sand (5.01 and 16.7 days); Silt soam (2.67 and 8.85 days); Sandy loam (1.65 and 5.48 days).	Rapidly aerobic degradation in soil	Lynn, R., McCorquodale, G., Paterson, K. (2003a/b)
Rate of dissipation of Phosmet and its degradation products, in soil, water, indoors and micro field experiments: (guideline not mentioned)	DT_{50}= 3.6 d and DT_{90} = 11.9 d, in soil under aerobic conditions.	No accumulation of Phosmet is expected in soil	McBain, J.B.; Hoffman, L.J., Menn J.J. (1973)
Hydrolysis: US EPA guideline 161-1	At 25ºC, pH 5 DT_{50}=180hours; pH 7 DT_{50}=7.8 hours; pH 9 DT_{50}=4.5min.	Supplementary information. Hydrolysis under neutral or alkaline conditions will be a major dissipation pathway for Phosmet.	Chang, L.L. (1987)
Photodegradation in water: US EPA guideline 161-2	photolytic DT_{50}= 4.5 days (pH 5)	-	Robinson, R.A. (1992)
Ready biodegradability: OECD guideline 301D	Biodegradation after 28 days=19.5 %	Not readily biodegradable	Kelly, C.R.; Paterson, K. (2003)
Degradation in aquatic systems: OECD Guideline 308	Degradation pathway	An interim data summary	Kidd, Gordon G., Davidson, J. (2005)

5.1.1 Stability

Hydrolysis.

McBain, J.B.; Hoffman, L.J., Menn J.J. (1973). Hydrolysis in buffered water.

Imidan ^{14}CO or Imdan ^{14}CH2 in acetone were mixed with water and buffered at pH 5, 7 or 9. The samples were capped and incubated at 23 ºC. At given intervals, aliquots were subjected to TLC analysis with reference standards. The quantification of the degradation products were made by LSC.

Imidan ^{14}CO or ^{14}CH2 dissolved in buffered water is hydrolysed to PI (phthalimide) and polar products. Hydrolysis occurs slowly at pH 5 (DT_{50}=225 h) but rapidly under neutral an alkaline conditions (DT_{50} at pH 7= 18 h, DT_{50} at pH 9 < 10min). A minor apolar product having an Rf similar to PI was observed in Imidan-14CH2.

After 4 months, TLC characterisation shows that the only product present at pH 9 was PAA (phthalamic acid); at pH 7 the principal products were PAA, smaller amounts of HMPAA (N-hydroxymethyl phthalamic acid) and PA (phthalic acid), and, at pH 5, the products were PAA and/or PA and a small quantity of PI.

The experiment was repeated at pH 7 in order to evaluate the hydrolysis products. Periodically, aliquots were analysed by TLC. The products detected were HMPAA, PAA, PA and PI. After 2 days incubation only a small amount of unchanged Imidan remained.

Table 76: ^{14}C-products derived from Imidan-CO upon hydrolysis

pH	Time (hr)	Imidan	PI	Polar products
5	0	100	0	
	25	94.6		5.4
	50	88.5	0.5	11.0
	235	47.6	7.9	44.5
	670	17	22	61.0
7	0	100	0	0
	5	77.6	8.1	14.3
	25	37.2	21.9	40.9
	50	11.2	26.3	62.5
	165	0.7	21.2	78.1
	235	0	6.9	93.1
9	0	51.0	10.4	38.6
	0.5	0.5	13.7	85.8
	1.5	0	3.9	96.1
	5	0	0.6	99.4

Table 77: ^{14}C-products derived from Imidan-CO upon hydrolysis in water at pH 7

days	Unknown	HMPAA	PAA	PA	PI	Imidan
2	9.8	20.9	**32.9**	3.6	**27.9**	5.0
5	trace	**25**	50.4	5.8	16.3	2.6
12	0	23.3	63.3	5.2	6.1	2.0
30	0	20.1	70.4	4.3	3.0	2.2
50	1.4	18.3	**71.9**	3.1	2.1	3.3

The hydrolysis study shows that this process occurs slowly at pH 5 but rapidly after neutral and alkaline conditions. At pH 7 accumulation of phthalamic acid was observed after 50 d of incubation.

Chang, L.L. (1987).

The hydrolysis rate of Phosmet was determined in three buffered aqueous solutions (pH 5, 7 and 9) at 25 °C and 40 °C (kinetic study). Test solutions with unlabelled Phosmet were prepared and were incubated for up to 232 hours (= 9.6 days) under sterile conditions in the dark.

Identification of hydrolysis products. Qualitative characterization of the hydrolysis products was performed using a portion of the non-labelled test solutions. NMR analysis of the methylene chloride extract from pH5 test solutions showed the presence of Phosmet; however, as would be expected from the results of the kinetic study, the extract from pH 9 test solutions showed no Phosmet. GC/MS analysis of a methylene chloride extract of the pH 9 test solution, which was first acidified to pH 2, showed the presence of Phthalimide (Pi) and N-methylphthalimide (PiM) and phthalic anhydride. As the latter cannot form in aqueous solution, it likely arose from loss of ammonia by phthalamic acid (PaA) and or dehydration of phthalic acid (Pa) in the heated GC injection port. HPLC analysis of the radiolabelled pH 5 test solutions confirmed the presence of phthalic acid (Pa), Phthalamic acid (PaA), Phthalimide (Pi), N-methylphthalimide (PiM) by comparison of retention times to those of authentic references standards. In addition, trace amounts of N-hydroxymethyl phthalimide (PiMOH) was detected by HPLC/RAM.

The aqueous phase contained mainly O,O-dimethyl phosphorodithioic acid with a small amount of O-methyl phosphorodithioic acid.

DT_{50} values were re-estimated in order to validate them (Table 78).

Table 78: Phosmet hydrolysis DT_{50} values in sterile solutions buffered at pH 5, pH 7 and pH 9 (25°C and 40°C)

Temperature [20 ºC]	pH	DT_{50}
25	5	180 hr
25	7	7.8 hr
25	9	4.5 min
40	5	50.04 hr
40	7	140.7 min
40	9	1.5 min

The major degradation products determined at pH 5 (day 11, 25 °C) in the organic phase were Phthalamic acid (34.3 % ^{14}C), Phthalic acid (8.8 % ^{14}C), N-Hydroxymethyl phthalimide (2.6 % ^{14}C), Phthalimide (9.8 % ^{14}C) and N-Methyl phthalimide (2.1 % ^{14}C). The aqueous phase contained mainly O,O-Dimethyl phosphorodithioic acid (79.4 % of original Phosmet) with a small amount of O-Methyl phosphorodithioic acid (4.1 %).

Hydrolysis under neutral or alkaline conditions will be a major dissipation pathway for Phosmet. No information of metabolites under these conditions were provided and it cannot be conclude if under neutral and alkaline conditions the amount of the hydrolytic products are equal major or minor than under acidic conditions since degradation of these products might occur producing major quantities of some hydrolytic products rather than others.

Summarising the available data on hydrolysis, the results of the two hydrolysis studies (Chang, 1987 and McBain, 1973) were compared and it was explained that the pattern of metabolites will be the same at higher pH values concluding that the pH value affects only the speed of degradation, but has no impact on the degradation pathway. *In acidic conditions the hydrolysis will be preceded by protonation of the most susceptible molecular entities such as hetero-atoms in the molecule, followed by hydrolytic attack on the neighbouring carbon atom and bond cleavage. In the cases of basic conditions, a reaction will take place directly between the readily available hydroxyl ions and a carbon activated by an adjacent hetero-atom, followed by bond cleavage.*

At pH 9 phthalimide (Pi) is rapidly degraded (0.6 % at 5 hours after the treatment) and after 4 months of incubations the main metabolite is phthalamic acid (PAA).

At pH 7: the main metabolites (> 10 %) are:

- phthalamic acid (PAA after 50 days 72 %),
- phthalimide (Pi 21.2 % after 7 days) and hydroxymethyl phthalamic acid (PaAMOH, 25 % after 5 days)

Smaller amounts of phthalic acid (PA 6 % after 5 days) were also identified. Nevertheless, after 4 months of incubation the principal product was PAA, and smaller amounts of PaAMOH, and PA were also identified.

At pH5: according to *Chang 1987*, the main metabolites are Phthalamic acid (PAA) at 11 DAT (34.3 %) and Phthalimide (Pi) (9.8 %) and Phthalic acid (8.8 %). it should be said that at the end of the study there was a 43.9 % identified as Phosmet. Therefore, the amounts of the metabolites can vary. N-hydroxymethyl phthalimide (PiMOH) was identified at low levels at pH 5.

Thus, according to *McBain (1973)* the amount of phthalimide (Pi) at the end of the study (28 days) was 21 %. After 4 months of incubation, the products were PAA and/or PA (in this particular case, the resolution of these two products were imperfect) and small quantity of phthalimide (Pi) but no N-hydroxymethylphthalamic acid (PAaMOH). In buffered water solution the order of stability of phthalimide (Pi) is acidic>>neutral>alkaline. Phthalamic acid (PAA) is the likely product of phathalimide (Pi). PAA in water solution undergoes little, if any, hydrolysis at pH 7 and pH 9, but at pH 5, it slowly converts to phthalic acid (PA). PaAMOH in water solution hydrolyzes, probably to PAA, slowly at neutral pH but at a more rapid rate at acidic and basic pH

Photolysis

Robinson, R.A. 1992

The photolytic stability of Carbonyl-^{14}C-Phosmet in aqueous solution was studied in sterile test solutions buffered at pH 5. The half-life of Phosmet was calculated from a plot of the log percentage of Phosmet remaining versus incubation time by linear regression.

According to material balance, the average total recovery of radioactivity for irradiated, dark control, and high dose samples was 97.42 %, indicating negligible losses due to volatility. The proportion of radioactivity in the methylene chloride fractions decreased with time from 97.3 % at day 0 to 59.7 % by day 10 which corresponded to an increase in the formation of water-soluble radioactivity.

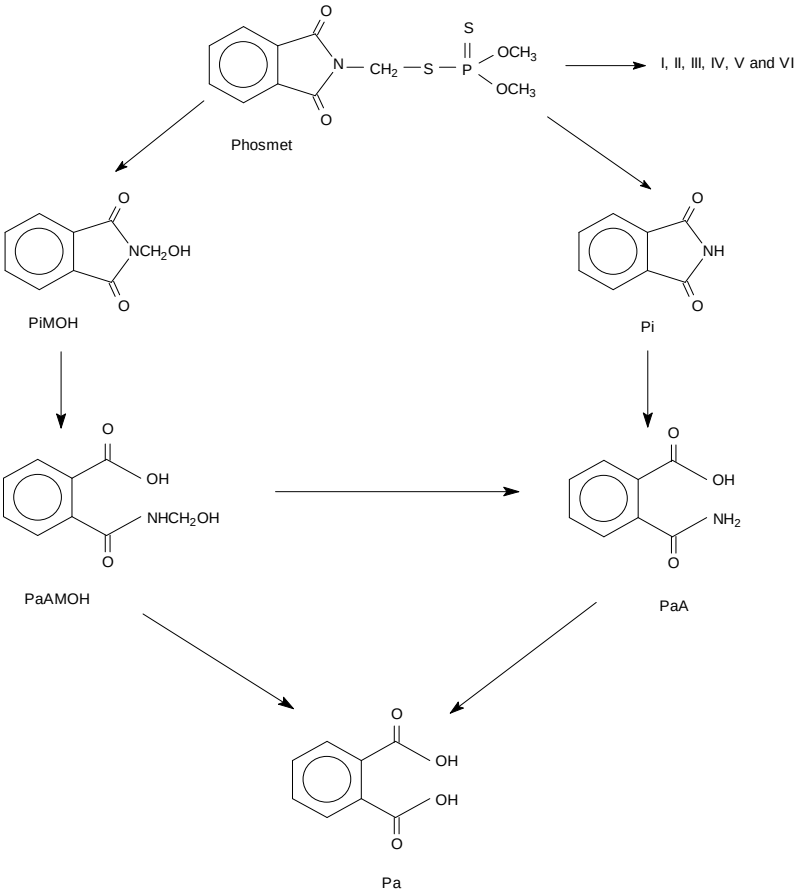
In other hand, according to the photolytic degradation, in the irradiated samples the amount of Phosmet in the organic extracts decreased steadily from 95.5 % on day 0 to 5.7 % by day 10 and this loss of Phosmet was accompanied by an increase in the formation of the major degradation product Phthalimide up to 46 % by day 10. Analysis of the dark control samples showed that very little Phthalimide was formed indicating that the formation of this product is impacted primarily by photodegradation.

In the aqueous extracts N-Hydroxymethyl phthalamic acid was the major degradation product increasing in the irradiated samples (up tp 19.5 %). The product was also formed in the dark and at the 15 mg/l concentration level with maximum amounts of 35.6 % and 14.41 % respectively. Phthalamic acid and Phthalic acid also increased steadily in the irradiated samples (7.6 % and 6.1 % respectively by day 10).

The photolytic half-life of Phosmet was determined by linear regression analysis assuming pseudo-first-order kinetics, i.e. the log of the percentage of Phosmet remaining in the sample versus incubation time.

A proposed hydrolytic and photochemical degradation pathway of Phosmet in water is shown in Figure 3. The major degradates from the photodegradation study in water (Phthalimide, N-Hydroxymethyl phthalimide, Phthalamic acid and Phthalic acid) closely tracked with the results of the hydrolysis study. However, the rates of formation were somewhat different for several of degradates.

Figure 3: Proposed hydrolytic and photochemical degradation pathway of Phosmet in water.



Pi: Phthalimide

PiMOH: N-Hydroxymethyl phthalimide

PaAMOH: N-Hydroxymethyl phthalamic acid

PaA: Phthalamic acid

Pa: Phthalic acid

I: O,O-Dimethyl phosphorodithioic acid (detected in hydrolysis study)

II: O-Methyl phosphorodithioic acid (detected in hydrolysis study)

III: O,O-Dimethyl phosphorothioic acid (detected in photolysis study)

IV: O,O-Dimethyl phosphoric acid (detected in photolysis study)

V: O-Methyl phosphoric acid (detected in photolysis study)

VI: Phosphoric acid (detected in photolysis study)

The rate estimated has into the account the both processes photolysis and hydrolysis, so it must carefully considered. For a more accurate estimation the hydrolysis losses should be taken away in the estimation. Taking into account this and following correction for hydrolysis the photolytic DT_{50} value for phosmet at pH 5 was 4.5 d with an r^2 of 99.6 %.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

Laboratory studies. Soil

Lynn, R., McCorquodale, G., Paterson, K. (2003a/b).

The objective of this study was to investigate the rate of breakdown of [^{14}C]-Phosmet in three fresh field soils. A metabolism study was also performed in one of the soils in which the rate of evolution of volatiles, the profile of degradation products and the formation of bound residues was investigated. The results demonstrate that, except for the sterile samples, the microbial viability during the study was representative of microbial active systems.

Soil samples were extracted and radioactivity in the extracts was quantified by liquid scintillation counting (LSC). Concentrated extracts were analysed by high pressure liquid chromatography (HPLC) and thin layer chromatography (TLC). Extracted soil samples were combusted and analysed by LSC to determine the amount of non-extractable residues.

The rate of degradation of Phosmet in soil was determined applying first-order kinetics and varying between 1.65 days (sandy loam) and 5.01 days (loamy sand).

Table 79: DT_{50} and DT_{90} values for Phosmet in soils under aerobic conditions

	DT_{50}	**DT_{90}**	**R^2**	**Kinetic**
Loamy sand pH(KCl_2) = 5.7	5.01	16.7	0.989	1st order; non-linear regression
Silt loam pH(KCl_2) = 6.2	2.67	8.85	0.973	1st order; non-linear regression
Sandy loam pH(KCl_2) = 7.6	1.65	5.48	0.994	1st order; non-linear regression

The experimental results show that microbial degradation and hydrolysis play an important role in the degradation of Phosmet in soil in which a high pH provokes a more rapid degradation, as it is shown in the rate of degradation.

McBain, J.B.; Hoffman, L.J., Menn J.J. (1973).

The objective of this study was to evaluate the impact of key environmental factors on the fate and ultimate disposition of Phosmet in nature. The rate of dissipation of Phosmet and the nature of its degradation products were studied in soil, water and under controlled photolytic conditions indoors and in micro field experiments.

This study was addressed in order to evaluate the metabolism of ^{14}C-phosmet in a fourth soil, according to the Directive 95/36/CE. In this assay two labelled ^{14}C-phosmet were used (carbonyl-^{14}C-phosmet and methylene-^{14}C-phosmet). The same several minor metabolites were identified in cases, carbonyl.-^{14}C-phosmet and methylene-^{14}C- Phosmet. No metabolites exceeding 10 % TAR were identified. CO_2 production increased throughout the study and a maximum of 39.2-42 % TAR was identified as bounded residue at 14 DAT, which decreased at the end of the study. This indicates that part of the bounded residues can be available for further degradation.

The DT_{50} and DT_{90} values of Phosmet in soil under aerobic conditions were calculated on the basis of the data obtained for the carbonyl label assuming simple first order kinetics (Table 80). No accumulation of Phosmet is expected in soil.

Table 80: DT_{50} and DT_{90} values for Phosmet in soil under aerobic conditions (days)

	DT_{50}	**DT_{90}**	**R^2**	**Kinetic**
Sorrento loam **pH(H_2O) = 7.4**	3.6	11.9	0.985	1st order; non-linear regression

This study let understand better the mechanisms of degradation of Phosmet in the environment. Thus, hydrolysis seems to play a dominant role in degrading Phosmet in soil, being microbial metabolism the key factor in further degradation to CO_2 and bounded residues. The runoff study showed that Phosmet and the degradation products had little tendency to be transported by water. Photodegradation is other factor in limiting the persistence of Phosmet in the environment.

This study gives additional information to understand the behaviour of Phosmet in the environment.

Biodegradability.

The biodegradability of Phosmet was investigated according to the OECD 301 D (EC Method C.4-E. Part VI: Closed Bottle Test).

Kelly, C.R., Paterson, K. (2003).

Phosmet was exposed to activated sludge micro-organisms with culture medium in sealed vessels in the dark at 20 to 22 °C for 28 days. The nominal test concentration was 3.62 mg a.i./l equivalent to a theoretical biological oxygen demand ($ThOD_{NH3}$) of 1.56 mg O_2/mg test item. The degradation of the test material was assessed by determination of dissolved oxygen concentration over time expressed as percent of the theoretical oxygen demand with respect of the test material. The oxygen concentration in the test vessels were corrected for nitrification. The degradation of the blank control was taken into account at each sampling point (degradation of Phosmet minus degradation of blank control).

The test material attained up to 19.5 % biodegradation after 28 days whereas the control substance sodium benzoate was > 60 % degraded within 4 days and > 80 % within 14 days. The toxic control prepared with Phosmet and sodium benzoate showed a mean biodegradability of 49.7 % within 14 days at concentrations of 3.62 mg/L thereby confirming that Phosmet at these concentrations was not toxic to sewage treatment micro-organisms used in the study. The results of the degradation are presented below in Table 81.

Table 81: Biodegradation of Phosmet, reference item and toxicity control (results expressed in % of applied substance)

Time [d]	Phosmet	Sodium Benzoate (Reference Item)	Toxicity Control
2	0.0	35.6	nd
4	0.0	68.6	nd
7	0.0	72.2	27.1
10	0.0	74.3	nd
14	11.3	83.5	49.7
21	23.4	69.2	43.7
28	19.5	90.4	41.5

Due to the fact that only 19.5 % of Phosmet was degraded during the 28 days incubation period, Phosmet cannot be classified as readily biodegradable under the conditions of this study. Phosmet was not toxic to the sewage treatment micro-organisms used in the study at concentrations of 3.62 mg/L.

5.1.2.3 Simulation tests

Water/Sediment

McCorquodale, G. Y. et al. (2003).

The degradation of ^{14}C-Phosmet in aquatic systems under aerobic conditions was studied in two water/sediment systems.

The DT_{50} value of Phosmet in the water column was < 1 d (sandy silt loam system) Moreover, the rate of dissipation of the sand system was not possible to estimated since the number of points was not sufficient. The pH of the water sediment study was above 7, and, as the hydrolysis study showed, under these conditions the hydrolytic degradation of Phosmet was so rapid (4.5 min). Moreover, in the sand system the pH at the sampling time was of 6.2 and after 28 days of acclimation this value fell to 4.9, and after 3 days of the test application the pH was above 8. This fluctuation on the pH value can be due to the hardness of the water that was only 34 mg/l

Due to the pH dependence of the degradation of Phosmet, the conditions of the water sediments system are not considered as the worst case and a new water sediment study at a pH below 7 is required with the identification of all metabolites.

Regarding to the identification of metabolites, the polar fraction was the most relevant with approximately 80 % TAR although the route of degradation of Phosmet was not clear.

It was considered that an accurate identification of the polar fraction must be re-done since this is the most relevant fraction (> 70 % at 7 days after application) found during the study and a new water sediment study done in two systems at PH 6-7 was required.

Regarding to the DT_{50} values calculated, they were re-estimated taking into account simple first order kinetics. The DT_{50} and DT_{90} values obtained from the k values are given in the following table:

Table 82: DT_{50} and DT_{90} for Phosmet in two water sediment systems

	Sand System			Sandy Silt Loam		
Compartment	**Whole System**	**Water Column**	**sediment[1]**	**whole system**	**water column**	**sediment[1]**
DT 50 (d)	0.91	0.4	-	0.1	0.092	-
DT 90 (d)	3.045	1.5	-	0.34	0.3	

(1) r^2<70%

Sven P, Gross, R (2005).

The previous water/sediment study comprised one slightly acidic system with an initial pH value of ca. 5. The pH value of this system increased during the test, which was probably a natural phenomenon.

Since hydrolysis studies showed that the hydrolytic degradation of Phosmet is faster under alkaline than under acidic conditions, the condition of the water sediment systems, both slightly alkaline after the test period, was not considered as a worst case and a new water sediment study at a pH below 7 was required.

In order to clarify these points, a statement was provided and was accepted by the EFSA demonstrating that the pH in abiotic aquatic systems has an influence on the speed of hydrolytic degradation but has no impact on the degradation pathway (i.e. the metabolite pattern is comparable at acidic and alkaline pH values). The same observation was made in biotic aquatic systems: there was no effect of the pH on the degradation pathway of Phosmet.

The identification of metabolites in water sediment systems has been completed and the same major metabolites as in the hydrolysis studies, Phthalamic acid, Phthalic acid and N-Hydroxymethyl phthalimide were identified.

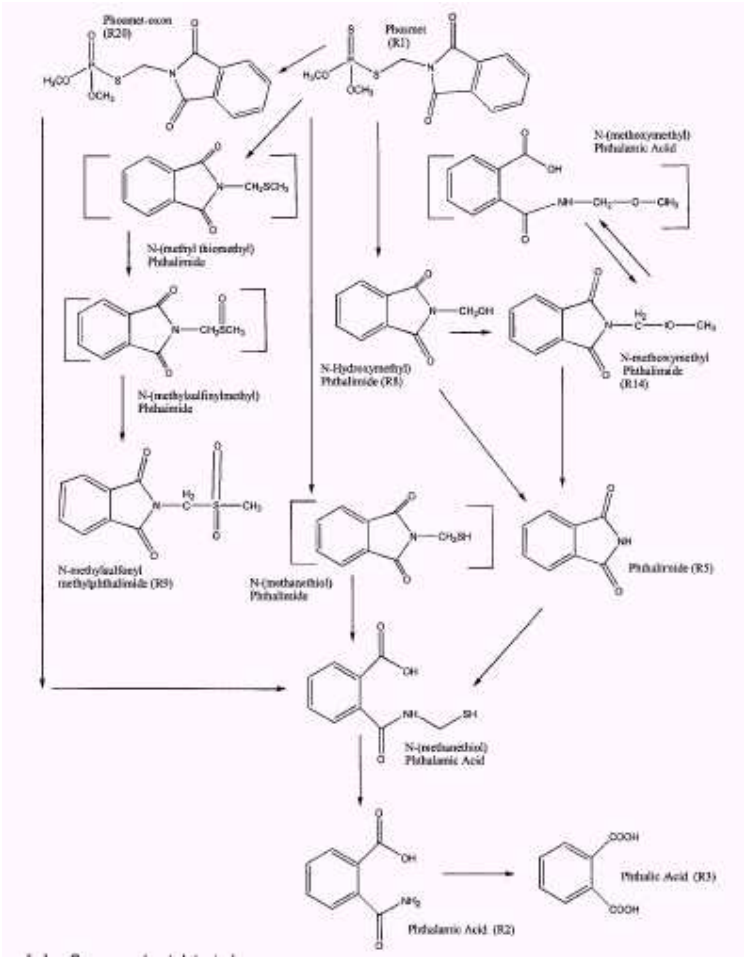
The degradation pathway of Phosmet in aquatic systems is independent of the pH and of the nature of the degradation, hydrolytical or microbial or both. Only the speed of degradation is influenced by the pH value.

Despite the increase of the pH in the acidic water/sediment system during test the assumption that Phosmet would not biodegrade in surface can be considered as a worst case. The rate of disappearance of Phosmet would then be equivalent to the rate of hydrolysis of Phosmet at the corresponding pH

Kidd, Gordon G; Davidson, Jennifer (2005).

During the aerobic degradation of ^{14}C-phosmet in two water/sediment systems, the analysis of surface waters and sediments extracts confirmed the presence of a number of radiolabelled components. The purpose of this study was to further investigate the identity of these components in the surface waters and sediment extracts using an optimized radio HPLC-method, and the proposed pathway of Phosmet in the water/sediment system is given in the figure below.

Figure 4: Proposed degradation pathway of Phosmet in sediment water systems



5.1.3 Summary and discussion of degradation

The results obtained in the ready biodegradability test indicate that Phosmet is not readily biodegradable in nutrient media inoculated with active sewage sludge.

In the soil compartment, it has been found during the soil degradation study of Phosmet under laboratory conditions (Lynn, R. et. all, 2003 a/b) that hydrolysis and microbial degradations play an important role and that high pH provokes a more rapid degradation, as the degradation rate shows that varies between 1.65 days (pH 7.6) and 5.0 days (pH 5.7).

All metabolism/degradation studies consistently show that Phosmet was extensively and rapidly metabolised in soil under aerobic, non-sterile conditions initially to many minor metabolites and ultimately to carbon dioxide and soil bound residues. There was no pre-exposure of the soil micro-organism, an environmentally realistic concentration of substance was tested (2 mg Phosmet/kg soil) and the degradation rate of each soil tested is > 0.043 day^{-1} (assuming simple first order kinetics).

On the other hand, in the water/sediment compartment, Phosmet shows a rapid degradation in both the water phase (DT_{50} = 0.546 days, mean) and in the total system (DT_{50} = 0.505 days, mean). In laboratory (20 °C) natural sediment water systems Phosmet both dissipated rapidly by partitioning to sediment and degraded rapidly.

The identification of metabolites in water/sediment show that the same major metabolites as in the hydrolysis studies, Phthalamic acid, Phthalic acid and N-Hydroxymethyl phthalimide (i.e. exceeding the trigger of 10 % of applied radioactivity or were observed with 5 % of applied radioactivity at 2 consecutive sampling points) were identified in both biotic systems: as well as in both sterile water sediment systems. Taking into account the results of the ecotoxicology studies conducted with these metabolites, it can be considered that these degradation products shall not be classified as hazardous to the aquatic environment and Phosmet is defined as the only residue in water.

Regarding the abiotic degradation, Phosmet undergoes fairly rapid hydrolysis at 25 ºC. Hydrolysis under neutral or alkaline conditions will be a major dissipation pathway for Phosmet. In one study, metabolite identification was performed only at pH 5. But in another submitted study the results of two hydrolysis studies are compared, and it concludes that the pH value affects only the speed of degradation, but has no impact on the degradation pathway.

Finally, taking into account all the abiotic and biotic degradation studies it can be conclude that Phosmet is considered as rapidly degradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The adsorption/desorption characteristics of Phosmet were investigated in four different soils and no linear relationship between the organic matter or clay content and the Koc values were found (Koc 3212 ml/g (mean)). However, the Koc values increased with increasing pH value. Phosmet was inmobile in one type of soil, and showed low mobility in the other three soils.

The leaching behaviour of aged residues of Phosmet was also investigated in aged column leaching studies. In these studies most of the applied Phosmet remained associated with the upper soil column layers. Analysis of the leachate characterised the residue as polar metabolites.

A satisfactorily completed lysimeter study carried out in Switzerland was available. This study indicates that for intended uses when applications are made in the summer, that leaching to groundwater of Phosmet or its breakdown products containing the carbonyl moiety above the parametric dinking water limit of 0.1 µg/l is unlikely even under vulnerable geoclimatic conditions.

5.2.2 Volatilisation

Concentrations of Phosmet in the air compartment are expected to be negligible, due to its very slightly volatile nature. Any Phosmet that was o reach the upper atmosphere would not be expected to be subject to long range transport.

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

There is no indication for bioaccumulation potential of Phosmet (log Pow = 2.95) and its metabolites (Table 83).

Table 83: log of the octanol/water partition coefficient (log P_{ow}) for Phosmet metabolites

METABOLITES	Log P_{ow} [1]
O,O-Dimethylphosphoric acid	- 0.66
Dimethylphosphorodithioic acid	1.26
Phthalamic acid	0.28
Phthalic acid	1.07
N-(Hydroxymethyl) Phthalimide	0.04

[1] calculated with KOWWIN version 1.66

5.3.1.2 Measured bioaccumulation data

Since the octanol-water partition coefficient of Phosmet and its metabolites as log Pow < 3, there is no indication for a bioaccumulation potential of Phosmet and its metabolites. Therefore studies with Phosmet and its metabolites investigating the aquatic bioaccumulation are not required.

5.3.2 Summary and discussion of aquatic bioaccumulation

The bioconcentration potential of active substance should be determined, when the log Pow is > 3. This criteria is not met because log Pow of Phosmet is 2.95 (pH 7, 25 ºC), well below the trigger value of 4. Long term exposure in the aquatic system is unlikely at pH 8 or higher because the DT_{90} value in the whole system is < 10 days as determined in a water/sediment study.

No bioconcentration of metabolites in fish is expected neither, since all log Pow values of Phosmet metabolites are lower than 3.

5.4 Aquatic toxicity

Table 84: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Fish			
Acute toxicity to fish: (no guideline was followed)	LC_{50} = 0.23 mg/l (*O.mykiss*)		Beliles, R.P. (1965)
Acute toxicity to fish: OECD 203; EPA 712 C 98-118	LC_{50} = 0.241 mg/l (*O.mykiss*) LC_{50} = 0.0197 mg/l (*L. macrochirus*)		Knight B. (2003)
Acute toxicity to fish: EPA 72-1 A	LC_{50} = 0.122 mg/l (*L. macrochirus*)	Additional information, not used in RA	Sleight, B.H. (1972)
Acute toxicity to fish: EPA 72-3	LC_{50}=0.170 mg/l (*C. variegatus)*		Bowman, J. H. (1987)
Acute toxicity to fish: Guide of Committee on Methods of toxicity tests with aquatic organisms (1975)	LC_{50} = 0.07 mg/l (*L. macrochirus*) LC_{50} = 0.150 mg/l (*O. tskawytscha*)	Additional information, not used in RA	Julin, A.M., Sanders, H.O. (1977)
Early life stage of rainbow trout: EPA-FIFRA 40 CFR 72-4	NOEC(96d) = 0.0032 mg/l (mm) (*O. mykiss*)		Cohle, P. (1988)
Aquatic invertebrates			
Acute toxicity to the water flea: EPA 660/3-75-009	EC_{50} = 0.0085 mg/l (*D.magna*)	Additional information, not used in RA	Vilkas, A.G. (1977)
Acute toxicity to *D.magna*: OECD 202	EC_{50} = 0.00211 mg/l (*D. magna*)		Knight B. (2003)
Acute toxicity to Daphnia: Committee on Methods of Toxicity Tests with Aquatic Organisms (1975)	EC_{50} = 0.0056 mg/l (*D. magna*)	Additional information, not used in RA	Julin, A.M., Sanders, H.O. (1977)
Aquatic toxicity to crustaceans: (no guideline was followed)	LC_{50} = 0.002 mg/l (*Gammarus fasciatus)*		Sanders, H.O. (1972)
Aquatic toxicity to crustaceans and insects: Committee on Methods of Toxicity Tests with Aquatic Organisms (1975)	EC_{50} = 0.0024 mg/l (*Gammarus pseudolimnaeus*); EC_{50} = 0.1 mg/l (*Asselus brevicaudus*); EC_{50} = 3.2 mg/l (*Chironomus plumosus*)	Additional information, not used in RA	Julin, A.M., Sanders, H.O. (1977)

Chronic toxicity to *D. magna:* EPA-FIFRA 40 CFR 72-4	NOEC(21d) = 0.00078 mg/l (*D. magna*)		Burgess, D., Hamer, M.J. (1988)
Algae			
Algae growth inhibition in a static system: OECD 201	$ErC_{50\ (24h)}$ = 1.2 mg/l NOErC = 0.36 mg/l (mm); $EbC_{50(48h)}$ = 0.51 mg/l NOEbC = 0.14 mg/l (mm) (*Selenastrum capricornutum*)		Knight, B. (2003)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Beliles, R.P. (1965).

Guidelines: none, not GLP

Imidan safety evaluation on fish and wild life (mallard ducks, rainbow trout).The purpose of this study was to determinate the acute toxicity of Phosmet (97 % purity) on *Oncorhynchus mykiss* under static 96 hour test. The test did not follow any guideline and it was not conducted under GLP. The study is valid.

Deviations: The stability of the compound was not verified along the duration of test and pH value of water is not detailed.

10 fishes (0.55 g weight and 3.92 cm length in mean) were exposed to each treatment concentration and control. In addition, a positive control of p,p´-DDT (77 % purity) was carried out. Treatments consisted of a dilution water control, a 0.004 µg/L solvent control (acetone) and measured initial concentrations of 0.10, 0.32, 0.56 and 1.0 mg/L. Mortality occurred in all treated groups.

Findings: The **96-hour LC_{50}** for the rainbow trout **was 0.23 mg a.i/L**.

Table 85: Mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to Phosmet for 96 hours in an unaerated, static acute test

Nominal concentration of Phosmet [mg/L]	**Cumulative mortality (No. dead / No. at test start)**			
	24 h	**48 h**	**72 h**	**96 h**
Water Control	0/10	0/10	0/10	0/10
Solvent Control	0/10	0/10	0/10	1/10
1.0	8/10	8/10	10/10	10/10
0.56	2/10	6/10	8/10	10/10
0.32	1/10	2/10	3/10	5/10
0.10	0/10	1/10	1/10	2/10

Knight B. (2003).

Guidelines: OECD 203, EPA 712 C 98-118; GLP

The purpose of this study is to determinate the acute toxicity of Phosmet technical (97 % purity) along 96 hours to Rainbow trout (*Oncorynchus mykiss*) under continuous flow conditions. The study is valid.

Deviation: Some concentrations differed by a factor higher than 2.2.

Seven fish (length between 4-6 cm) were exposed to one of the follow nominal concentrations, 0, 12.8, 28.2, 62.0, 136 and 300 µg a.i./L. Phosmet technical was added to the test system with acetone as a solvent. During the test period (0-96 h), duplicate aliquots (50 ml) were removed from each tank at 24 h prior to the addition of fish, at 0 h and at 24 and 96h after introduction of fish and analysed by HPLC for phosmet. The measured values were 12.6, 27.6, 75.3, 175 and 286 µg a.i./L. The overall mean measured concentrations deviated from nominal by -5 % to 29 %.

After 96 h the surviving fish at 286 µg/L were lethargy and on the tank base. All fish at 175 µg/L were on the tank base and appeared lethargic. All fish at 75.3 µg/L appeared lethargic. Fish at 27.6 and 12.6 µg/L and in both control appeared active and healthy throughout the duration of test. At the end of study fish were within the range 4.01-5.99 cm in length.

Findings: LC_{50} was calculated by probit method. The **96 h LC_{50}** of Phosmet to rainbow trout was **0.241mg a.i/L** and the 96h NOEC was 27.6 µg/L a.i based both on overall mean measured concentrations of Phosmet.

Table 86: Mortality and toxicity values of Phosmet on rainbow trout

Nominal (µg a.i./L)	**Measured (µg a.i./L)**	**Deviation from nominal**	**Mortality (7 fish/ concentration)**			
			24 h	**48 h**	**72 h**	**96 h**
0 control	ND		0	0	0	0
0 solvent	ND		0	0	0	0
12.8	12.6	-2%	0	0	0	0
28.2	27.6	-2%	0	0	0	0
62.0	75.3	21%	0	0	0	0
136	175	29%	0	0	1	1
300	286	-5%	2	4	5	5
LC_{50} (µg a.i./L)			>286	286	241.2 (541.9-161.6)	**241.2** (541.9-161.6)

Knight B. (2003).

Guidelines: OECD 203, EPA 712 C 98-118; GLP

The purpose of this study is to determinate the acute toxicity of Phosmet technical (97 % purity) along 96 hours to Bluegill sunfish (*Lepomis macrochirus*) under continuous flow conditions. The study is valid.

Deviation: Some concentrations differed by a factor higher than 2.2.

The assay was carried out just in the same way detailed above.

Findings: LC_{50} was calculated by probit method. The **96 h LC_{50}** of Phosmet to blue sunfish **was 19.7 µg a.i/L** and the 96 h NOEC was 9.4 µg/L a.i based both on overall mean measured concentrations of Phosmet.

Table 87: Mortality and toxicity values of Phosmet on *Lepomis macrochirus*

Nominal (µg a.i./L)	**Measured (µg a.i./L)**	**Deviation from nominal**	**Mortality (7 fish/ concentration)**			
			24 h	**48 h**	**72 h**	**96 h**
0 control	ND		0	0	0	0
0 solvent	ND		0	0	0	0
4.3	3.9	-9%	0	0	0	0
9.4	8.7	-7%	0	0	0	0
20.7	20.0	-3%	0	0	3	7
45.5	45.2	-1%	5	7	7	7
100	106.6	7%	7	7	7	7
LC_{50} (µg a.i./L)			39.2	30.7	21.7	**19.7**

ND: no detected (limit of detection 0.73µg/L)

Sleight, B.H. (1972).

Guidelines: EPA 72-1A; no GLP

The aim of this study was to determinate the acute toxicity of Imidan technical (96 %purity) to bluegill *(Lepomis macrochirus*) under static conditions for a 96 hours period. It did not follow GLP. This study will be considered as additional information and it will be not used for the risk assessment.

Deviations: The number of fish was not reported. No solvent control was assayed. The concentration of the test item in the test media was not measured and the stability of test concentration along the assay was not demonstrated. The quality of dose-response curve is not good since any in-between (0-90 %) effect has been identified; hence the Rapporteur has doubts about the precision of the obtained LC_{50} value.

Two bioassays were conducted. The first “unaged”, Imidan was dissolved in acetone and added to the test vessels, which contain the fish. The second “aged”, Imidan was in the test vessels for 96 h before introducing the fish.

Mortality (96 h) occurred at the following concentrations: 0.14 (90 %), 0.24 (100 %) and 0.42 mg/L (100 %) in the case of “non aged” Phosmet, and at 1.4 (20 %), 2.4 (50 %), 4.2, 7.5 and 14.0 mg/L (all 100 %) in the case of “aged” substance. The NOEC was determined to be 0.075 and 0.75 mg/L for the “unaged” and “aged” test item, respectively.

Findings: The **96-hour LC_{50}** for the bluegill **was 0.122 mg** “unaged” Phosmet/L and 1.83 mg “aged” Phosmet/L.

Table 88: Mortality and toxicity values of Phosmet "unaged" and Phosmet "aged"on bluegill sunfish

Unaged nominal concentration (mg a.i./L)	% mortality		Aged nominal concentrations (mg a.i./L)	% mortality	
	24 h	96 h		24 h	96 h
control	0	0	control	0	0
0.0075	0	0	0.14	0	0
0.014	0	0	0.42	0	0
0.024	0	0	0.75	0	0
0.042	0	0	1.4	0	20
0.075	0	0	2.4	10	50
0.14	10	90	4.2	100	100
0.24	80	100	7.5	100	100
0.42	100	100	14	100	100

Bowman, J. H. (1987).

Guidelines: EPA-FIFRA 72-3; GLP

The purpose of this study was to assess the acute toxicity of Imidan Technical (purity not specified) to sheephead minnow (*Cyprinodon variegatus)* along 96 hours static test. The study is valid.

Deviation: An analytical verification of Phosmet concentrations was not conducted.

Juvenile sheephead minnow (*Cyprinodon variegatus)* were exposed to 6 treatment concentrations, water control and the solvent control (DMF). Ten unfed fish, with a mean weight of 0.11g and mean length of 16 mm, were used per treatment. These consisted of 0.032, 0.056, 0.10, 0.18, 0.32, and 0.56 mg Phosmet/L, nominal concentrations.

Test solution parameters were measured at the beginning and at 24 hours interval in all tanks. Temperature was 22 ºC, pH ranged from 8.0 to 8.4, dissolved oxygen from 5.0 to 7.7 mg/l (65 and 100 % of air saturation) and conductivity from 156.6-161.3µS. The light cycle was 16L/8D.

Findings: 96-h LC_{50} for sheephead minnow (*Cyprinodon variegatus*) was **0.170 mg Phosmet/L**, based on nominal concentrations and obtained by probit method. The 96h-NOEC was determined to be 0.056 mg/L nominal concentrations, based on lack of mortality and abnormal effects.

Julin, A.M., Sanders, H.O. (1977).

Guidelines: Committee on Methods of Toxicity Tests with Aquatic Organisms (1975); no GLP

Toxicity and Accumulation of the Insecticide Imidan in Freshwater Invertebrates and Fishes. The objective of this study was to determinate the acute toxicity of Phosmet (95.8 % purity) and Imidan 50WP (50 % wettable power) to several fish along 96 hours and under static conditions. Data from this study will be considered as additional and these results will be not used for the risk assessment.

Deviations: Concentration range is not specified. The concentration of the test item in the test media was not measured. The temperature is slightly lower than recommended according to OECD guidelines.

Findings: LC_{50} was calculated by the method of Litchfield and Wilcoxon (1949) and 95 % confidence limits. The most sensitive warmwater fish is the bluegill sunfish, the **96-hour LC_{50}** for *Lepomis macrochirus* was **0.07 mg Phosmet/L**. Channel catfish and fathead minnow were roughly

an order of magnitude less sensitive. The most sensitive coldwater fish is *O. tskawytscha* **LC50** was 0.150 mg Phosmet/L (0.540 – 1.1 mg Phosmet/L).

Table 89: Toxicity values of Imidan to fish

Specie	Substance	Temp (°C)	LC_{50} µg/L	
			24 h	96 h
Chinook salmon *O. tskawytscha*	Technical	10	180	150
Bluegill *L. macrochirus*	Technical	20	230 (180 – 300)	70 (50 – 100)
Smallmouth bass *M. dolomieui*	Technical	20	400	150
Rainbow trout *S. gairdneri*	Technical	10	760	560
Fathead minnow *P. promelas*	Technical	20	10000 (6700 – 15000)	7300 (4700 – 11000)
Channel catfish *I. punctatus*	Technical	20	13000 (10000 – 16000)	11000 (8400 – 130000)

5.4.1.2 Long-term toxicity to fish

A chronic toxicity study on juvenile rainbow trout, following exposure of 28 days is not available. A chronic test should be required if the DT_{50} from the water sediment study of parent compound in the water column is > 2 days at an environmentally relevant pH in the range of 6 – 9. From the available water/sediment study, the dissipation half-life in surface water was calculated at pH 8 (lower than 2 days) but not at lower pH (worst case situation).

Considering that at the fate section a new water sediment study at pH < 7 was required, this study could be used to calculate the DT_{50}. If from fate section a DT_{50} lower than 2 in the water column is validated, then the long term study to fish would not be necessary.

In addition, the following higher tier fish early life stage toxicity test addresses this data requirement.

Cohle, P. (1988).

Guidelines: EPA-FIFRA 72-4; GLP

The aim of this study was to determinate the effects of ^{14}C-Phosmet (purity not specified, radiochemical purity of Imidan-methoxy-^{14}C was 97-99 %) to early life stage of rainbow trout (*Oncorhynchus mykiss)* under unaerated, continuous-flow conditions for 96 days. The study is valid.

A dilution water control, a 12.5 µl/L solvent control (N,N-dimethylformamide), and nominal test item concentrations of 3.0, 6.0, 12.0, 25.0, and 50.0 µg/L were used during the study. Analytical verification of ^{14}C-Phosmet concentrations was made on test solutions sampled on day 0, 1, 7, 14 and every 7 days thereafter until test end (day 96). Concentrations were measured by liquid scintillation counting (LSC). Mean measured concentrations of 14C-Phosmet were 3.2, 6.1, 12.0, 24.0 and 51 µg/L and ranged from 96 to 107 % of nominal concentrations.

Test solutions were supplied to each replicate test chamber by a continuous flow metering system at a rate of approximately 53.7 ml per hour (replacing a replicate volume approx. 6.6 times per 24 h).

120 embryos were exposed per concentration at test start (30 embryos per embryo cup, 1 cup per replicate, 4 replicates per concentration). Post-hatch period began on study day 36 (hatch >95 %). On day 39 all unhatched eggs were removed and the number of fry per replicate was reduced to 15 (60 per concentration). After hatching of embryos into fry all aquaria were held under a photoperiod of 16 hours light. The vessels were maintained at approx. 10°C. Starting with day 15 post-hatch, the fry were fed brine shrimp (Artemia salina) and dry pellet food as the fish grew.

All chemical and physical parameters (dissolved oxygen 74 % saturation, hardness 38-52 mg $CaCO_3$/L, pH 7.5-8.4, temperature 9.2-10.6, total organic carbon<1-9.2 ppm) in the definitive test were within expected ranges.

Hatchability of eyed rainbow trout eggs after 39 days of continuous exposure to Phosmet was higher than 97% in both controls and the five test levels. Survival of fry, 60 days post hatch was significantly reduced in the two highest test concentrations of 24 and 51µg/L.

Length was significantly reduced at 12, 24 and 51 µg/L after 35 days post hatch and at 6.1 and 51 µg/L at 60 post hatch. Weight was reduced after 60 days post hatch at 12, 24 and 51 µg/L.

Some of these parameters are summarised in the following table:

Table 90:

Nominal conc. (µg a.i/L)	**Mean, measured conc. (µg/L)**	**Egg Hatch[a] >95 %**	**Fry Survival**	**Mean standard length (mm)**	**Fry Survival**	**Mean standard length (mm)**	**Mean wet weight (g)**
			35 days post hatch		**60 days post-hatch**		
	Water Control	99	100	27	98	39	0.87
	Solvent C.	98	98	27	98	39	0.85
3	3.2	99	98	27	97	38	0.84
6	6.1	99	98	26.3	98	37*	0.78
12	12	99	93*	25.8*	92	34*	0.57*
25	24	97	98	25*	82*	31*	0.43*
50	51	97	97	23*	41*	28*	0.33*

[a] Based on day 36 data

* Significantly different (P <0.05) from control

Findings: The **96-day NOEC** was **3.2 µg a.i/L** and the LOEC 6.1 µg a.i/L of Phosmet in a trout early life stage test, based on mean measured concentrations. MATC (maximum Acceptable Toxicant Concentration) defined as the geometric mean of NOEC and LOEC was calculated to be 4.4µg a.i/L.

It was considered not necessary to present a Fish Full Life Stage (FFLS) due to a fish early life stage toxicity test that was conducted, and DT_{90} would be unlikely higher than 100 days. In addition a high tier study (mesocosm) has been provided.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Vilkas, A.G. (1977).

Guidelines: EPA-660/3-75-009; no GLP

The objective of this study was to determinate the acute toxicity of Phosmet (92.5 % purity) to the water flea *Daphnia magna* Straus under static conditions along 48 hours. This information is additional and data cannot be considered for the risk assessment due to the following comment: The concentration of the test item in the test media was not measured. Considering that the DT_{50} value at the pH of the assay is lower than 8 hours, the stability of the Phosmet concentration in solution should be measured. There is no a good quality dose-response curve because the highest mortality recorded at the highest concentration is 50 %.

Unfed *Daphnia magna* (< 20 hours old) were exposed to one of the next treatments consisted of a water control, a solvent control (10 µg/L acetone) and nominal concentrations of 1.0, 1.8, 3.2, 5.6, and 10 µg a.i/L each consisting of four replicates without aireation. Five daphnids were used per replicate.

Table 91: Summary of observed immobility and sublethal effects of unfed *Daphnia magna* exposed to Phosmet for 48 hours in an unaerated, static acute test

Nominal conc. Phosmet (µg a.i/L)	Mean Mortality [%]	
	24 Hours	48 Hours
Water control	0	0
Solvent control	0	0
1.0	0	0
1.8	0	0
3.2	0	5
5.6	0	25
10.0	20	50

Findings: The **48-hour LC_{50}** with 95 % confidence limits in *Daphnia magna* **was 8.5 µg a.s/L** (7.0 – 10.2 µg/L). The 48-hour NOEC was 1.8 µg/L.

Knight B. (2003).

Guidelines: OECD 202; GLP

The purpose of this study was to determinate the acute toxicity of Phosmet (purity 97.0 % w/w) to *Daphnia magna* under continuous flow conditions during 48 hours. The study is valid.

Four replicates with 5 daphnids (less than 24 hours old) each were exposed to one of the next nominal concentrations of 0 (negative), 0 (acetone 0.1 ml/L) 0.034, 0.75, 0.165, 3.64 and 8 µg a.i/L. The overall mean measured concentrations ranged from 20 to 203 % of nominal concentrations, hence result are based on mean measured concentrations. The test system was allowed to equilibrate for 2 days prior to the addition of the *Daphnia*. Daphnias were not fed during the study.

Water quality parameters like temperature, dissolved oxygen and pH were within acceptable limits.

After 24h exposure 20 (100 %) daphnia were immobile at 8.45µg/L and 7 (35 %) at 3.32 µg/L. After 48 hours, the number of immobile Daphnia was 18 (90 %) at 3.30, 6 (30 %) at 2.55, 2 (10 %) at 0.79 and 1 (5 %) at 0.37 µg a.i/L based on measured values.

Findings: The EC_{50} was estimated by maximum likehood estimation compared to the probit method. Based on the test results, the **48-hour EC_{50}** for *Daphnia magna* for Phosmet **was 2.11 µg a.i/L**. This is the lowest acute toxicity value from all the acute tests conducted, so this EC50 is the value which establishes the classification for short term environmental hazards of Phosmet.

The no observed effect concentration (NOEC) was 0.79 µg a.i/L based both on overall mean measured concentrations.

Table 92: Immobilised daphnids, *Daphnia magna*, exposed to Phosmet for 48 hours in a continuous-flow test system

Nominal concentration of Phosmet [µg/L]	**Mean, measured concentrations of Phosmet [µg/L]**	**Cumulative number of immobilised Daphnia 20 *Daphnia* per group**	
		Time	
		24 h	**48 h**
Control	Control	0	0 (0 %)
Solvent control	Solvent control	0	0 (0 %)
0.34	0.37*	0	1 (5 %)
0.76	0.79	0	2 (10 %)
1.65	2.55	0	6 (30%)
3.64	3.32	7	18 (90%)
8	8.45	20	20 (100 %)

* Limit of detection 0.73µg a.i/ for direct injected or diluted samples and 0.47µg/L for concentrated samples.

Julin, A.M., Sanders, H.O. (1977).

Guidelines: Committee on Methods of Toxicity Tests with Aquatic Organisms (1975); no GLP

Toxicity and Accumulation of the Insecticide Imidan technical (95.8 % purity) in Freshwater Invertebrates and Fishes. The aim of this study was to determinate the acute toxicity of Phosmet (05.8 % purity) to *Daphnia magna*. The results will be considered as additional information non-included for risk assessment due to the lack of essential data.

Deviations: The concentration range of the test item was no indicated. Number of animals and replicates were not detailed. The dose-response curve is not included.

The test was carried out along 48-hour with first instar daphnids at 20 ºC under static conditions. Standard reconstituted water was employed with a pH 7.2 (daily checked), alkalinity 35 mg/L and Hardness 40 mg/L $CaCO_3$.

Findings: The **48 h-EC_{50}** was **5.6 µg Phosmet/L** (4.2 –8.4 mg Phosmet/L).

Sanders, H.O. (1972).

Guidelines: none, no GLP

Toxicity of Some Insecticides to Four Species of Malacostracan Crustaceans. The objective of this study was to evaluate the effect of Imidan (technical) to the amphipod (*Gammarus fasciatus*) along 96 hours in a static test. The study is valid.

Comment: The concentration of the test item in the test media was measured at start but not at the end of the assay. Having in mind the DT_{50} of Phosmet in water at pH of the assay, this data would be very interesting.

Test animals were collected from local freshwater stream and ponds, they were transferred to an indoor simulated stream simulating the physical and chemical characteristics to that from which they were collected. The temperature was maintained at 21 ºC, pH 7.1-7.4. Two replicates of 10 organisms each were set up for each concentration tested. All animals were held under test conditions for 24 h acclimation period, after this time, the surviving animals were exposed to one of five concentrations tested of the insecticide. The concentration of toxic compound in the reconstituted water was determined at start but not at the end of the study.

Findings: The 96 h- **LC_{50} of *Gammarus fasciatus* was 2 µg a.i/L** (1.4 – 2.8 µg a.i/L) and. 5 µg a.i/L (4 – 6 µg a.i/L) after 24 hours.

Julin, A.M., Sanders, H.O. (1977).

Guidelines: Committee on Methods of Toxicity Tests with Aquatic Organisms (1975); no GLP

Toxicity and Accumulation of the Insecticide Imidan (purity: 95.8 %) in Freshwater Invertebrates and Fishes. The purpose of this study was to determinate the acute effect of Phosmet to the amphipod (*Gammarus pseudolimnaeus*), aquatic sowbug (*Assellus brevicaudus*), and the midge (*Chironomus plumosus*) in a 48-hour test. Data from this study will be considered as additional and these results will be not used for the risk assessment due to the lack of essential data.

Deviations: The concentration of the test item in the test media was not measured. Number of animals and replicates were not detailed.

Table 93: Acute toxicity of Phosmet to fresh water invertebrates during 48 hours exposure

Species	Stage	EC_{50} or LC_{50} [µg/L]
Amphipod (*Gammarus pseudolimnaeus*)	Adult	2.4 (1.9 – 3)
Aquatic sowbug (*Asselus brevicaudus*)	Adult	100 (78 – 120)
Midge (*Chironomus plumosus*)	4th instar	3200 (2300 – 4200)

Findings: The method of Litchfield and Wilcoxon was used to estimate the EC_{50} and LC_{50} values. The most sensitive aquatic invertebrate was the amphipod (*Gammarus pseudolimnaeus*), showing an EC_{50} of **2.4 µg/L** (1.9 – 3). Aquatic sowbug (*Asselus brevicaudus*) with EC_{50} of **100 µg/L** (78 – 120) and the chironomid (*Chironomus plumosus*) showing an EC_{50} of **3200 µg/L** (2300 – 4200) were roughly two to three orders of magnitude less sensitive.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Burgess, D. (1988) initial study

Hamer, M.J. (1988) definitive study

Guidelines: EPA-FIFRA 72-4, similar to OECD 202 Part II; GLP

The aim of this study was to evaluate the chronic toxicity of ^{14}C-Imidan technical (94.3 % purity) to *Daphnia magna* under flow through test conditions along 21 days. The study is valid.

Deviations: water hardness 206 – 275 mg/L as $CaCO_3$ (recommended 160 – 180 mg/L). The deviation is not considered to invalidate the study.

A total of 40 daphnids (age < 24 h) were exposed to each treatment (10 daphnids per replicate). Treatments consisted of a dilution water control, a solvent control (acetone) and nominal test compound concentrations of 0.19, 0.38, 0.75, 1.5, and 3.0 µg a.i /L. Analytical verification of ^{14}C-Phosmet concentrations was made on test solutions sampled on day 0, 4, 7, 14 and 21. Concentrations were measured for Phosmet by liquid scintillation counting (LSC) and were 0.17, 0.43, 0.78, 1.6, and 3.6 µg a.i/L and ranged from 89 to 120 % of nominal concentrations.

The daphnids were fed with *Selenastrum capricornutum* three times daily and once daily a suspension of tetramin, cereal leaves, vitamins and yeast. Test solutions were maintained between 20 and 21 °C.

All chemical and physical parameters (dissolved oxygen concentration, pH and temperature) in the definitive test were within expected ranges.

Table 94: Analytical determination of concentrations tested and summary of test endpoints following exposure of *Daphnia magna* to Phosmet for 21 days

Nominal Phosmet concentr.	**Measured ^{14}C-Phosmet concentr. (µg/L)**						**Mean adult survival[a]**	**Mean adult length**	**Mean young/adult/ reproduction day**	**Mean length to first brood**
(µg/L)	**0 d**	**4 d**	**7 d**	**14 d**	**21 d**	**Mean**	**%**	**(mm)**	**number**	**(days)**
Water Control							92	3.6	4.7	8.0
Solvent Control							92	3.6	4.7	8.0
0.19	0.172	0.178	0.178	0.165	0.167	0.17	95	3.6	4.7	8.0
0.38	0.452	0.454	0.498	0.395	0.374	0.43	95	3.6	4.6	8.0
0.75	0.799	0.791	0.846	0.747	0.701	0.78	95	3.6	4.8	8.0
1.5	1.60	1.63	1.82	1.52	1.48	1.6	85	3.4*	3.4*	8.2
3.0	3.72	3.59	3.79	3.48	3.35	3.6	0*	-	0.63*	8.5*

[a] Percent of adult daphnids alive at the end of the test

* significantly different (α = < 0.05) from pooled controls

Findings:

The mean young/adult/reproduction day for 21 days was significantly affected in the mean measured exposure levels of 1.6 and 3.6 µg/L test levels.

To assess the effect on reproductive output of *D. magna*, this test showed an overall **21-day NOEC** value of **0.78 µg a.i/L** (mean measured concentration). This is the lowest chronic toxicity value from all the tests conducted, so this NOEC should be taken into consideration as the value which establishes the classification for short term environmental hazards of Phosmet, because the study is well conducted, followed GLP and it is considered valid.

Other parameters also reported in this study are: adult survival, adult length, first day of reproduction and time to production of first brood. Based on the survival, mean adult length, and time until first brood and young/adult/reproduction day, the overall NOEC value was 0.78 µg a.i/L (mean measured concentration).

Based on the survival of the adults at day 21 the EC_{50} was calculated to be 2.0 µg/L (but this data is considered additional due to its low quality). Effects on parental daphnids occur in the same order of magnitude as the first reproductive effects were observed.

5.4.3 Algae and aquatic plants

Knight, B. (2003).

Guidelines: OECD 201; GLP

The objective of this study was to determinate the effects of Phosmet Technical (97.0 % purity) on Alga Growth inhibition (*Selenastrum capricornutum*) in a static test system along 72 hours test. The study is valid.

Deviation: The concentration spacing factor is 2.5 (greater than recommended in the guideline 2.0).

Cultures of the freshwater green algae *Selenastrum capricornutum* were exposed to one of the follow nominal test concentrations 1.0, 2.6, 6.4, 16.0, 40.0 and 100.0 mg Phosmet/L. Three replicate flasks at each test concentration and six control replicate flasks (Algal Growth Medium alone) were included in the test.

Analyses of test material concentrations were performed using a validated HPLC method with an UV/VIS absorbance detector. Measured concentrations were not maintained at initial concentrations over the test period, the 24 h measured concentrations were between 3 % and 12 % of the initial 0 h measured concentrations in the samples with algae, and between 0 % and 10 % in the samples without algae. For the reason of this rapid degradation, results will be related to initial measured values, in spite of geometric mean measured concentrations (0-24 h) proposed by the Notifier.

The average specific growth rate (µave) was estimated for each replicate flask during the experimental period, using daily cell counts. Cell growth inhibition started at 2.6 mg /L (nominal) and was almost completely inhibited at the highest test concentration (100.0 mg /L nominal); see following table.

Table 95: Areas under growth curves and average specific growth rates determined from daily cell counts at 0, 24, 48 and 72 h during the definitive test

Nominal conc. of Phosmet [mg/L]	**Mean measured initial conc. [mg a.i/L]**	**Area under growth curve (AUC) [x10^5 cells*h*mL-1]**			**Average Specific Growth Rate [μ_{ave}*day^{-1}]**		
	0 h	**0-24**	**0-48**	**0-72**	**0-24**	**0-48**	**0-72**
Control	ND	0.29	1.27	4.26	2.52	2.19	1.94
1.0	0.14	0.29	1.30	4.48	2.52	2.20	1.96
2.6	0.36	0.29	0.62	2.45	2.54	1.65	1.80
6.4	0.99	0.14	0.44	1.0	1.86	1.62	1.40
16.0	2.31	0.01	0.04	0.44	0.39	0.55	1.29
40.0	5.43	0.01	0.02	0.43	0.16	0.35	1.30
100.0	15.5	0.00	0.00	0.01	0.00	0.00	0.00
EC^*_{50} (mg/L)		1.0	**0.51**	0.60	**1.2**		

* Values referred to initial measured concentration of a.i.

The Notifier proposes results based on geometric mean (0-24 hours) measured test concentrations. The 72 h EC_{50} values for growth rate (E_rC_{50}) and for the area under growth curve (AUC = E_bC_{50}) were 1.3 mg/L and 0.14 mg/L, respectively. The 72 h NOEC values were estimated to be 0.04 mg/L, respectively, for both growth rate and AUC.

However, the differences on the measured Phosmet concentrations at 0 hours and after 24 hours are so big (the degradation is very quick) that the geometric mean no describes the concentration of the active ingredient along the 72 hours. Hence, the conclusion is that is simpler to use the measured initial concentration of the compound to express the toxic effects (biomass and growth rate). In addition, effects on growth rate will be assessed at 0-24 hours and effects on biomass will be evaluated at the three intervals (24, 48 and 72 hours) and the most sensitive inhibitory value will be chosen as endpoint.

Findings:

Based on initial measured concentrations, the 24 h inhibitory value for growth rate (E_rC_{50}) was **1.2 mg** a.i/L and for the area under growth curve (biomass AUC = E_bC_{50}) was **0.51 mg** a.i/L**,** obtained at 48 h exposure. The **NOEC** values were estimated to be **0.36 mg/L** and **0.14 mg/L** for both growth rate and AUC, respectively, again this value is based on initial measured concentration at the time interval which acute exposure was more toxic.

5.4.4 Other aquatic organisms (including sediment)

Since the acute toxicity studies with daphnids and chironomids showed that the toxicity of Phosmet to daphnids might be three orders of magnitude higher, and since Phosmet is applied only once per season to citrus, pome fruits and potatoes repeated exposure is unlikely to occur. Therefore, additional studies concerning the chronic toxicity to aquatic insects are not required.

Effects on sediment dwelling organisms are required where environmental fate and behaviour data report that an active substance is likely to partition to and persist in aquatic sediments. In the conducted water/sediment study the percentage of the parent compound in sediment was in a range of 9.25-11.51 % application rate at 0-1 days. Due to the fact that the acute toxicity of Phosmet to sediment dwelling organisms (*Chironomus plumosus*) has shown to be lower than the acute toxicity to daphnids (1600 fold), a study on chronic toxicity to chironomids is not triggered.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Phosmet is considered not readily biodegradable according to the result of the biodegradation test presented, following OECD 301 D guideline, EC method C.4-E. The ready biodegradability criterion stated in this guideline considers substances readily biodegradable when 70% biotic degradation takes place in the 10 days window within the 28 days long duration test.

Phosmet has a log K_{ow} of 2.95 (at pH 7, 25 ºC) below the cut-off value of log K_{ow} > = 4, so no potential for bioaccumulation is expected, regarding CLP criteria. No experimentally BCF was established, since this type of studies are required if log K_{ow} > 3.

Phosmet can be considered as rapidly degradable in the aquatic environment from the water/sediment system studies carried out, because in this test very short DT_{90} values were registered for the whole system (in sand system DT_{90} = 3,045 days; in sandy silt loam DT_{90} = 0,34 days). So, 90 % of the substance degradation is undergoing in a degradation time far below the 28 days period. This clearly means complying with the criterion established by CLP (Annex I, point 4.1.2.9.2): “the substance did actually degrade biotically or abiotically in the aquatic environment

by > 70 % in 28 days. Thus, if degradation is demonstrated under environmentally realistic conditions, then the criterion of “rapid degradability” is met”; and also complying with the fact stated under point 4.1.2.9.5. c).

Due to the results summarized above, Phosmet can be considered as a rapidly degradable substance in the environment, according to the CLP criteria.

Phosmet is assessed as very toxic to aquatic life with long lasting effects, based on the following acute and chronic ecotoxicity data to invertebrates: lowest acute toxicity endpoint is *D.magna* (48h) EC_{50} = 0.00211 mg/l, and lowest chronic toxicity endpoint is *D.magna* (21d) NOEC = 0.00078 mg/l. These two endpoints for *D. magna* will also establish the M factors needed for CLP environmental classification categories.

Taking into account the lowest ecotoxicity endpoints obtained from the acute and chronic ecotoxicity studies carried out with invertebrates, Phosmet should be classified according to Regulation (EC) No 1272/2008 criteria as:

Aquatic Acute 1 with M factor of **100**; CLP criteria for EC_{50} acute toxicity values below or equal to 1 mg/l (EC_{50} = 0.00211 mg/l < 1 mg/l), and

Aquatic Chronic 1 with M factor of **10**; CLP criteria for NOEC chronic values below or equal to 0,01 mg/l (NOEC = 0.00078 mg/l < 0,01mg/l), plus the fact that this substance is rapidly degradable.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Due to all the results summarized in the previous sections, the following classification categories and M factors can be concluded for this active substance:

Phosmet meets the CLP Regulation criteria for being classified as Aquatic Acute 1 with M factor of 100.

Phosmet meets the CLP Regulation criteria for being classified as Aquatic Chronic 1 with M factor of 10.

The current entry in Annex VI Table 3.1 of CLP Regulation should not be changed, only the chronic M factor of 10 should be added to this entry.

6 OTHER INFORMATION

None.

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7.1 Physico-chemical properties

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Myers, H.W.	1987	PHOSMET-PHYSICAL PROPERTIES Stauffer Chemical Company, Richmond, CA Report-no.: RRC 87-67 GLP: No Published: No
Pessin, G.	1998	PARTICLE SIZE DISTRIBUTION OF IMIDAN TECHNICAL Bioagri Laboratorios Ltda., Brazil GLP: - Published: No
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7.2 Toxicology and metabolism

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Andrews, A.H.	1998	STATEMENT TO THE BSE INQUIRY, STATEMENT NO. 269A GLP: No Published: No
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Meyer, L.S., Walberg, J.A.	1990	TWO-GENERATION REPRODUCTION STUDY IN RATS WITH R-1504 CIBA-Geigy Corporation, Farmington, USA Report-no.: T-13260 GLP: Yes Published: No
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Nielsen E., Ostergaard G., Larsen J.C.	2008	TOXICOLOGICAL RISK ASSESSMENTS OF CHEMICALS: A PRACTICAL GUIDE Informa Healthcare, New York, pp. 140-143. GLP: No Published: Yes
OECD	2009	SERIES ON TESTING AND ASSESSMENT NUMBER 39: GUIDANCE DOCUMENT ON ACUTE INHALATION TOXICITY TESTING ENV/JM/MONO(2009)28 (21 July 2009). GLP: No Published: Yes
Pauluhn, J.	2008	INHALATION TOXICOLOGY: METHODOLOGICAL AND REGULATORY CHALLENGES. Exp Toxicol Pathol. 60(2-3):111-24. GLP: No Published: Yes
Pestana, C.B.	1999	MICRONUCLEUS TEST FOR IMIDAN 500 PM TECAM - Tecnologia Ambiental Ltda., S. Paulo, Brasil Report-no.: RL908561 GLP: No Published: No
Pinto, P.J.	1991	PHOSMET: EMBRYOTOXICITY STUDY IN THE RABBIT ICI Central Toxicology Laboratory, Alderley Park, Cheshire, United Kingdom Report-no. : RB0538 GLP: No Published: No
Proudlock, R.J	1998	RAT LIVER DNA REPAIR (UDS) TEST Huntingdon Life Sciences Ltd., Cambridgeshire, United Kingdom Report-no.: GPP 013/983935 GLP: Yes Published: No
Purdey, M.	1996	THE UK EPIDEMIC OF BSE: SLOW VIRUS OR CHRONIC PESTICIDE-INITIATED MODIFICATION OF THE PRION PROTEIN? Med Hypotheses. 1996 May;46(5): 429-43 and 445-54 GLP: No Published: Yes
Purdey, M.	1998	HIGH-DOSE EXPOSURE TO SYSTEMIC PHOSMET INSECTICIDE MODIFIES THE PHOSPHATIDYLINOSITOL ANCHOR ON THE PRION PROTEIN: THE ORIGINS OF NEW VARIANT TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES? Med Hypotheses. 1998 Feb;50(2):91-111 GLP: No Published: Yes
Ray, D.E.	1998	STATEMENT TO THE BSE INQUIRY GLP: No Published: No
Rosenberg, Quenon	1988	ORGANOPHOSPHATE TOXICITY ASSOCIATED WITH FLEA-DIP PRODUCTS – CALIFORNIA Epidemiologic Notes and Reports 37(21): 329-337 GLP: No Published: Yes
Roucou, X., Gains, M., LeBlanc, A.C.	2003	NEUROPROTECTIVE FUNCTIONS OF PRION PROTEIN GLP: No Published: Yes

Author(s)	Year	Title Testing Facility Report No. GLP or GEP status (where relevant) Published or not
Scientific Steering Committee	1998	OPINION ON POSSIBLE LINKS BETWEEN BSE AND ORGANOPHOSPHATES USED AS PESTICIDES AGAINST ECTP- AND ENDOPARASITES IN CATTLE GLP: No Published: No
Scientific Steering Committee	2004	OPINION ON ORGANOPHOSPHATE (OP) POISONING AND HYPOTHETICAL INVOLVEMENT IN THE ORIGIN OF BSE GLP: No Published: No
Serota, D.G., Thakur, A.K., Ulland, B.M., Kirschman, J.C., Brown, N.M., Coots, R.H., Morgareidge, K.	1986	A TWO-YEAR DRINKING WATER STUDY OF DICHLOROMETHANE IN RODENTS. II MICE. Fd. Chem. Toxic. Vol. 24, No. 9, pp 959-963 GLP: No Published: No
Shaw I., Berry,C., Lane, E., Fitzmaurice, P., Clarke, D., Holden, A.	2002	STUDIES ON THE PUTATIVE INTERACTIONS BETWEEN THE ORGANOPHOSPHORUS INSECTICIDE PHOSMET AND RECOMBINANT MOUSE PRPC AND ITS IMPLICATION IN THE BSE EPIDEMIC Veterinary Research Communications; June 2002, Volume 26, Issue 4, pp 263-271 GLP: No Published: Yes
Shirasu, Y.	1976	MUTAGENICITY SCREENING OF PESTICIDES IN THE MICROBIAL SYSTEM Mutation Research, 40 (1976) 19-30 GLP: No Published: Yes
Shirasu, Y Moriya, M. Tezuka, H. Teramoto, S. Ohta, T. Inoue, T.	1984	MUTAGENICITY OF PESTICIDES Institute of Environmental Toxicology, Kodeira, Tokyo, Japan GLP: No Published: Yes
Shirasu, Y. Moritani, M. Koyashiki, R	1979	MUTAGENICITY EVALUATION OF JAPANESE-MADE PHOSMET IN MICROBIAL ASSAY Institute of Environmental Toxicology, Kodeira, Tokyo, Japan GLP: No Published: No
Snyder, R.D.	1986a	REPORT NO. T-12821, MUTAGENITY EVALUATION IN MOUSE LYMPHOMA MULTIPLE ENDPOINT TEST CYTOGENETIC ASSAY The *In Vitro* Toxicology Section, Environmental Health Center, Stauffer Chemical Company, Farmington, USA Report-no. : T-12821, Lot No. 10201-41-1 GLP: No Published: No
Snyder, R.D.	1986b	EFFECTS OF IMIDAN ON HUMAN FIBROBLAST DNA The *In Vitro* Toxicology Section, Environmental Health Center, Stauffer Chemical Company, Farmington, USA Report-no.: T-12823 GLP: No Published: No
Sprague, G. L.	1982	ACUTE DELAYED NEUROTOXICITY STUDY WITH IMIDAN TECHNICAL IN ADULT HENS DeGuigne Technical Center, Richmond Research Center, Richmond, USA Report-no. : T-10910 GLP: No Published: No

Author(s)	Year	Title Testing Facility Report No. GLP or GEP status (where relevant) Published or not
Sprague, G.L, Turnier, J.C.	1988	2-YEAR DIETARY ONCOGENICITY STUDY IN MICE WITH IMIDAN TECHNICAL - ADDENDUM II ICI Americas Inc., Environmental Health Center, Farmington, USA Report-no. : T-10719 Addendum I and II GLP: Yes Published: No
Staff	1966	IMIDAN HUMAN EXPERIENCE DATA Stauffer Chemical Company GLP: No Published: No
Staples, R.E. Kellam, R.G. Haseman, J.K.	1976	DEVELOPMENTAL TOXICITY IN THE RAT AFTER INGESTION OR GAVAGE OF ORGANOPHOSPHATE PESTICIDES (DIPTEREX, IMIDAN) DURING PREGNANCY Environ. Health Persp. 13: 133-140 GLP: No Published: Yes
Sunder-Plassmann, N., Moxer, M.	2005	91/414/EEC REVIEW OF PHOSMET - ALLEGED CORRELATION BETWEEN BSE INCIDENCE AND PHOSMET EXPOSURE GLP: No Published: No
Takemoto, T., Suzuki, T., Miyama, T.	1975	AN UNEXPECTED INCIDENCE OF CONVULSIVE ATTACK IN MALE MICE AFTER LONG-TERM ISOLATED CONDITION. Tohoku J Exp. Med. 115 pp 97-98 GLP: No Published: Yes
Thiel, A., Hofer, M.	2004a	91/414/EEC REVIEW OF PHOSMET – DEDUCTION OF ARFD Scientific Consulting Company, Wendelsheim, Germany Report-no.: 272-034 GLP: No Published: No
Thiel, A., Hofer, M.	2004b	91/414/EEC REVIEW OF PHOSMET – EVALUATION OF THE TWO-YEAR CHRONIC TOXICITY/CARCINOGENICITY FEEDING STUDY IN SPRAGUE DAWLEY CRL:CD SD BR RATS WITH PHOSMET Scientific Consulting Company, Wendelsheim, Germany Report-no.: 272-034 GLP: No Published: No
Thiel, A., Hofer, M.	2004c	91/414/EEC REVIEW OF PHOSMET – ACUTE INHALATION TOXICITY OF IMIDAN 50 WP Scientific Consulting Company, Wendelsheim, Germany Report-no.: 272-034 GLP: No Published: No
Thiel, A., Hofer, M.	2004d	91/414/EEC REVIEW OF PHOSMET – ACUTE INHALATION TOXICITY OF PHOSMET Scientific Consulting Company, Wendelsheim, Germany Report-no.: 272-034 GLP: No Published: No
Thiel, A., Hofer, M.	2005	91/414/EEC REVIEW OF PHOSMET – EVALUATION OF GENOTOXICTY, LONG TERM / CARCINOGENICITY STUDIES IN RATS AND MICE, DEDUCTION OF ADI, AND CLASSIFICATION AND LABELLING WITH REGARD TO CARCINOGENICITY Scientific Consulting Company, Wendelsheim, Germany Report-no.: 272-034 GLP: No Published: No

Author(s)	Year	Title Testing Facility Report No. GLP or GEP status (where relevant) Published or not
Tracey, J.A. Gallagher, H	1990	USE OF GLUCOPYRROLATE AND ATROPINE IN ACUTE ORGANOPHOSPHORUS POISONING Human & Experimental Toxicology 9: 99-100 GLP: No Published: Yes
Tsukamoto M., Casida J.E.	1967	METABOLISM OF METHYLCARBAMATE INSECTICIDES BY THE NADPH2-REQUIRING ENZYME SYSTEM FROM HOUSEFLIES. GLP: No Published: Yes
Vlcková, V. Miadoková, E. Podstavková, S. Vlcek, D.	1993	MUTAGENIC ACTIVITY OF PHOSMET, THE ACTIVE COMPONENT OF THE ORGANOPHOSPHORUS INSECTICIDE DECEMTIONE EK 20 IN SALMONELLA AND SACCHAROMYCES ASSAYS GLP: No Published: Yes
Werner, M., Hofer, M.	2006	CLASSIFICATION AND LABELLING OF PHOSMET WITH RESPECT TO HEALTH HAZARDS. POSITION PAPER ON THE JUSTIFICATION FOR THE CLASSIFICATION OF PHOSMET AS “HARMFUL IF SWALLOWED” (Xn, R22) BASED ON DATA AVAILABLE FOR RELEVANT MATERIAL USED IN PLANT PROTECTION PRODUCTS Scientific Consulting Company, Wendelsheim, Germany Report-no.: 272-034 GLP: No Published: No
Werner, M., Hofer, M., Koehl, W.	2007	CLASSIFICATION AND LABELLING OF PHOSMET WITH RESPECT TO HEALTH HAZARDS. POSITION PAPER ON THE JUSTIFICATION FOR THE CLASSIFICATION OF PHOSMET AS “HARMFUL IF SWALLOWED” (Xn, R22) AND FOR THE ABSENCE OF AN ACUTE INHALATION AS WELL AS REPRODUCTION HAZARD Scientific Consulting Company, Wendelsheim, Germany Report-no.: 272-034 GLP: No Published: No
Zimmermann, F.K. Kern, R. Rasenberger, H.	1975	A YEAST STRAIN FOR THE SIMULTANEOUS DETECTION OF INDUCED MITOTIC CROSSING OVER, MITOTIC GENE CONVERSION AND RESERVE MUTATION GLP: No Published: Yes
Zimmermann, F.K. et al.	1984	TESTING OF CHEMICALS FOR GENETIC ACTIVITY WITH SACCHAROMYCES CEREVISIAE: A REPORT OF THE U.S. ENVIRONMENT PROTECTION AGENCY GENE-TOX PROGRAM GLP: No Published: Yes

7.3 Environment

Authors	Date	Title	Testing Facility	GLP	Published
Lynn, R., McCorquodale, G., Paterson, K.	2003	The route and rate of degradation of (^{14}C)-Phosmet in 3 soils types under aerobic conditions.	na	Yes	No
McBain, J.B.	1973	Environmental behaviour of Imidan.	na	No	No
Peter, S. Gross, R. Hofer, M.	2005	91/414/EEC Review of Phosmet statement related to the relevance of field DT50 values for the calculation of the predicted environmental concentrations of Phosmet in soil.	na	No	No
Chang, L.L.	1987	Phosmet – Hydrolisis and photolysis studies.	na	No	No
Robinson, R.A.	1992	Aqueous photolysis of ^{14}C-Phosmet	na	Yes	No
Kelly, C.R.	2003	Determination of ready biodegradability of Phosmet by the closed bottle test	na	Yes	No
McCorquodale, G.Y. et al.	2003	The aerobic degradation of ^{14}C-Phosmet in two natural sediment/water systems	na	Yes	No
Kidd, G. G. Davidson, J.	2005	Characterization of polar radiolabelled components present in sediments/water systems treated with ^{14}C-Phosmet.	na	Yes	No
Yeh, S.M.	1988	Phosmet batch equilibrium adsorption and desorption in four soils and protocol.	na	Yes	No
Beliles, R.P	1965	Imidan safety evaluation on fish and wild life (mallard ducks, rainbow trout)	na	No	No
Knight, B.	2003	Acute toxicity to fish (Oncorynchus mykiss)	na	Yes	No
Knight, B.	2003	Acute toxicity to fish (*Lepomis macrochirus*)	na	Yes	No
Sleight, B.H.	1972	Acute toxicity to fish (*Lepomis macrochirus*)	na	No	No
Bowman, J.H.	1987	Acute toxicity to fish (*Cyprinodon variegates*)	na	Yes	No
Julin, A.M., Sanders, H.O.	1977	Toxicity and Accumulation of the Insecticide Imidan in Freshwater Invertebrates and Fishes	na	No	No

Authors	Date	Title	Testing Facility	GLP	Published
Cohle, P.	1988	Fish early life stage toxicity	na	Yes	No
Vilkas, A.G.	1977	Acute toxicity to Daphnia magna	na	No	No
Knight, B.	2003	Acute toxicity to Daphnia magna	na	Yes	No
Julin, A.M., Sanders, H.O	1977	Toxicity and Accumulation of the Insecticide Imidan technical (95.8% purity) in Freshwater Invertebrates and Fishes.	na	No	No
Sanders, H.O.	1972	Toxicity of Some Insecticides to Four Species of Malacostracan Crustaceans	na	No	No
Julin, A.M., Sanders, H.O.	1977	Toxicity and Accumulation of the Insecticide Imidan (purity: 95.8 %) in Freshwater Invertebrates and Fishes.	na	No	No
Burgess D., Hamer M.J.	1988	Chronic toxicity to Daphnia magna	na	Yes	Yes
Knight B.	2003	Effects on algal growth	na	Yes	No

8 ANNEXES