Committee for Risk Assessment

RAC

Annex 1

Background document

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

Salicylic acid

EC Number: 200-712-3
CAS Number: 69-72-7

CLH-O-0000001412-86-110/F

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

Adopted

10 March 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Salicylic acid

EC Number: **200-712-3**

CAS Number: **69-72-7**

Index Number: **None**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Salicylic acid***
EC number:	***200-712-3***
CAS number:	***69-72-7***
Annex VI Index number:	***No***
Degree of purity:	***≥ 99 %***
Impurities:	***No impurity ≥ 0.1 %***

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	No
Current proposal for consideration by RAC	Acute Tox. 4 - H302: Harmful if swallowed Eye Damage 1 – H318: Causes serious eye damage
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

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. 4 H302: Harmful if swallowed			

	Acute toxicity - dermal				conclusive but not sufficient for classification
	Acute toxicity - inhalation				conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation				conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Eye Damage 1 H318: Causes serious eye damage			
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				conclusive but not sufficient for classification
3.10.	Aspiration hazard				conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment				conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer				conclusive but not sufficient for classification

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

Labelling:

Signal word: Danger

Hazard statements:
H302: Harmful if swallowed
H318: Causes serious eye damage

Precautionary statements:
P264: Wash with water thoroughly after handling
P270: Do no eat, drink or smoke when using this product
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell

P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310: Immediately call a POISON CENTER or doctor/physician.
P330: Rinse mouth.
P501: Dispose of contents/container to licensed facility

Proposed notes assigned to an entry:

No

2 BACKGROUND TO THE CLH PROPOSAL

Salicylic acid has never been a DSD Annex I, nor CLP Annex VI entry. The substance has been REACH registered in 2010 by two manufacturers, including the above concluded self-classification notification. When the C&L inventory has been published by ECHA, a significant number (33) of C&L notifications appeared to be different, including Reproductive toxicity C&L.

Salicylic acid is mainly used in industrial applications such as resins, rubber, dye, concrete, pharmaceutical intermediate, remedy itself (keratolytic; e.g. aginst warts, in preparations against acne etc as an OTC product, cosmetic industry and a marginal part of biocide use in whole quantities manufactured (< 0.1 %). During preparation of biocide dossier, the dossier rapporteur raised to the Biocide consortium a potential Reproductive toxicity C&L.

Therefore the manufacturers decided to improve the 2010 REACH dossier by deeper analysis of the Reproductive toxicity endpoint, including launching a new epidemiology literature analysis by an external expert. Then they concluded with the same C&L as notified in 2010, and submitted in 2013 a dossier update including these improvements and new data analysis.

Apart from salicylic acid, the Lead manufacturer has been involved in four REACH registration dossiers for substances having a common metabolite: salicylic acid. Therefore it owns the most up-to-date and extensive dossier concerning salicylic acid and would like to guarantee as soon as possible proper and comprehensive information provision to consumers and workers on salicylic acid.

Meanwhile, as manufacturers had concerns about different C&L notified by some companies, they launched a discussion on the ECHA website C&L platform, in order to get arguments having lead to different C&L, and discuss them to try to reach an agreement and an harmonised self-classification; with a proposed deadline of 31st July 2013. Only one answer was received from one notifier who submitted the same C&L as manufacturers, whereas there were more than 2000 notifications submitted with 33 different C&L.

2.1 History of the previous classification and labelling

The only discussion on salicylic acid classification and labelling was that initiated by the Biocide NL Competent Authority. The rapporteur discussed about a potential Reproductive toxicity classification proposal.

While one manufacturer initiated salicylic acid registration under Biocide Directive in 2002, given the marginal part of biocide use in whole quantities manufactured (< 0.1 %) and potential cost of the dossier, the manufacturer decided to withdraw from the Biocide dossier. So the manufacturer transmitted the OECD HPV IUCLID dossier to downstream users in 2006 in order to allow such downstream users to carry on with the dossier. Meanwhile, thanks to the REACH registration performed in 2010, involvement in several other registrations related to this substance, and registration update in 2013, the IUCLID dossier was **extensively updated since** 2002, and it is unlikely that such updated information had been included in the Biocide dossier.

2.2 Short summary of the scientific justification for the CLH proposal

2.2.1. HUMAN HEALTH: Acute toxicity

Acute oral toxicity:

LD50 = 891 mg/kg from key study similar to OECD 401. : This is in the range of 300 -2000 mg/kg and therefore meets the criteria for acute toxicity category 4 according to the CLP Regulation.

Salicylic acid is classified Acute toxicity, oral, category 4 (Harmful if swallowed)

2.2.2. HUMAN HEALTH: Irritation

Eye irritation:

Under the conditions of a key study according to Draize method, salicylic acid induced severe irritation not recovering within 21 days of treatment. If, when applied to the eye of an animal, a substance produces at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days, it meets the criteria for category 1 (irreversible effects on the eye) according to the CLP Regulation.

Salicylic acid is classified Eye Dam. 1; H318.

2.2.3. HUMAN HEALTH: Repeated dose toxicity

The repeated dose toxicity data are reported here because they are relevant for the assessment of reproductive toxicity developed further.

Read across NOAEL from methyl salicylate was 45.4 mg/kg bw/day as salicylic acid (50 mg/kg bw/day as methyl salicylate). Slight effects on bone density were reported at the LOAEL of 500 mg/kg bw/day in the subchronic study, not seen in human juvenile arthritis treatment with o-acetylsalicylic acid (aspirin, having salicylic acid as common metabolite). The 2 year oral toxicity data for methyl salicylate are consistent with the oral subchronic toxicity data from the same laboratory.

2.2.4. HUMAN HEALTH: Toxicity for reproduction

Fertility:

Not classified for effects on reproduction (fertility) according to CLP criteria.

Results in animal studies

No fertility studies are available on salicylic acid itself. Assessment of the potential of salicylic acid to impair fertility has been based on read-across data from published data on related salicylates. Multi-generation studies on the effects of the read-across substance methyl salicylate on fertility in rats and mice, indicate that salicylic acid does not adversely affect fertility. Comparison of the relative pharmacological potency of o-acetylsalicylic acid and salicylic acid, its metabolite, indicate that salicylic acid has negligible potential for maternal or fetal perinatal hemorrhagic effects.

Reduced embryo-foetal viability was reported at high maternally toxic dose levels, when parental toxicity refers to the systemic NOAELs: the NOAEL fertility = 225 mg/kg bw/day is distinctly higher than the chronic NOAEL of 45.4 mg/kg bw/day.

This means that there is no effect on fertility at doses that show no chronic general toxicity.

Evidence from humans

A weight of evidence was based on above animal studies, and human information, which supports the results in animal studies.

Well-designed epidemiological studies (Slone, 1976; Shapiro, 1976; Kozer, 2002) on the use of aspirin (o-acetylsalicylic acid) at up to the maximum recommended therapeutic dose of 4000 mg/day (equivalent to 66.7 mg/kg bw/day as o-acetylsalicylic acid or 56 mg/kg/day as salicylic acid) have largely demonstrated an absence of increased risk of adverse pregnancy outcome in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay, despite widespread self-administration of aspirin during pregnancy. A meta-analysis of studies on the use of low-dose aspirin at 50-150 mg/day (Kozer, 2003) has demonstrated that this dose range is not associated with any adverse pregnancy outcomes, in terms of perinatal mortality, birth complications, congenital malformations or adverse effect on subsequent development. For pregnancies where there was moderate or high risk of pre-eclampsia and/or premature delivery, adverse pregnancy outcome rate was reduced with low-dose aspirin. There was no increased risk of early miscarriage with this dose regime.

These data have been reviewed and evaluated by an Epidemiologist (Pr. D. BARD report to Novacyl, 2012, key study) with the conclusion of no link between o-acetylsalicylic acid use during pregnancy and reprotoxic effects.

Overall, it can be concluded that o-acetylsalicylic acid, and its metabolite, salicylic acid, do not adversely affect fertility in human and animals. Therefore the substance does not meet the criteria for reproductive toxicity category 1 or 2 (i.e. evidence from humans and/or animal studies for effects on sexual function and fertility).

Development of offspring:

Assessment based on a weight of evidence approach assessing appropriate data from animal studies and human data on o-acetylsalicylic acid (its metabolite being salicylic acid).

Results in animal studies

The results of the key and supporting studies in rats demonstrate that salicylic acid has an embryofoetotoxic effect in rats at doses causing clear maternal toxicity in systemic assays, with evidence of malformations only at high maternal toxic doses.

The effect of o-acetylsalicylic acid (aspirin) on development has been studied in rats, mice and rabbits with results leading to the conclusion that there are considerable species differences in sensitivity, with the rat being a specifically sensitive species. Data on the effect of aspirin in human pregnancy (Bard, 2012) has been used to assess the relevance of the animal data for risk assessment. These data indicate that humans are far less sensitive than rats to the effect of o-acetylsalicylic acid and more comparable to rabbits in several points including ADME or protein binding. Results from all studies showed that o-acetylsalicylic acid is embryotoxic at moderate maternal toxic doses and induces malformations at high maternally toxic doses.

This made a weight of evidence that the rat is not a relevant species to extrapolate developmental effect to humans. This is supported by results showing that the bone effects seen in rat are in contradiction with Human juvenile arthritis treatment (Abbott and Harrisson, 1978).

For effects in rabbits, the key study is Cappon et al (2003). There were no adverse effects on development at doses not causing severe maternal toxicity: the NOAEL development = 268 mg/kg bw/day and the maternal NOAEL of 96 mg/kg/d are higher than the general chronic NOAEL of 45.4 mg/kg bw/day.

This means that there is no effect on development of the offspring at doses that show no chronic general toxicity.

Evidence from humans

A weight of evidence was based on above animal studies, and human information, which supports the results in animal studies.

As decribed in Fertility chapter, human data in IUCLID dossier have been completed and all reviewed by an Epidemiologist (Pr. D. BARD, 2012) with the conclusion of no link between o-acetylsalicylic acid use during pregnancy and reprotoxic effects.

This absence of any clear evidence of adverse effects from aspirin on human development demonstrated in well-designed epidemiological studies despite widespread prescribed use and self-medication with aspirin at all stages of pregnancy over a period spanning several decades appears to indicate that humans are considerably less sensitive than rats to the developmental toxicity of salicylate, which is confirmed in mouse (NTP, 1984) and rabbit (Cappon, 2003).

Overall, it can be concluded that o-acetylsalicylic acid, and therefore, salicylic acid, does not adversely affect development of offspring in humans, and that the developmental toxicity reported in the rat is of no relevance for humans.

Therefore the substance does not meet criteria for reproductive toxicity category 1 or 2 (i.e. evidence from humans and animals relevant for toxicity assessment in humans, of effects on development).

2.3 Current harmonised classification and labelling

No current harmonised classification and labelling exist, except those between three manufacturers/notifiers out of more than 2000.

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

No

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

No

2.4 Current self-classification and labelling

No harmonised classification and labelling exist. Therefore self-classification has to apply. However, among more than 2000 registrants/notifiers, there is no harmonisation, as explained in chapter 3.

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

Acute Tox. 4 - H302: Harmful if swallowed

Eye Damage 1 – H318: Causes serious eye damage

2.4.2 Current self-classification and labelling based on DSD criteria

RAC general comment

Background: Toxicokinetics and read-across

Supplementing the database with read-across from structural analogues

The class of salicylic compounds has been widely studied but limited information is available for salicylic acid itself. In particular, no study could be identified specifically addressing the adverse effects of salicylic acid on sexual function and fertility in adult males and females. To overcome the information gaps, the Dossier Submitter (DS) performed a read across to salicylic acid from sodium salicylate (NaS), methyl salicylate (MeS), and o-acetyl salicylic acid (ASA). The justification provided relies on toxicokinetic data.

Short description of key information

Upon oral administration in rats, salicylic acid, MeS, NaS and ASA are all rapidly absorbed even at high concentrations. A publication by Davison (1961) has compared the oral absorption and metabolism of MeS and NaS in rats and humans with that of ASA.

Several publications demonstrated that salicylic acid is the initial metabolite (hydrolysis product) for the related salicylates: ASA, NaS, MeS. Salicylic acid is found in blood both bound to plasma albumin and as the unbound (free) moiety. Therefore, many studies use the term "plasma (serum) salicylate" as an equivalent. Unless specified otherwise, the plasma (serum) salicylate levels refer to the total (*i.e.* bound and free) concentration. This expression will be used also in this document.

Plasma analyses in rats showed rapid hydrolysis to free salicylate for MeS, NaS and ASA, resulting in comparable plasma concentrations of salicylate at 60 minutes post dosing. However, in humans hydrolysis of MeS to salicylic acid was slower and less complete. The publications of Rainsford *et al.* (1980) and Tjalve *et al*. (1973) revealed that salicylic acid was found in the stomach, liver, kidney, lungs, bone marrow, intestine, inflamed paws and spleen of rats; the *in vivo* distribution of ASA and the methyl ester of ASA (AME) were very similarly to that observed with salicylic acid. Tjalve *et al.* (1973) confirmed that there was no difference between the distribution of salicylic acid compared to ASA in mice after injection. In mice, rats, monkeys and humans, salicylic acid was found in the placenta and readily passed into the foetus.

Studies reported by Emudianughe (1988) and McMahon *et al.* (1989), both performed on rats, demonstrated that salicylic acid is metabolised to two major urinary metabolites, salicyluric acid and salicyl-glucuronic acid and oxidative metabolites (2,3- and 2,5 - dihydroxybenzoic acid) and other conjugated salicylic acid compounds (salicyl ester glucuronide or salicyl ether glucuronide). All these metabolites as well as unchanged salicylic acid are eliminated almost entirely via the urine.

In summary, the toxicokinetics of salicylic acid and the selected salicylates indicate that following absorption, the initial metabolic step for MeS, NaS and ASA is hydrolysis to free salicylate. Since salicylate is the principal species circulating in plasma at comparable concentrations, it follows that data from the selected salicylates are acceptable for read across to salicylic acid.

RAC notes that read across from close structural analogues to salicylic acid has been previously used by The Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers (SCCNFP).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Article 36 (3) of the CLP regulation states that a harmonised classification and labelling proposal can be submitted if a justification is submitted demonstrating the need for such action at EU level. There is a need for action at EU level because differences in self-classification between different notifiers in the C&L Inventory and the registration dossier have been discovered, and notifiers are not able to agree, as explained hereafter.

The C&L inventory displays 33 different C&L proposals, which have been notified by more than 2000 companies. Table 1 shows these proposals (displayed in bold and red is the one of the 2010 REACH registrants) that will lead to different risk management measures at the EU level. This table has been proposed to the C&L platform in an attempt to reach an harmonisation. However, no answer from any of these more than 2000 companies has been received as stated in background.

Table 1: SA classifications displayed in the C&L inventory

Acute Toxicity		**Cat 4 H302**				
		Cat 4 H312		No Classification		
		Cat 4 H332				
Skin corrosion/irritation		Cat 2 H315		**No Classification**		
Eye damage/irritation		**Cat 1 H318**		Cat 2 H319		No classification
Reproductive toxicity		Cat 2 H361		**No classification**		
STOT Single Exposure		Cat 2 H371		Cat 3 H335		**No Classification**
STOT Repeated Exposure		Cat 1 H372		**No Classification**		

The REACH registrants consider there is a need for harmonisation for all the endpoints where there was no agreement, in order to harmonise risk management of this substance at the European community level. This seems also very important given the broad range of uses of salicylic acid across end-users, with the substance being handled in various types of industries and being used in various product types covered by several regulations

Part B.

SCIENTIFIC EVALUATION OF THE DATA

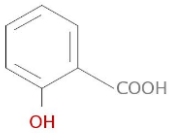
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 2: Substance identity

EC number:	200-712-3
EC name:	salicylic acid
CAS number (EC inventory):	69-72-7
CAS number:	69-72-7
CAS name:	Benzoic acid, 2-hydroxy-
IUPAC name:	2-Hydroxybenzoic acid
CLP Annex VI Index number:	None
Molecular formula:	C7H6O3
Molecular weight range:	138.1207

Structural formula:



1.2 Composition of the substance

Table 3: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
salicylic acid EC no.: 200-712-3	99.8 % (w/w)	>= 99.0 — <= 100.0 % (w/w)	No impurity >= 0.1 %

Current Annex VI entry: None

Table 4: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

Table 5: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

1.2.1 Composition of test material

The substance salicylic acid as registered is a ≥ 99 % pure substance. Therefore all the studies used have been performed on test material at most as pure as the registered substance.

1.3 Physico-chemical properties

Table 6: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	Solid	Cosmetic Ingredients Review Expert Panel (Fiume MZ) (2003), Int J Toxicol 22S3:1-108	Review of existing handbooks
Melting/freezing point	157-160 °C	Several references	Weight of evidence
Boiling point	256 °C	Verschueren K (1983), Handbook of environmental data on organic chemicals. 2nd ed, Van Nostrand Reinhold Co. Inc., New York, NY, USA	Handbook
Relative density	1.44	Three different values from reference handbooks	Handbook
Vapour pressure	at 25°C: 0.000208 hPa	Mackay D. et al. (1998), Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals : Vol IV, 550-551, CRC Lewis Publishers	Handbook
Surface tension	surface activity is not expected or predicted.		
Water solubility	**1.5 to 2.6 g/L** at 20°C or 25°C	Different values from reference handbooks	
Partition coefficient n-octanol/water	logKow: -1 to 3	Different values from reference handbooks and publications	Measured
Flash point	Flash point does not need to be carried out as the substance is a solid at room temperature (melting point 158-160 deg C).		
Flammability	Under the conditions of the procedure N1 of UN manual for burning rate measure, cannot be considered as flammable.	Keldenich HP (2010), Process and Plant Safety Laboratory, Bayer HealthCare AG	Measured

Explosive properties	does not contain any chemical groups associated with inherent explosive properties. categorised as Class St 3 for dust explosion hazard.		
Self-ignition temperature	does not need to be carried out since the melting point is 158-160 deg C		
Oxidising properties	the chemical structure indicates that the substance is incapable of reacting exothermically with combustible materials.		
Granulometry	D50: 29.9 to 50.1 µm With < 5% below 4 µm	Gras G. (2009), OSIRIS GIE. Roussillon, Service controle analytique, Rhodia	
Stability in organic solvents and identity of relevant degradation products	The conditions for criticality described in REACH Guidance R.7.1.16 are not applicable.		
Dissociation constant	pKa: ca. 3.12	Mackay D. et al. (1998), Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals : Vol IV, 550-551, CRC Lewis Publishers	
Viscosity	the study does not need to be conducted as the substance is a solid.		

2 MANUFACTURE AND USES

2.1 Manufacture

Table 7. Manufacture

Identifiers	Use descriptors	Other information
M-1: Salicylic acid	**Environmental release category (ERC):** ERC 1: Manufacture of substances **Process category (PROC):** PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 15: Use as laboratory reagent	Number of sites: 1-10

2.2 Identified uses

Table 8. Formulation

Identifiers	Use descriptors	Other information
F-5: Formulatio,	**Environmental release category (ERC):** ERC 2: Formulation of preparations **Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 5: Mixing or blending in batch processes for formulation of preparations and articles (multistage and/or significant contact)	Number of sites: 1-10 Substance supplied to that use: As such

Identifiers	Use descriptors	Other information
	PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 15: Use as laboratory reagent **Product Category formulated:** PC 12: Fertilisers PC 35: Washing and cleaning products (including solvent based products) PC 39: Cosmetics, personal care products PC 31: Polishes and wax blends PC 3: Air care products **Technical function of the substance during formulation:** Intermediates	

Table 9. Uses at industrial sites

Identifiers	Use descriptors	Other information
IW-2: Intermediate	**Environmental release category (ERC):** ERC 6a: Industrial use resulting in manufacture of another substance (use of intermediates) **Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation)	Number of sites: 1-10 Substance supplied to that use: As such Subsequent service life relevant for that use: no

Identifiers	Use descriptors	Other information
	PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 15: Use as laboratory reagent **Product Category used:** PC 19: Intermediate **Sector of end use:** SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: Manufacture of fine chemicals SU 0: Other: SU3 Industrial **Technical function of the substance during formulation:** Intermediates	
IW-3: Use for manufacture of resins	**Environmental release category (ERC):** ERC 6d: Industrial use of process regulators for polymerisation processes in production of resins, rubbers, polymers **Process category (PROC):** PROC 3: Use in closed batch process (synthesis or formulation) PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing)	Number of sites: 1-10 Substance supplied to that use: As such Subsequent service life relevant for that use: no

Identifiers	Use descriptors	Other information
	PROC 15: Use as laboratory reagent **Product Category used:** PC 19: Intermediate **Sector of end use:** SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: Manufacture of fine chemicals SU 0: Other: SU3 Industrial **Technical function of the substance during formulation:** Intermediates	
IW-4: Use for separation of salts	**Environmental release category (ERC):** ERC 6d: Industrial use of process regulators for polymerisation processes in production of resins, rubbers, polymers **Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 5: Mixing or blending in batch processes for formulation of preparations and articles (multistage and/or significant contact) PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities **Product Category used:** PC 19: Intermediate **Sector of end use:** SU 2a: Mining (without offshore industries) SU 0: Other: SU3 Industrial	Number of sites: 1-10 Substance supplied to that use: As such Subsequent service life relevant for that use: no

Identifiers	Use descriptors	Other information
	Technical function of the substance during formulation: Intermediates	
IW-10: Tyre manufacturing and retreading	**Environmental release category (ERC):** ERC 5: Industrial use resulting in inclusion into or onto a matrix **Process category (PROC):** PROC 14: Production of preparations or articles by tabletting, compression, extrusion, pelletisation PROC 21: Low energy manipulation of substances bound in materials and/or articles **Product Category used:** PC 32: Polymer preparations and compounds **Sector of end use:** SU 11: Manufacture of rubber products **Technical function of the substance during formulation:** Binding agents	Number of sites: 1-10 Substance supplied to that use: As such Subsequent service life relevant for that use: no

Table 10. Uses by professional workers

Identifiers	Use descriptors	Other information
PW-6: Use of fertilizer formulations	**Environmental release category (ERC):** ERC 8e: Wide dispersive outdoor use of reactive substances in open systems **Process category (PROC):** PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to	Substance supplied to that use: In a mixture Subsequent service life relevant for that use: no

Identifiers	Use descriptors	Other information
	vessels/large containers at dedicated facilities **Product Category used:** PC 12: Fertilisers **Sector of end use:** SU 1: Agriculture, forestry and fishing **Technical function of the substance during formulation:** Intermediates	
PW-8: Use in cleaning agents	**Environmental release category (ERC):** ERC 8a: Wide dispersive indoor use of processing aids in open systems **Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 10: Roller application or brushing PROC 11: Non industrial spraying	Substance supplied to that use: In a mixture Subsequent service life relevant for that use: no

Identifiers	Use descriptors	Other information
	PROC 13: Treatment of articles by dipping and pouring **Technical function of the substance during formulation:** Intermediates	

Table 11. Consumer uses

Identifiers	Use descriptors	Other information
C-7: Use in cosmetics	**Environmental release category (ERC):** ERC 8a: Wide dispersive indoor use of processing aids in open systems **Product Category used:** PC 39: Cosmetics, personal care products **Technical function of the substance during formulation:** Intermediates	Substance supplied to that use: In a mixture Subsequent service life relevant for that use: no
C-9: Use in cleaning agents	**Environmental release category (ERC):** ERC 8a: Wide dispersive indoor use of processing aids in open systems **Product Category used:** PC 3: Air care products PC 31: Polishes and wax blends PC 35: Washing and cleaning products (including solvent based products) **Technical function of the substance during formulation:** Intermediates	Substance supplied to that use: In a mixture Subsequent service life relevant for that use: no

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

No classification requested

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The results of studies on absorption, metabolism, distribution and elimination are summarised in the following table:

Table 12. Studies on absorption, metabolism, distribution and elimination

Method	Results	Remarks	Reference
mouse female intravenous Exposure regime: 24 hour(s) Doses/conc.: Females: 2.3 mg/kg b.w. (salicylic acid) Pregnant mice were injected i.v. with 14C-labelled salicylic acid. The distribution of radioactivity was then studied with whole-body autoradiography at different time intervals up to 24 hr after the injection.	Metabolites identified: no Evaluation of results: bioaccumulation potential cannot be judged based on study results	2 (reliable with restrictions) key study experimental result **Test material (EC name): salicylic acid**	Tjalve H, Sjostrand SE, Hansson E (1973)
rat (Sprague-Dawley) male oral: gavage Exposure regime: 180 minute(s)	Metabolites identified: yes Details on metabolites: Small quantities of salicylic acid and 2,5-dihydroxybenzoic acid were present in the blood of rats dosed with salicylic acid.	2 (reliable with restrictions) key study experimental result	Rainsford KD, Schweitzer A, Green P et al (1980)

Method	Results	Remarks	Reference
Doses/conc.: Males: 10 and 100 mg/kg The radioactively labelled substances were orally administered to rats. Then, the distribution and metabolism of radioactively labelled test substance were investigated using whole body autoradiography and chemical analyses.	Evaluation of results: bioaccumulation potential cannot be judged based on study results	**Test material (EC name): salicylic acid**	
rat (Wistar) female intraperitoneal Exposure regime: 18 day(s) Doses/conc.: 60 mg/kg (females) The metabolism of salicylic acid was investigated in pregnant rats over the whole gestational period: salicylic acid was administered via i.p. route in pregnant rat. Urine and faeces were collected at fixed days of pregnancy and analysed.	Metabolites identified: yes Details on metabolites: Analysis of the urine revealed the presence of 2 major metabolites, salicyluric acid and salicyl-glucuronic acid in addition to the free unchanged salicylic acid. Evaluation of results: bioaccumulation potential cannot be judged based on study results	2 (reliable with restrictions) key study experimental result **Test material (EC name): salicylic acid**	Emudianughe TS (1988)
rat (Fischer 344) male oral and iv Exposure regime: 96 hour(s) Doses/conc.: 5, 50 and 500 mg/kg (oral doses)	Metabolites identified: yes Details on metabolites: The administered 14C-SA was excreted as the oxidative metabolites 2,3- and 2,5 dihydroxybenzoic acid (2,3- and 2,5-diOH), unmetabolized SA, salicyl ester glucuronide, ether glucuronide (SA-PC), or the	2 (reliable with restrictions) key study experimental result **Test material (EC name): salicylic acid**	McMahon TF, Diliberto JJ, Birnbaum LS (1990)

Method	Results	Remarks	Reference
5 and 50 mg/kg (iv doses) To examine age and dose-related changes in disposition and metabolism, male Fischer 344 rats aged 3, 12 and 25 months were administered single doses of 14C-salicylic acid (14C-SA) at 5, 50 and 500 mg/kg orally and 5 and 50 mg/kg iv.	glycine conjugate salicyluric acid (SUA). Evaluation of results: no bioaccumulation potential based on study results		
rabbit oral: unspecified Exposure regime: 72 hour(s) Doses/conc.: 100 mg (excretion study) 1.5 g/kg (clearance study) Method: other: comparison of the distribution of salicylates	Metabolites identified: yes Details on metabolites: salicylate, salicyluric acid, and other conjugated salicylic acid compounds. Evaluation of results: no bioaccumulation potential based on study results	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS number): 54-21-7 (See endpoint summary for justification of read-across)**	Dalgaard-Mikkelsen S (1951)
dog (mongrel) male oral: capsule Exposure regime: once Doses/conc.: 300 mg/kg method: other: Plasma analyses in dogs after oral	Metabolites identified: yes Details on metabolites: free salicylate	3 (not reliable) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): methyl salicylate**	Davison C, Zimmerman EF, Smith PK (1961a)

Method	Results	Remarks	Reference
administration of methyl salicylate.		**(See endpoint summary for justification of read-across)**	
rat (Wistar) male oral: gavage Exposure regime: only once. Doses/conc.: 500 mg/kg, calculated as free salicylic acid. method: other: - Plasma analyses in rats after oral administration of methyl salicylate, sodium salicylate and acetylsalicylic acid.	Metabolites identified: yes Details on metabolites: The results (see table 1) showed that MeS does not produce any higher plasma or brain concentrations than NaS and ASA, and is completely hydrolyzed to free salicylate in as little as 20 minutes.	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): methyl salicylate (See endpoint summary for justification of read-across)**	Davison C, Zimmerman EF, Smith PK (1961b)
Dermal absorption study monkey (macaca mulatta (rhesus)) female Exposure regime: 14 days Doses/conc.: Females: 4 mg/cm² The chemical was administered in a small volume to a lightly clipped area of the abdomen on a single- or multiple-dose exposure. Then, percutaneous absorption and urinary elimination were investigated.		2 (reliable with restrictions) key study experimental result **Test material (EC name): salicylic acid**	Bucks DAW, Hinz RS, Sarason R et al (1990)

Method	Results	Remarks	Reference
Dermal absorption study in vitro human skin male In vitro study:Male human skin was obtained frozen from skin banks. The sample had been cascade frozen and stored at liquid nitrogen temperatures at the skin bank. These sample were stored at -90°C until the epidermis was separated. The epidermis was peeled away from the dermis after exposure to 60°C water for 80 sec. It was rapidly rinsed with hexane to remove surface lipid, rinsed with water, and placed on aluminium foil. Modified static skin diffusion cells maintained at 32°C were used. The epidermis was mounted on the lower receptor compartment by floating it on the receiver solution (0.01% aqueous gentamicin). Either, a 1 mL reservoir of saturated solutions of the loaded hydrogel discs were the donor phase. Data were analyzed by a linear least-squares fit to the steady-state region of the cumulative		2 (reliable with restrictions) supporting study experimental result **Test material (EC name): salicylic acid**	Berner B, Wilson DR, Mazzenga GC et al (1989a)

Method	Results	Remarks	Reference
amount penetrated versus time curve. Male human skin was mounted on the lower receptor compartment in a modified static skin diffusion cells. Data were analyzed by a linear least-squares fit to the steady-state region of the cumulative amount penetrated versus time curve.			
Dermal absorption study in vitro rat (fuzzy) female Coverage (dermal absorption study): in vitro Exposure regime: 24 hours Doses/conc.: dose applied on the skin 5 µg/cm² Dorsal skin of female fuzzy rats was removed and a dermatome section of 200 µm was prepared for assembly in the diffusion cells. The test compounds were applied to skin and receptor fluid was collected in 6-hr intervals for a total of 24-hr at a flow rate of 1.5 ml/hr. Percutaneous absorption was		2 (reliable with restrictions) supporting study experimental result **Test material (EC name): salicylic acid**	Bronaugh RL, Stewart RF, Storm JE (1989)

Method	Results	Remarks	Reference
measured by determining the radioactivity in the receptor fluid and skin samples at the end of the experiment. In vitro test using dorsal skin of female fuzzy rats, in a diffusion cells. Receptor fluid was collected at 6-hr intervals for a total of 24-hr at a flow rate of 1.5 ml/hr. Percutaneous absorption was measured by determining the radioactivity in the receptor fluid and skin samples at the end of the experiment.			

4.1.2 Human information

The exposure-related observations in humans are summarised in the following table:

Table 13. Exposure-related observations on basic toxicokinetics and/or dermal absorption in humans

Method	Results	Remarks	Reference
Study type: Analysis of human urine for metabolites of ASA. Details on study design: 129 healthy human volunteers Endpoint addressed: basic toxicokinetics	68.1 % of administered dose was recovered in 12 h. Major metabolite: salicyluric acid: 19.8-65% of dose Salicyl glucuronides: 0.8-42% of dose Elimination of glucuronides was inversely related to that of salicyluric acid.	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint**	Hutt AJ, Caldwell J, Smith RL (1986)

Method	Results	Remarks	Reference
	Minor metabolites: gentisic acid and salicyluric acid phenolic glucuronide: 1 & 3% of dose, respectively.	**summary for justification of read-across)**	
Study type: Pharmacokinetics of acetylsalicylic acid and salicylic acid after intravenous administration. Endpoint addressed: basic toxicokinetics	The result and plasma concentration-time curves were described by bi-exponential equation. The half-life of the first exponent was 2-5 min. while that of the second exponent was 3.5-4.5 hr.	2 (reliable with restrictions) supporting study **Test material (EC name): salicylic acid**	Rowland M, Riegelman S (1968)
Study type: Dermal absorption in humans Details on study design: 28 healthy male volunteers with mean age 29 (18-36) years Endpoint addressed: dermal absorption	SA absorption (4h): 70.8 +/- 2.5	2 (reliable with restrictions) supporting study **Test material (EC name): salicylic acid**	Yano T, Nakogawa A, Tsuji M, Noda K (1986)
Study type: Metabolism : gender differences in excretion of salicylates in man Endpoint addressed: basic toxicokinetics	The results obtained showed that the female subjects had higher capacity for salicylurate formation than the male (P <= 0.025). The urinary hourly excretion ratio of salicylurate and salicylglucuronic acid was about equal to or greater than 1 while in the male this ratio is less than 0.50. A comparison of this ratio between female and male showed a highly significant difference (P<= 0.001). The high capacity of glucuronic acid pathway in male and the alternate pathway in female suggest a possible genetic influence in salicylic acid metabolism.	2 (reliable with restrictions) supporting study **Test material (EC name): salicylic acid**	Emudianughe TS (1998)

Method	Results	Remarks	Reference
Study type: Metabolic profile for ASA and other salicylates Endpoint addressed: basic toxicokinetics	The major metabolic pathway for elimination of salicylate is via conjugation. The principal metabolite in humans is salicyluric acid. A minor oxidative pathway leads to production of 2,5-dihydroxybenzoic acid (gentisic acid, 25DHBA) and 2,3-dihydroxybenzoic acid.	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Graham GG, Roberts MS, Day RO, Rainsford KD (2004)
Study type: Oral absorption and hydrolysis in humans Details on study design: 4 men, 2 women Endpoint addressed: basic toxicokinetics	After 15 min, the mean MeS and free salicylate values were 4.9 and 7.9 mg/l, respectively. After 90 min, these values were 2.8 and 10.5 mg/l, respectively. 30% MeS remained unhydrolysed at 15 minutes, and 21% at 90 minutes.	2 (reliable with restrictions) supporting study **Test material (EC name): methyl salicylate (See endpoint summary for justification of read-across)**	Davison C; Zimmerman EF, Smith PK (1961)

4.1.3 Summary and discussion on toxicokinetics

Abbreviations used:
SA: salicylic acid
ASA: o-acetylsalicylic acid (aspirin)
MeS: methyl salicylate
AME: o-acetylsalicylic acid, methyl ester
NaS: sodium salicylate

The toxicokinetic profile of salicylic acid (SA) has been investigated in a range of studies, none of which completely fulfill all the criteria of current study protocols. Nevertheless, acceptable

information from studies on SA itself and from related salicylates (methyl ester and sodium salt) as well as o-acetylsalicylic acid (ASA) covers absorption, distribution, metabolism and elimination.

Salicylic acid is rapidly absorbed after oral administration (Rainsford et al., 1980).

Rainsford and his colleagues (1980) compared the distribution of acetylsalicylic acid (ASA), salicylic acid (SA) and the methyl ester of ASA (AME) in rats. SA was found in the stomach, liver, kidney lungs, bone marrow, intestine, inflamed paws and spleen. The AME was distributed in vivo very similarly to that observed with ASA and SA. Tjalve et al. (1973) confirmed that there was no difference between the distribution of SA versus ASA in mice after injection of these compounds. Tjalve et al. (1973) also showed that after iv administration in mice, SA was found in the placenta and readily passed into the fetuses.

A study in rats (Emudianughe, 1988) revealed two major urinary metabolites, salicyluric acid and salicyl-glucuronic acid in addition to the free unchanged SA. Additionally, the results of this study showed also no increase in the metabolism of salicylic acid in the course of the various stages of gestation in rats. In another study in rats, McMahon et al. (1989) showed that salicylic acid or its sodium salt (NaS) is metabolized to oxidative metabolites (2,3- and 2,5 -dihydroxybenzoic acid), salicylicuric acid and other conjugated SA compounds (salicyl ester glucuronide or salicyl ether glucuronide). A study in rabbits (Dalgaard-Mikkelsen., 1951) demonstrated that the rate of excretion and proportion of urinary salicylate to conjugated SA metabolites depends on urinary pH.

Salicylate is the main metabolite produced from both MeS and ASA. Small quantities of 2,5-dihydroxybenzoic acid were also present in the blood of rats dosed with salicylic acid (Rainsford, 1980). The oral absorption and metabolism of Methyl Salicylate (MeS) and NaS in rats and humans have been compared with that of ASA (Davison, 1961). In rats, MeS, NaS and ASA are all rapidly absorbed on oral administration even at high concentrations. Plasma analysis in rats showed rapid hydrolysis to free salicylate for all three compounds, resulting in comparable plasma concentrations of salicylate at 60 minutes post dosing. On the other hand in humans, hydrolysis of MeS to SA was slower and less complete.

McMahon et al. (1989) showed that SA is excreted almost exclusively in the urine. Less than 1 % was found in bile (as unmetabolized SA), as exhaled carbon dioxide or in faeces. This study reported also a shift in urinary excretion at high concentrations, towards a higher proportion of oxidative metabolites in older rats.

Taken together these results show that SA is well absorbed in several species of animal and distributed through several organ systems. It is metabolized mainly to salicyluric acid and conjugated SA compounds, with a small proportion of oxidative metabolites. These metabolites and free unchanged salicylic acid are excreted almost entirely via the urine. SA is able to pass through the placenta to reach the foetus.

In the Rainsford book on aspirin and salicylates (2004), reported in the ASA dossier, the following results were gathered:

ASA, as SA, is rapidly absorbed after oral administration (Rainsford at al., 1980), they compared the distribution of acetylsalicylic acid (ASA), salicylic acid (SA) and the methyl ester of ASA in rats.

In vivo in the rat there is uptake of aspirin and salicylate into the stomach mucosa, with the acetyl moiety of aspirin binding covalently to proteins and other molecules in the stomach wall, indicating some presystemic metabolism in the stomach in this species (Morris et al., 1973; Rainsford et al., 1983). This gastric metabolism of aspirin is consistent with its gastric toxicity (Rainsford-1980: at

least at 100 mg/kg while Thromboxane inhibition is present at 10mg /kg (Hung, 1998). The major site of presystemic metabolism of aspirin in man is in the liver (Rowland et al., 1972). There is a marked species-dependence in the binding of salicylate to serum proteins, with high binding in man, rhesus monkey, rabbit and guinea pig, while several other species, including the rat, mouse and dog, have much lower binding (Sturman and Smith, 1967). There are considerable interspecies differences in the activity of plasma aspirin esterase, with cats and rabbits showing approximately the same esteratic activity as humans while rats have a higher and dogs a lower activity than man (Morgan and Truitt, 1965).

Pharmacokinetics of aspirin (ASA) :

Unchanged aspirin can be detected in plasma for about 1 hour after its intravenous or oral administration. Following its intravenous administration in man, it has a distribution half-life of about 3 minutes, an elimination half-life of 10 minutes and a clearance of about 800 ml blood/min (Rowland and Riegelman,1968). Aspirin is hydrolysed enzymatically in blood, but its clearance in blood accounts for only about 15 per cent of the total body clearance of the drug and the bulk of the clearance is considered to occur in the liver (Rowland et al., 1972). By contrast, the clearance of aspirin in the rat is dose-dependent and at a low dose (40 mg/kg) is slightly greater than hepatic blood flow, indicating significant extra hepatic hydrolysis (Wientjes and Levy, 1988). Although these differences, rat and rabbit have some common pathways.

All theses effects indicated that it is difficult to extrapolate from animals to human, nevertheless the rabbit is more in line with Epidemiology, with 2 major points:

- Binding to proteins.

- Non-ion trapping and no accumulation of SA in embryos at morphogenesis time.

This will make the rat a non-relevant species for developmental effects evaluation for human health protection. (See exposure related observations in developmental toxicity chapter)

When comparing human and rat blood levels (for details, see Annex 2), at equivalent doses (allometric scaling factor of 4), they are higher in human blood and far higher when comparing fetal blood levels. This further indicate that abnormalities seen in rat are not seen in humans, certainly due to different factors described in toxicokinetics and reprotoxicity sections.

Discussion on absorption rate:

An *in vivo* study by Bucks et al (1990) in rhesus monkey has been chosen as key study. This demonstrated that dermal application of SA is followed by significant absorption of SA (approximately 60% of a single dose and approximately 80% for 14 days of repeated doses). Two supporting *in vitro* percutaneous absorption studies (Bronaugh et al., 1989; Berner et al., 1990) with rat and human skin showed that SA is absorbed through skin without dermal metabolism.

Short description of key information on bioaccumulation potential result:

A publication by Rainsford et al (1980) has been chosen as key study for absorption, demonstrating that SA is readily absorbed. This publication and another by Tjalve et al (1973) have been chosen as key studies for distribution, demonstrating that SA is distributed in several organ systems, including via the placenta to the foetus. Publications by Emudianughe (1988) and McMahon et al (1989) have been chosen as key studies for metabolism and elimination, demonstrating that SA is metabolized to two major urinary metabolites, salicyluric acid and salicyl-glucuronic acid and oxidative metabolites and other conjugated salicylic acid compounds. All these metabolites as well as

unchanged SA are eliminated almost entirely via the urine. A supporting study be Dalgaard-Mikkelsen (1951) demonstrated that elimination rate depends on urinary pH. Several publications also demonstrate that SA is the initial metabolite (hydrolysis product) for related salicylates (ASA, methyl acteylsalicylate, NaS, MeS). In addition to the key study by Rainsford et al (1980) a publication by Davison (1961) reporting hydrolysis of MeS to SA in humans, rats and dogs has been chosen as supporting study.

Conclusions :

Bioaccumulation potential: no bioaccumulation potential

Absorption rate - dermal (%): 60

4.2 Acute toxicity

Table 14: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Equivalent to OECD Guideline 401 (Acute Oral Toxicity)	LD50: 891 mg/kg bw	Only male rats (strain not specified) were tested. No information on test substance purity. Insufficient detail on method in report to exclude other possible deviations.	Bio-Fax (Northbrook), 1971
Weight of evidence (acute inhalation toxicity)	No adverse effect observed	only one Klimisch 3 itself is available (BioFax, 1971), on Salicylic acid administered as a dust at 0.9 mg/l. A subacute inhalation toxicity study (Gage, 1970) on methyl salicylate vapour, which supports a conclusion of low potential for systemic toxicity by inhalation.	Bio-Fax, 1971 Gage, 1970
OECD Guideline 402 (Acute Dermal Toxicity)	LD50 > 2000 mg/ kg bw	Study report in 1989, published in 1996, Kimisch 1	Bomhard E (1996) J Am Coll Toxicol, Vol. 15, Suppl. 1, p. S81

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

The results of studies on acute toxicity after oral administration are summarised in the following table:

Table 15. Studies on acute toxicity after oral administration

Method	Results	Remarks	Reference
rat male	LD50: 891 mg/kg bw (male)	2 (reliable with restrictions)	Anonymous (1971)

Method	Results	Remarks	Reference
oral, probably gavage equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)		key study experimental result **Test material (EC name): salicylic acid**	
rat (6 laboratories used Sprague-Dawley rats, 3 used Wistar rats) male/female oral, probably gavage equivalent or similar to OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	LD50: 500 — 2000 mg/kg bw (male/female)	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (IUPAC name): Sodium salicylate (See endpoint summary for justification of read-across)**	Schlede E, Mischke U, Diener W, Kayser D (1995)
rat (Wistar) male/female oral: unspecified equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD50: 1580 mg/kg bw (male) LD50: 1250 mg/kg bw (female)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): salicylic acid**	Hasegawa R, Nakaji Y, Kurokawa Y, Tobe M (1989)

4.2.1.2 Acute toxicity: inhalation

4.2.2 Summary and discussion of acute toxicity

Abbreviations used:
SA: salicylic acid

Acute oral toxicity:

A study report (Bio-Fax, 1971) has been chosen as key study for this endpoint. This study did not follow a published guideline but was similar to OECD guideline 401. It gives a LD50 = 891 mg/kg. The signs of intoxication were hypoactivity and muscular weakness. At necropsy, inflammation of gastrointestinal tract was reported in dead animals. Publications by Hasegawa et al. (1989) and Schlede et al. (1995) on NaS were chosen as supporting studies. Both were performed with a protocol similar to OECD guidelines and give LD50 of 1580 mg/kg bw and between 500 and 2000 mg/kg bw respectively. All LD50 values were therefore in the range of 500 -2000 mg/kg, demonstrating that salicylic acid is harmful via the oral route.

Conclusions :

For acute oral toxicity, a study report (Bio-Fax, 1971; Rel. 2) has been chosen as key study, reporting oral LD50 891 mg/kg in rats. Publications by Hasegawa et al (1989) and Schlede et al. (1995) on NaS (both Rel. 2) were chosen as supporting studies.

4.2.3 Comparison with criteria

Acute oral toxicity:

LD50 = 891 mg/kg from key study similar to OECD 401. : This is in the range of 300 -2000 mg/kg and therefore meets the criteria for acute toxicity category 4 according to the CLP Regulation.

4.2.4 Conclusions on classification and labelling

Acute toxicity, oral, Category 4 (Harmful if swallowed)

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Summary of the studies on acute toxicity

A study report by BioFax (1971) was chosen as key study. This study did not follow a published test guideline (TG) but it was reported as being similar to OECD TG 401. The tests were performed in male rats with salicylic acid of unspecified purity, administered (probably by gavage) at a concentration of 25% in corn oil. The result was an LD_{50} of 891 mg/kg bw. The signs of intoxication were hypoactivity and muscular weakness. At necropsy, inflammation of the gastrointestinal tract was reported in the dead animals. Publications by Hasegawa *et al.* (1989) and Schlede *et al*. (1995) on NaS were chosen as supporting studies. Both were performed with a protocol similar to OECD TGs and gave values for LD_{50} of 1580 mg/kg bw and between 500 and 2000 mg/kg bw, respectively. In summary, all LD_{50} values were in the

range 500 - 2000 mg/kg bw, demonstrating that salicylic acid is harmful via the oral route (Acute Tox. 4; H302).

Discussion

All three studies have limitations as reflected by the Klimisch score of 2. The key study and one of the supporting studies (Hasegawa *et al.,* 1989) presented experimental results obtained with salicylic acid. The second supporting study, Schlede *et al.* (1995), is an acute toxic class method assay collaborative study using sodium salicylate; the study was included because read across from sodium salicylate to salicylic acid was considered justified. The DS chose a conservative value for comparison with the classification criteria.

Comments received during public consultation

Four out of five comments supported the DS proposal, i.e. Acute Tox 4 via the oral route. One comment disagreed with the proposal and recommended no classification. The reason for the disagreement was the low reliability of the key study.

Assessment and comparison with the classification criteria

Two salicylic acid and one NaS studies (all considered as "reliable with restrictions") have been included in the assessment; all the LD_{50} values are in the range of 300-2000 mg/kg. Therefore RAC considers that the argumentation presented by the DS supports the proposal and agrees with classification of salicylic acid as **Acute Toxicity Category 4, H302** (Harmful if swallowed) according to the CLP criteria.

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

4.4 Irritation

4.4.1 Skin irritation

4.4.2 Eye irritation

Table 22: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Draize Test	Highly irritating	Klimisch 2	Sugai S et al. (1991) Journal of Toxicological Sciences, 16, 111-130

4.4.2.1 Non-human information

The results of studies on eye irritation are summarised in the following table:

Table 23. Studies on eye irritation

Method	Results	Remarks	Reference
rabbit Vehicle: unchanged (no vehicle) Draize Test	highly irritating Cornea score: ca. 54.1 of max. 80 (mean) (Time point: not applicable) (not fully reversible within: 21 days) (Draize score : The sum of value recorded for cornea was divided by the number of observation times) Conjunctivae score: 10.3 of max. 20 (mean) (Time point: not applicable) (not fully reversible within: 21 days) (Draize score: The sum of values was divided by the number of observation times.)	2 (reliable with restrictions) key study experimental result **Test material (EC name): salicylic acid**	Sugai S, Murata K, Kitagaki T, Tomita I (1991)
rabbit Vehicle: unchanged (no vehicle) Draize Test	highly irritating	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): salicylic acid**	anonymous (1971)
in vitro study bovine cornea Vehicle: MEM + 1% FBS equivalent or similar to Bovine Corneal Opacity and	highly irritating	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): salicylic acid**	Gautheron P, Dukic M, Alix D, Sin JF (1992)

Method	Results	Remarks	Reference
Permeability (BCOP) Assay			
rabbit (New Zealand White) Vehicle: unspecified Method: Draize Test	highly irritating	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): sodium salicylate (See endpoint summary for justification of read-across)**	Ohno Y, Kaneko T, Inoue T et al (1999)

4.4.2.2 Human information

No relevant information available

4.4.2.3 Summary and discussion of eye irritation

Three *in vivo* studies (Rel 2) are available for evaluation of this endpoint. The publication by Sugai et al. (1991) has been chosen as key study. The primary eye irritation potential of salicylic acid was evaluated according to Draize method. Under the conditions of this study, salicylic acid induced severe irritation not recovering within 21 days of treatment. Draize scores for cornea and conjunctivae were 54.1 and 10.3 respectively. In the study report (Bio-fax, 1971), chosen as supporting study, the primary eye irritation potential of salicylic acid was evaluated with a method similar to a Draize test. In this study, salicylic acid induced also severe irritation. Mean scores for cornea, iris and conjunctivae were 51.5, 40.3 and 38.7 at 24 hr, 48 hr and 72 hr respectively. A publication by Ohno et al (1999), a draize eye irritation test was conducted with sodium salicylate. Average scores at 24 hr after application for cornea, iris and conjunctiva were 21.7, 3.3 and 12.7 respectively. This study is also considered acceptable as a supporting study.

An *in vitro* BCOP test evaluated SA as part of a program to develop alternatives for in vivo eye irritation tests (Gautheron, 1992). Results for opacity but not permeability were reported for SA tested at up to 10%. With opacity readings: 0.1%: 7.2 +/- 1.7; 1%: 70.2 +/- 8.4; 5%: 88.2 +/- 5.1; 10%: 98.7 +/- 7.4, SA was considered a severe irritant.

Taken together the *in vitro* and *in vivo* results indicate that salicylic acid is a severe eye irritant, due crystal mechanical irritation and chemical properties.

4.4.2.4 Comparison with criteria

Under the conditions of a key study according to Draize method, salicylic acid induced severe irritation not recovering within 21 days of treatment. If, when applied to the eye of an animal, a substance produces at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days, it meets the criteria for category 1 (irreversible effects on the eye) according to the CLP Regulation.

4.4.2.5 Conclusions on classification and labelling

Salicylic acid is classified Eye Dam. 1; H318.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier submitter's proposal

Summary of the studies on eye irritation

Three *in vivo* studies (all having a Klimisch reliability score of 2) were presented for evaluation of this endpoint.

The publication by Sugai *et al*. (1991) was chosen as the key study. The primary eye irritation potential of salicylic acid was evaluated according to the Draize method. Under the conditions of this study, salicylic acid induced severe irritation not recovering within 21 days of treatment. Draize scores for cornea and conjunctivae were 54.1 and 10.3, respectively.

In the study report (BioFax, 1971), chosen as a supporting study, the primary eye irritation potential of salicylic acid was evaluated with a method similar to a Draize test. In this study, salicylic acid induced severe irritation. Mean scores for cornea, iris and conjunctivae were 51.5, 40.3 and 38.7 at 24 h, 48 h and 72 h, respectively.

In a publication by Ohno *et al.* (1999), a Draize eye irritation test was conducted with NaS. Average scores at 24 h after application for cornea, iris and conjunctiva were 21.7, 3.3 and 12.7, respectively. This publication was considered acceptable as a supporting study.

An *in vitro* Bovine Corneal Opacity/Permeability (BCOP) test evaluated salicylic acid as part of a program to develop alternatives for *in vivo* eye irritation tests (Gautheron, 1992). Results for opacity but not permeability were reported for salicylic acid tested at up to 10%. Based on the following opacity readings, salicylic acid was considered a severe irritant: 0.1%: 7.2 +/- 1.7; 1%: 70.2 +/- 8.4; 5%: 88.2 +/- 5.1; 10%: 98.7 +/- 7.4.

Comments received during public consultation

All five comments received were in favour of the proposed classification, *i.e.* Eye Damage Category 1 (causes serious eye damage). However, one of the comments recommended that the DS complete the dossier with tables showing all the findings of the studies.

Assessment and comparison with the classification criteria

The assessment is based on three *in vivo* studies (two with salicylic acid and one with NaS) and one *in vitro* study (performed with salicylic acid). The NaS study was also taken into account. Taken together, the results indicate that salicylic acid causes severe eye damage; the DS mentions the crystal mechanical irritation and chemical properties as causes for the damage. The irritation did not recover within 21 days following treatment.
RAC considers that the argumentation presented by the DS supports the proposal and agrees with the classification of salicylic acid as **Eye Damage Category 1, H318** (causes serious eye damage) according to the CLP criteria.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

4.4.3.2 Human information

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

4.5 Corrosivity

4.6 Sensitisation

4.7 Repeated dose toxicity

The repeated dose toxicity data are reported here because they are relevant for the assessment of reproductive toxicity..

Table 24: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Methyl salicylate was blended with diet and administered daily to rats for a period of 2 years.	NOAEL: 45.4 mg/kg bw (diet) Increased amount of cancellous bone LOAEL: 454 mg/kg bw (diet)	Calculated for Salicylic acid from Methyl salicylate NOAEL 50 mg/kg bw (diet) Klimisch 2	Webb WK and Hansen WH (1963) Toxicol Appl Pharmacol 5: 576-687
MeS was administered in dogs orally in capsule daily for a period of 2 years.	NOAEL: 45.4 mg/kg bw (nominal) Hepatomegaly	Calculated for Salicylic acid from Methyl salicylate NOAEL 50 mg/kg bw. Results less relevant than in rats due to small number (4) of animal tested per dose	Webb WK and Hansen WH (1963) Toxicol Appl Pharmacol 5: 576-687
Human information: Restrospective studies of children receiving salicylate therapy in the management of juvenile rheumatoid arthritis	Did not reveal any cases in which either bone lesions or hepatomegaly, as seen in rats and dogs, could be associated with massive daily doses of salicylate over prolonged periods of time	Secondary reference	Abbott DD, Harrisson JWE (1978)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

The results of studies on repeated dose toxicity after oral administration are summarised in the following table:

Table 25. Studies on repeated dose toxicity after oral administration

Method	Results	Remarks	Reference
rat (Osborne-Mendel) male/female chronic (oral: feed)	NOAEL: 50 mg/kg diet (male/female) (Increased amount of cancellous bone from 250 mg/kg)	2 (reliable with restrictions) key study read-across from supporting	Webb WK, Hansen WH (1963a)

Method	Results	Remarks	Reference
0, 0.1, 0.5, 1 and 2% (0, 50, 250, 500, and 1000 mg/kg bw/day) (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 2 years (daily) MeS was blended with diet and administered daily for a period of 2 years.		substance (structural analogue or surrogate) **Test material (CAS name): Methyl Salicylate (See endpoint summary for justification of read-across)**	
dog (Beagle) male/female chronic (oral: capsule) 0, 50, 150 and 350 mg/kg/day Vehicle: unchanged (no vehicle) Exposure: 2 years (daily for 6 days a week) MeS was administered in dogs orally in capsule daily for a period of 2 years.	NOAEL: 50 mg/kg bw/day (nominal) (male/female)	2 (reliable with restrictions) key study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): methyl salicylate (See endpoint summary for justification of read-across)**	Webb WK, Hansen WH (1963b)
rat (Osborne-Mendel) male/female subchronic (oral: feed) 0, 0.1 and 1% (0, 50, 500 mg/kg bw/day) (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 17 weeks (once/day)	NOAEL: 50 mg/kg diet (male/female)	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): methyl salicylate (See endpoint**	Webb WK, Hansen WH (1963a)

Method	Results	Remarks	Reference
MeS was blended with diet and adminstered daily for a period of 17 weeks.		**summary for justification of read-across)**	
rat (Sprague-Dawley) male/female subchronic (oral: feed) 1.2% MeS and 1.2% MeS + 0.3% Calcium carbonate (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 12 weeks (daily) The aim of this study was to investigate the effect of the addition of calcium to the diet on the appearance of bone lesions. MeS was administered in 5 rat/sex in diet at 1.2% and a group of 10 rat/sex received 1.2% MeS+ 0.3% calcium over a 12 week test period.	No detailed results given	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): Methyl salicylate (See endpoint summary for justification of read-across)**	Abbott DD, Harrisson JWE (1978)
rat (Sprague-Dawley) male/female subchronic (oral: feed) 0.2%, 0.36%, 0.63%, 1.13%, and 2.0% in the diet (equivalent to 100, 180, 320, 560 and 1000 mg/kg/day) (The animals received 50% of the dose during weeks 1 to 2, 75% of the dose during weeks 3 to 4, and 100% of the dose thereafter) (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 12 weeks (daily) The authors conducted a series of six experiments (S1-S6) to assess the body weight and	NOAEL: 180 mg/kg bw/day (nominal) (male/female)	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): Methyl salicylate (See endpoint summary for justification of read-across)**	Abbott DD, Harrisson JWE (1978)

Method	Results	Remarks	Reference
bone formation effects of MeS fed to SD rats of both sex at various dietary concentrations and exposure periods. In this study (S1), MeS was fed in the diet at levels ranging from 0.2% to 2.0% for a period of 12weeks.			
rat (Sprague-Dawley) male/female subchronic (oral: feed) 300 (0.6% in the diet); 300 (0.6% in the diet in feed portions equivalent to the 1000 mg/kg/day/group); 1000 (2.0% in the diet) Ad libitum and pair fed controls. (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 6 weeks (daily) The "bone effect" observed in s1 and s2 by Abbott and Harrisson, 1978 was accompanied by reduced food intake and body growth. To study the possible nutritional implications, groups of 10 male rats were fed 0.6%; 2% MeS on a ad libitum basis and on a paired basis for 11 weeks.	No detailed results given	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): Methyl salicylate (See endpoint summary for justification of read-across)**	Abbott DD, Harrisson JWE (1978)
rat (Sprague-Dawley) male/female subchronic (oral: feed) 2.0% in the diet, equivalent to 1000 mg/kg/day. (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 11 weeks (daily)	No detailed results given	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate)	Abbott DD, Harrisson JWE (1978)

Method	Results	Remarks	Reference
The aim of this study was to determine whether or not the bone lesions, observed in S1 were specific for MeS. Materials with chemical structures similar to that of MeS were fed in the diet as follows: 1- Methyl p-OH benzoate at 2.0% 2 -Methyl m-OH benzoate at 2.0 % 3- Acetylsalicylic acid at 2.36% 4- Sodium salicylate at 2.1% All test materials were fed at 50% of their final level during weeks 1 and 2 and at 75% during weeks 3 and 4. Each test group contained 6 rats/sex/dose.		**Test material (CAS name): Methyl salicylate (See endpoint summary for justification of read-across)**	
rat (Sprague-Dawley) male/female subchronic (oral: feed) 0.6, 0.9, 1.2 and 2.0% in the diet (equivalent to 300, 450, 600 and 1000 mg/kg/day) (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 11 weeks (daily) The results of study 1 (Abbott and Harrisson, 1978) had shown 1.13% and 2.0% of MeS in the diet to cause accumulation of cancellous bone at the metaphyses of the long bones. This bone effect was not observed at levels of 0.2%, 0.36% or 0.63%. This study was undertaken to evaluate the progression of the bone change and to determine whether or not an intermediate level between 0.6% and 1.2% wouold lead to an increase in	No detailed results given	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): Methyl salicylate (See endpoint summary for justification of read-across)**	Abbott, D.D., Harrisson, J.W.E. (1978)

Method	Results	Remarks	Reference
cancellous bone. Therefore 10/rats/dose were administred MeS at 0.6%, 0.9%, 1.2% and 2.0% in diet over a period of 11 weeks.			
rat (Sprague-Dawley) male subchronic (oral: feed) 0.6% and 2.0% in the diet (equivalent to 300 and 1000 mg/kg/day) Vehicle: unchanged (no vehicle) Exposure: 12 weeks (daily) The aim of this study was to determine whether or not ASA and / or NaS were capable of producing bone lesions at dietary levels where MeS had been found no to produce lesions. 5/males/dose were administered 0.6% , 2.0% MeS, 0.7%, 2.3% ASA and 0.7%, 2.1% NaS in the diets over a 12 week test period.		2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): Methyl salicylate (See endpoint summary for justification of read-across)**	Abbott DD, Harrisson JWE (1978)
dog (Beagle) male/female subchronic (oral: capsule) 0, 50, 100 and 167 mg/kg/day (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 6 months in duration with a 2 month recovery period. (the daily amount for each animal was given in divided doses following morning and	NOAEL: 167 mg/kg bw/day (nominal) (male/female)	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): Methyl salicylate (See**	Abbott DD, Harrisson JWE (1978)

Method	Results	Remarks	Reference
afternoon feedings; 6 days/week.) In dogs, MeS was administered in capsule form to provide doses of 0, 50, 100 and 167 mg/kg/day 6 days/week for 6 months with a 2 month recovery period.		**endpoint summary for justification of read-across)**	
dog (Beagle) male/female subchronic (oral: capsule) 0, 150, 300, 500 and 800 mg/kg/day (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 6.5-7.5 months in duration with a 6 week recovery period for 3 dogs at 300 mg/kg/day. (the daily amount for each animal was given in divided doses following morning and afternoon feedings; 6 days/week.) In dogs, MeS was administered in capsule form to provide doses of 150, 300, 500 and 800 mg/kg/day 6 days/week for 6.5 -7.5 months with a 6 week recovery period.	NOAEL: 300 mg/kg bw/day (nominal) (male/female)	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): Methyl salicylate (See endpoint summary for justification of read-across)**	Abbott DD, Harrisson JWE (1978)
dog male/female subchronic (oral: capsule) 50, 100, 250, 500, 800 and 1200 mg/kg/day Vehicle: unchanged (no vehicle) Exposure: 59 days (daily for 6 days/ weeks)	NOAEL: 250 mg/kg diet (male/female)	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate)	Webb WK, Hansen WH (1963c)

Method	Results	Remarks	Reference
MeS was administered orally in capsule form daily 6 days per week for up to 59 days.		**Test material (EC name): methyl salicylate (See endpoint summary for justification of read-across)**	

4.7.1.2 Repeated dose toxicity: inhalation

4.7.1.3 Repeated dose toxicity: dermal

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

Restrospective studies of children receiving salicylate therapy in the management of juvenile rheumatoid arthritis did not reveal any cases in which either bone lesions or hepatomegaly, as seen in rats and dogs, could be associated with massive daily doses of salicylate over prolonged periods of time. The reviews of human case histories (secondary reference, Abbott and Harrisson, 1978) suggest that the salicylate-related bone lesion in rats and hepatomegaly in dogs are not relevant for human risk assessment.

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

Abbreviations used:
SA: salicylic acid
ASA: o-acetylsalicylic acid (aspirin)
MeS: methyl salicylate
NaS: sodium salicylate

No valid repeated dose toxicity studies on salicylic acid are available. A read-across approach is therefore proposed from studies on Methyl salicylate (MeS) which is readily metabolised to salicylic acid.

Justification for Read-across from Methyl salicylate:

The oral absorption, distribution and metabolism of MeS, NaS and ASA have been compared in rats, dogs and humans (Davison, 1961). In rats and dogs, MeS, NaS and ASA were all rapidly absorbed following oral administration even at high concentrations. Absorption of MeS in humans was somewhat slower than for ASA, with total salicylate plasma concentration at 90 minutes approximately half that from ASA. Salicylic acid has also been shown to be rapidly absorbed after oral administration in rats (Rainsford at al., 1980).

Plasma analysis in rats showed rapid hydrolysis to free salicylate for MeS, NaS and ASA, resulting in comparable plasma concentrations of salicylate at 60 minutes post dosing, with no measurable parent compound. In humans, hydrolysis of MeS was slower and less complete, with 30% MeS remaining unhydrolysed at 15 minutes, and 21% at 90 minutes (Davison, 1961).

These results indicate that following absorption, the initial metabolic step for all these salicylates (MeS, NaS and ASA) is hydrolysis to free salicylate. Since free salicylate is the principal species circulating in plasma following absorption of MeS and SA, it follows that data from methyl salicylates are acceptable for read across to SA for all systemic toxicological endpoints.

Methyl salicylate Subchronic toxicity studies

Subchronic toxicity oral studies have been conducted on MeS: two in rats and two in dogs. In the 17 -week study in Osborne-Mendel rats reported by Webb and Hansen, 1963, a NOAEL of 0.1% in the diet, equivalent to ~ 50 mg/kg bw/day, was identified. Bone lesions and growth retardation were observed in rats fed MeS at 1% and 2% in the diet. The results of 6 to 12 week experiments in SD rats reported by Abbott and Harrisson (1978), suggested a NOAEL of 0.3% in the diet (180 mg/kg/bw/day) based on reduced bodyweight at 0.63%. In dogs, administered MeS orally in capsules, a NOAEL of 50 mg/kg bw/day was identified from a study of duration 59 days (Webb & Hansen, 1963) and a NOAEL of 170 mg/kg bw/day was reported from studies of duration approximately 6 months (Abbott and Harrisson, 1978). Liver enlargement and growth retardation were reported in dogs given capsules with 150 and 350 mg/kg/day of MeS. Although these studies were limited in endpoints evaluated, they were well conducted and reported (reliability: 2).

Given these results, the lowest systemic NOAEL of 50 mg/kg bw/day MeS was selected, equivalent to 45.4 mg/kg bw/day salicylic acid.

Methyl salicylate Chronic toxicity studies

Chronic toxicity studies have been conducted on MeS in rats and in dogs for 2 years (Webb and Hansen, 1963). Although the studies are relatively old and are limited in the endpoints evaluated, the protocol and results were reported in adequate detail and included hematological studies (reliability: 2)

Webb and Hansen (1963) administered methyl salicylate in rats at dietary concentrations of 0, 0.1%, 0.5%, 1.0% or 2.0% in the diet (equivalent to 0, 50, 250, 500, and 1000 mg/kg bw/day) for two years. Body weight of both sexes were significantly decreased in both the 500 and 1000 mg/kg group body weight/day groups and an increased amount of cancellous bone was present in the metaphyses in rats treated at either 500 or 1000 mg/kg body weight/day.

In dogs, the same authors administered MeS in capsule form at doses of 0, 50, 150, or 350 mg/kg body weight/day, 6 days/week for 2 years. One high dose animal died of hepatitis unrelated to MeS. Hematological analyses and necropsy examination were normal, except that dogs treated at 150 and 350 mg/kg body weight/day had enlarged livers with hepatocellular swelling. No other pathology was reported in any of the animals. Reduced body weight was reported in the 350 and 150 mg/kg body weight/day groups.

The 2 year oral toxicity data for MeS are consistent with the oral subchronic toxicity data from the same laboratory.

NOAEL value for MeS is 50 mg/kg bw/day in both rats and dogs, equivalent to 45.4 mg/kg bw/day as SA.

Human information

Restrospective studies of children receiving salicylate therapy in the management of juvenile rheumatoid arthritis did not reveal any cases in which either bone lesions or hepatomegaly, as seen in rats and dogs, could be associated with massive daily doses of salicylate over prolonged periods of time. The reviews of human case histories (secondary reference, Abbott and Harrisson, 1978) suggest that the salicylate-related bone lesion in rats and hepatomegaly in dogs are not relevant for human risk assessment.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

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

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

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

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

4.9 Germ cell mutagenicity (Mutagenicity)

4.10 Carcinogenicity

4.11 Toxicity for reproduction

Table 26: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Equivalent or similar to OECD Guideline 416 (Three-Generation Reproduction Toxicity Study)	NOAEL fertility: 225 mg/kg bw/day	Substance tested: Methyl salicylate Several currently recommended parameters were not assessed, but the study 2 years/oral/rat (Webb and Hansen, 1963 (reliability: 2) was used to supplement some observations.	Collins TFX, Hansen WH and Keeler HV (1971) Toxicol Appl Pharmacol 18:755-765
Cohort studies (retrospective) Endpoint addressed: toxicity to reproduction / fertility Endpoint addressed: developmental toxicity / teratogenicity	There is no link between o-acetylsalicylic acid use during pregnancy and reprotoxic effcts. No adverse effect of aspirin treatment can be considered as established, either at low or high dose.	Study on aspirin (o-acetylsalicylic acid) effects at therapeutic doses	Bard D. (2012), unpublished report (attached)
ICH Topic S 5(R2)	NOAEL development: 268 mg/kg bw/day	Substance tested: o-acetylsalicylic acid	Cappon GD, Gupta U, Cook JC, Tassinari MS, Hurtt ME (2003) Birth Defects Research (part B) 68:38-46

4.11.1 Effects on fertility

4.11.1.1 Non-human information

The results of studies on fertility are summarised in the following table:

Table 27. Studies on fertility

Method	Results	Remarks	Reference
rat (Osborne-Mendel) male/female three-generation study oral: feed 0, 500, 1500, 3000 and 5000 ppm (equivalent to 25, 75, 150, 250 mg/kg bw as MeS, or	NOAEL (P): 250 mg/kg bw/day (male/female) NOAEL (reproduction): 250 mg/kg bw/day (male/female) LOAEL (development): 150 mg/kg bw/day	2 (reliable with restrictions) key study read-across from supporting substance (structural	Collins TFX, Hansen WH, Keeler HV (1971) Gross MA, Fitzhugh OG (1970)

Method	Results	Remarks	Reference
22.5, 67.5, 135, 225 mg/kg bw as SA) (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 100 days before the first mating and then throughout the experiment. (once/day) equivalent or similar to OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)	NOAEL (development): 75 mg/kg bw/day	analogue or surrogate) **Test material (EC name): methyl salicylate (See endpoint summary for justification of read-across)**	
rat (Sprague-Dawley) male/female one-generation study oral: feed 4000 ppm and 6000 ppm equivalent to 200 and 300 mg/kg bw MeS, or 180, 27 mg/kg bw as SA). (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 60 days before the first mating and then throughout the experiment (daily.) equivalent or similar to EU Method B.34 (One-Generation Reproduction Toxicity Test)	NOAEL (F1): 300 mg/kg bw/day (male/female)	4 (not assignable) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): methyl salicylate (See endpoint summary for justification of read-across)**	FDA (1966) Cosmetic Ingredients Review Expert Panel (Fiume MZ) (2003)
rat (Wistar) male/female two-generation study oral: feed 0.25% and 0.5% (2500 ppm and 5000 ppm equivalent to 125 and 250 mg/kg bw MeS,	No detailed results given	2 (reliable with restrictions) supporting study read-across from supporting substance (structural	Abbott, D.D and Harrisson. J.W.E. (1978)

Method	Results	Remarks	Reference
or 113, 225 mg/kg bw as SA). (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 60 days before the first mating and then throughout the experiment (daily.) equivalent or similar to OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)		analogue or surrogate) **Test material (EC name): Methyl salicylate (See endpoint summary for justification of read-across)**	
rat (Holtzman) male/female fertility oral: feed 0.4 % in the diet, equivalent to 210 mg/kg for female and 209 mg/kg for male (nominal conc.) Exposure: Exposure period: prior to mating Premating exposure period (males): treated: 63 days prior to mating Premating exposure period (females): treated: 14 days prior to mating and up through weaning Duration of test: The dams, inseminated by treated male prior to mating, were sacrificed on day 21 of gestation (1 day prior to term). The dams, treated prior to mating, were allowed to bear and wean a single litter. (daily) To determine the effect on male fertility, groups of 20 male rats were given	NOAEL parental males : < 210 mg/kg bw/day NOAEL parental females : < 210 mg/kg bw/day NOEL (F1): < 210 mg/kg bw/day	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Schardein J.L., Blatz A.T., Woosley E.T., Kaump D.H., (1969)

Method	Results	Remarks	Reference
acetylsalicylic acid (ASA) in the diet for 2 months (63 days), and then were exposed in a 1:1 ratio (overnight) to untreated females until at least 10 inseminated females were obtained. The dams were sacrificed on day 21. All pups were dissected for determination of external and internal gross abnormalities. To assess the effect on female fertility, groups of 30 females rats were given ASA in the diet for 14 days prior to mating and through weaning. They were exposed to untreated males (overnight) in a 1:1 ratio until at least 20 animals were inseminated. These dams were allowed to bear and wean a single litter.			
mouse (CD-1) male/female one generation+ fertility oral: gavage 100, 250 and 500 mg/kg/day. (nominal conc.) Vehicle: corn oil Exposure: Task2: 7 days prior to mating then for 14 weeks of cohabitation period and 3 weeks thereafter. (daily) In this study, MeS was administered in CD-1 Mice by gavage according to the NTP continuous breeding protocol at dose levels of 100, 250 and 500 mg/kg bw/day MeS (90, 225 and 450 mg/kg bw/day as SA) for 7 days prior to mating then for 14 weeks of cohabitation and 3 weeks thereafter. The FACB consists	NOAEL (reproductive effect): 100 mg/kg bw/day NOAEL (P): 500 mg/kg bw/day	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): Methyl salicylate (See endpoint summary for justification of read-across)**	National Toxicology Program (Gulati DK, Choudhury H, Chambers R, Sabharwal (1984) Morrissey RE, Lamb IV JC, Morris RW et al (1989) Chapin RE, Sloane RA (1997)

Method	Results	Remarks	Reference
of four related tasks, not all of which are necessarily performed for a given compound. These tasks include, Task 1, dose finding; Task 2, continuous breeding phase, Task 3, identification of the affected sex and Task 4, offspring assessment.			
mouse male/female two-generation study oral: feed 0.25% and 0.5% (2500 ppm and 5000 ppm, equivalent to 357 and 714 mg/kg bw MeS, or 324 and 628 mg/kg bw as SA) (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 30 days before the first mating and then through the experiment (daily) equivalent or similar to OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)	NOAEL (reproduction): 250 mg/kg bw/day NOAEL (development): 250 mg/kg bw/day	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): Methyl salicylate (See endpoint summary for justification of read-across)**	Abbott, D.D and Harrisson. J.W.E. (1978)
mouse (CD-1) male/female two-generation study oral: gavage 0, 25, 50 and 100 mg/kg/day. (nominal conc.) Vehicle: corn oil Exposure: For 7 days prior to mating then for a 98 day cohabitation period and 21-day segregation periods. (daily)	NOAEL (F1): 100 mg/kg bw/day NOAEL (reproductive effects): 100 mg/kg bw/day	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): Methyl salicylate (See endpoint**	National Toxicology Program (Reel JR, Wolkowski-Tyl R, Lawton AD, (1984) Chapin RE, Sloane RA (1997) Morrissey RE, Lamb IV JC, Morris RW et al (1989)

Method	Results	Remarks	Reference
In this 2-generation study, MeS was administered to CD-1 Mice by gavage according to the NTP continuous breeding protocol at dose levels of 25, 50 and 100 mg/kg bw/day MeS (22.5, 45 and 90 mg/kg bw/day as SA) for 7 days prior to mating then for a 98 day cohabitation period and 21-day segregation periods. The FACB consists of four related tasks, not all of which are necessarily performed for a given compound. These tasks include, Task 1, dose finding; Task 2, continuous breeding phase, Task 3, identification of the affected sex and Task 4, offspring assessment.		**summary for justification of read-across)**	Lamb IV JC, Reel JR, Tyl R, Lawton AD (1997)

4.11.1.2 Human information

The exposure-related observations in humans are summarised in the following table:

Table 28. Exposure-related observations on toxicity to reproduction / fertility in humans

Method	Results	Remarks	Reference
Study type: cohort studies (retrospective) Endpoint addressed: toxicity to reproduction / fertility Endpoint addressed: developmental toxicity / teratogenicity	No link in pregnancy with aspirin medication.	key study **Test material (EC name): O-acetylsalicylic acid**	Bard, D (2012)
Study type: cohort study (prospective) Type of population: pregnant women Details on study design: HYPOTHESIS TESTED : To assess the value of low-dose	FINDINGS INCIDENCE / CASES - Incidence/ Number of cases for each disease / parameter under consideration:	2 (reliable with restrictions) supporting study **Test material (EC**	Rai R, Backos M, Baxter N et al (2000)

Method	Results	Remarks	Reference
aspirin (75 mg daily) in improving the livebirth rate in women with either unexplained recurrent early miscarriage or unexplained late pregnancy loss. STUDY POPULATION - Total population : not specified - Selection criteria: unexplained recurrent early miscarriage or unexplained late pregnancy loss. - Total number of subjects participating in study: 1055 - Sex/age/race: female, childbearing age, race not specified - Smoker/nonsmoker: no data - Total number of subjects at end of study: 1055 - Matching criteria: no data COMPARISON POPULATION - Type: Control or reference group - Details: pregnant women not taking aspirin HEALTH EFFECTS STUDIED - Disease(s): early or late miscarriage Endpoint addressed: toxicity to reproduction / fertility	Recurrent early miscarriage (Aspirin / no aspirin): Livebirth (%): 251 (68.4) / 278 (63.5) Median gestational age (range: weeks): 39.6 (27-41.8) / 39.5 (30.1-41.8) Median birthweight (range: kg): 3.4 (0.8-5.0) / 3.4 (1.4-4.8) Miscarriage (%): 116 (31.6) / 160 (36.5) Early miscarriage (%): 108 (93.1) / 153 (95.6) Late miscarriage (%): 8 (6.9) / 7 (4.4) No significant difference between groups Previous late miscarriage (Aspirin / no aspirin) (significance): Livebirth (%): 122 (64.6) / 30 (49.2) (P=0.03) Median gestational age (range: weeks): 38.6 (24.1-42.3)/38.4 (26.1-41.1) (NS) Median birthweight (range: kg): 3.4 (0.55-4.45) / 3.22 (0.86-4.2) (NS) Miscarriage (%): 67 (35.4) / 31 (50.8) Early miscarriage (%): 29 (43.4) / 31 (50.8) Late miscarriage (%): 38 (56.7) / 5 (16.1) (P<0.001)	**name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	

Method	Results	Remarks	Reference
Study type: cohort study (prospective) Type of population: pregnant women Details on study design: HYPOTHESIS TESTED : To test whether prenatal use of NSAIDs is associated with increased risk of miscarriage METHOD OF DATA COLLECTION - Type: Interview: - Details: Interview soon after confirmation of pregnancy STUDY PERIOD: 1998-2002 SETTING: Kaiser Permanente Medical Care program, Califormia STUDY POPULATION - Total population : - Selection criteria: Use of non-steroidal anti-inflammatory drugs (NSAIDs) including aspirin during early pregnancy - Total number of subjects participating in study: 17231 - Sex/age/race: Female - Smoker/nonsmoker: both - Total number of subjects at end of study: with miscarriage: 1554 - Matching criteria: NSAID use with or without miscarriage COMPARISON POPULATION - Type: Control or reference group:	FINDINGS INCIDENCE / CASES (NSAID use, including aspirin / non-users): Number: 1554/ 15677 Total miscarriages: 45 (2.9%) / 313 (2.0%) No. miscarriages with NSAID use 1 week before miscarriage: 3/8 OR 3.35 (95%C.I. 0.88-11.79) No. miscarriages with NSAID use 2-3 weeks before miscarriage: 5/33 OR 1.50 (95%C.I. 0.58-3.86) No. miscarriages with NSAID use 4-6 weeks before miscarriage: 18/122 OR 1.50 (95%C.I. 0.91-2.47) No. miscarriages with NSAID use 7-9 weeks before miscarriage: 16/100 OR 1.59 (95%C.I. 0.93-2.70) No. miscarriages with NSAID use 10-12 weeks before miscarriage: 3/50 OR 0.58 (95%C.I. 0.18-1.85)	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Nielsen GL (2004)

Method	Results	Remarks	Reference
- Details: NSAID users without miscarriage (15677) HEALTH EFFECTS STUDIED - Disease(s): Miscarriage Endpoint addressed: toxicity to reproduction / fertility			
Study type: cohort study (prospective) Type of population: pregnant women at risk of pre-eclampsia or intrauterine growth retardation Details on study design: HYPOTHESIS TESTED: To determine any benefits or risks to mothers and babies of low dose aspirin treatment in pregnancies at high risk of complications due to pre-eclampsia or intrauterine growth retardation (IUGR). METHOD OF DATA COLLECTION - Type: Cohort study - Details: Randomised double-blind placebo-controlled trial of low dose aspirin. Rationale for enrollment into trial: 74.4% for prophylaxis of pre-eclampsia 12% for prophylaxis of IUGR 12% for treatment of pre-eclampsia 3% for treatment of IUGR STUDY PERIOD: not stated SETTING: STUDY POPULATION	FINDINGS INCIDENCE / CASES The use of aspirin was associated with a non-significant 12% reduction in the incidence of proteinuric pre-eclampsia. There was no effect on the incidence of IUGR, stillbirth or neonatal death. Asprin significantly reduced the likelihood of preterm delivery (19.7% for aspirin versus 22.2% for control). Aspirin was not associated with a significant increase in placental haemorrhages or in bleeding during preparation for epidural anaesthesia, but there was a slight increase in the use of blood transfusion after delivery. There was no evidence of increased likelihood of bleeding or any other adverse effect in foetuses or newborn infant.	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) Collaborative (1994)

Method	Results	Remarks	Reference
- Total population: not stated - Selection criteria: women at risk of pre-eclampsia or IUGR - Total number of subjects participating in study: 9364 - Sex/age/race: female, childbearing age, race not stated - Smoker/nonsmoker: both - Total number of subjects at end of study: 4659 who received aspirin COMPARISON POPULATION - Type: Control or reference group - Details: 4650 women receiving placebo HEALTH EFFECTS STUDIED - Disease(s): perinatal outcome, congenital malformations Endpoint addressed: toxicity to reproduction / fertility			
Study type: cohort study (prospective) Type of population: pregnant women Details on study design: HYPOTHESIS TESTED : To test whether prenatal use of NSAIDs is associated with increased risk of miscarriage METHOD OF DATA COLLECTION - Type: Interview: - Details: Interview soon after confirmation of pregnancy STUDY PERIOD: 1996-1998	FINDINGS INCIDENCE / CASES (aspirin / non-users): Number: 22/ 980 Miscarriage (%): 5 (23) / 149 (15) No miscarriage (%): 17 (77) / 831 (85) Miscarriage with use from conception (%): 3 (50) / 3 (50) OR 4.3 (95%C.I. 1.3-14.2) Miscarriage with use after conception (%): 2 (14) / 12	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Li D-K, Liu L, Odouli R (2003)

Method	Results	Remarks	Reference
SETTING: Kaiser Permanente Medical Care program, California STUDY POPULATION - Total population : not stated - Selection criteria: Use of non-steroidal anti-inflammatory drugs (NSIDs) including aspirin during early pregnancy - Total number of subjects participating in study: 22 - Sex/age/race: Female - Smoker/nonsmoker: both - Total number of subjects at end of study: 22 - Matching criteria: NSAID v non-drug use COMPARISON POPULATION - Type: Control or reference group: - Details: Non-drug users (980), paracetamol users HEALTH EFFECTS STUDIED - Disease(s): Miscarriage OTHER DESCRIPTIVE INFORMATION ABOUT STUDY: Median gestational age at entry to the study: 40 days Endpoint addressed: toxicity to reproduction / fertility	(86) OR 1.1 (95%C.I. 0.3-4.5) Miscarriage with use <= 1 week (%): 3 (19) / 13 (81) OR 1.4 (95%C.I. 0.4-4.5) Miscarriage with use > 1 week (%): 2 (40) / 3 (60) OR 3.0 (95%C.I. 0.7-12.9)		
Study type: case control study (prospective) Type of population: pregnant women	FINDINGS INCIDENCE / CASES For women with miscarriage, 29% had taken aspirin during	2 (reliable with restrictions) supporting study	Keim SA, Klebanoff MA (2006)

Method	Results	Remarks	Reference
Details on study design: HYPOTHESIS TESTED: Relationship of aspirin use during pregnancy to increased risk of miscarriage. METHOD OF DATA COLLECTION - Type: Review of data from interviews - Details: information on drug use, maternal illnesses, pregnancy complications was recorded at each antenatal visit. STUDY PERIOD: 1959-1965 SETTING: The Collaborative Perinatal Project, 12 hospitals in USA STUDY POPULATION - Total population (Total no. of persons in cohort from which the subjects were drawn): approx. 54000 - Selection criteria: women who had miscarriages with use or non-use of aspirin during pregnancy - Total number of subjects participating in study: with miscarriage: 542 - Sex/age/race: female, race not stated - Smoker/nonsmoker: smoker/non-smoker (status known) - Total number of subjects at end of study: - Matching criteria: without miscarriage: 2587 - Other:	pregnancy versus 34% who had not. OR: 0.64-0.92 (95% C.I. 0.48-1.38) for individual lunar months and combinations of lunar months. STATISTICAL RESULTS There were no statistically significant differences	**Test material (EC name): O-acetylsalicylic acod (See endpoint summary for justification of read-across)**	

Method	Results	Remarks	Reference
HEALTH EFFECTS STUDIED - Disease(s): miscarriages (spontaneous pregnancy loss at less than 140 days from last menstrual period) Endpoint addressed: toxicity to reproduction / fertility			
Study type: cohort study (prospective) Type of population: pregnant women Details on study design: HYPOTHESIS TESTED: Relationship of aspirin use during pregnancy to reduced birth-weight and increased risk of perinatal death. METHOD OF DATA COLLECTION - Type: Interview - Details: information on drug use, maternal illnesses, pregnancy complications was recorded at each antenatal visit. STUDY PERIOD: not stated SETTING: The Collaborative Perinatal Project, 12 hospitals in USA STUDY POPULATION - Total population (Total no. of persons in cohort from which the subjects were drawn): 50282 - Selection criteria: use or non-use of aspirin in at least 6 (5) lunar months during pregnancy (for pregnancies lasting 8 (7) lunar months)	FINDINGS INCIDENCE / CASES Stillbirth: Rates were similar for each exposure category for the population as a whole or for black and white ethnic groups separately (no statistical significance) Incidence of stillbirth (all): - Heavy aspirin exposure: 21/1515 (1.4%) - Other aspirin exposure: 296/24866 (1.2%) - Non-exposed: 203/14956 (1.4%) Incidence of stillbirth by ethnic group: White (590): - Heavy aspirin exposure: 1.3% - Other aspirin exposure: 1.1%) - Non-exposed: 1.3% Black (883): - Heavy aspirin exposure: 0.9% - Other aspirin exposure: 1.2%)	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acod (See endpoint summary for justification of read-across)**	Shapiro S, Siskind V, Monson RR, et al (1976)

Method	Results	Remarks	Reference
- Total number of subjects participating in study: 41337 - Sex/age/race: female, race not stated - Smoker/nonsmoker: smoker/non-smoker (status known) - Total number of subjects at end of study: 26381 subjects exposed to aspirin - Matching criteria: 14956 subjects not exposed - Other: COMPARISON POPULATION - Type: Control or reference group - Details: pregnant women attending the same antenatal clinics HEALTH EFFECTS STUDIED - Disease(s): malformations in offspring Endpoint addressed: toxicity to reproduction / fertility	- Non-exposed: 1.4% Neonatal Death: Rates were similar for each exposure category for the population as a whole or for black and white ethnic groups separately (no statistical significance) Incidence of neonatal death (all): - Heavy aspirin exposure: 17/1515 (1.1%) - Other aspirin exposure: 252/24866 (1.0%) - Non-exposed: 168/14956 (1.1%) Incidence of neonatal death by ethnic group: White (590): - Heavy aspirin exposure: 1.7% - Other aspirin exposure: 0.9%) - Non-exposed: 0.8% Black (883): - Heavy aspirin exposure: 0.8% - Other aspirin exposure: 1.1%) - Non-exposed: 1.4% Birthweight: White children who were heavily exposed weighed less than non-exposed white children. However, the reverse was the case for black children (no statistical significance)		

Method	Results	Remarks	Reference
	Birthweight (g) by ethnic group (unadjusted): White (590): - Heavy aspirin exposure: 3212 g - Other aspirin exposure: 3275 g - Non-exposed: 3256 g Black (883): - Heavy aspirin exposure: 3089 g - Other aspirin exposure: 3058 g - Non-exposed: 3024 g Birthweight by ethnic group (standardised): White (590): - Heavy aspirin exposure: 3223 +/- 20.4g - Other aspirin exposure: 3268 +/- 4.6 g - Non-exposed: 3296 +/- 6.1 g Black (883): - Heavy aspirin exposure: 3074 +/- 17.0 g - Other aspirin exposure: 3047 +/- 4.6 g - Non-exposed: 3046 +/- 6.2 g STATISTICAL RESULTS There were no statistically significant differences		

Method	Results	Remarks	Reference
Study type: Analysis of 2 studies Type of population: pregnant women Endpoint addressed: toxicity to reproduction / fertility	FINDINGS INCIDENCE / CASES Neither of the studies showed a benefit of one treatment over the other (ASA or heparin). Therefore, the use of anticoagulants in this setting is not recommended. No adverse effects of ASA treatment were reported.	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acod (See endpoint summary for justification of read-across)**	Kaandorp S, Di Nisio M, Goddijin M, Middeldorp S (2009)
Study type: Meta-analysis of published studies Type of population: pregnant women Details on study design: HYPOTHESIS TESTED: Association of improved pregnancy outcome with aspirin use during moderate to high risk pregnancies. METHOD OF DATA COLLECTION - Type: other: Meta-analysis from literature review. - Details:1904 citations identified 182 studies selected for detailed review 38 of these met the inclusion criteria HEALTH EFFECTS STUDIED risk of pre-term delivery, rate of perinatal mortality	FINDINGS Miscarriage rate: Aspirin started during first or second trimester (7 studies): Risk of miscarriage: OR 0.92, 95% C.I.: 0.71-1.10 (NS) Miscarriage rate: Aspirin started during third trimester (2 studies): Risk of miscarriage: OR 1.3, 95% C.I.: 0.63-2.69 (NS) Rate of prematurity (22 studies): Aspirin v no exposure: OR 0.92, 95% C.I. 0.86-0.98 (P=0.21) Rate of prematurity with aspirin before 24 weeks (14 studies): Aspirin v no exposure: OR 0.92, 95% C.I. 0.84-1.0 (significant)	2 (reliable with restrictions) weight of evidence **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Kozer E, Costei A, Boskovic R et al (2003)

Method	Results	Remarks	Reference
OTHER DESCRIPTIVE INFORMATION ABOUT STUDY: A search of the literature was carried out for studies that involved the effects of aspirin on the outcome of human pregnancy. Controlled studies of human populations, both prospective and retrospective, were included for data analysis if they examined maternal exposure to aspirin during the second and third trimester of pregnancy and reported outcomes. Only full publications were considered. Endpoint addressed: toxicity to reproduction / fertility	Rate of prematurity with aspirin after 24 weeks (6 studies): Aspirin v no exposure: OR 0.66, 95% C.I. 0.41-1.04 (NS) Rate of prematurity with 75 mg aspirin: Aspirin v no exposure: OR 0.92, 95% C.I. 0.88-0.97 Rate of prematurity with >75 mg aspirin: Aspirin v no exposure: OR 0.55, 95% C.I. 0.31-0.99 Pregnancy duration (27 studies) Aspirin v no exposure: pregnancy about 2 days longer: 1.82 days OR 0.55, 95% C.I. 0.31-0.99 Rate of perinatal mortality (20 studies): Aspirin v no exposure: OR 0.92, 95% C.I. 0.81-1.05: No significant difference whther timing was taken into account or not, or whether dose was 75 mg or higher. Birthweight (31 studies): Aspirin v no exposure: slightly heavier, mean increase 43g, 95% C.I. 18-67g Neonatal bleeding (12 studies): Aspirin v no exposure: OR 1.03, 955 C.I. 0.85-1.25 (NS)		

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

The results of studies on developmental toxicity are summarised in the following table:

Table 29. Studies on developmental toxicity

Method	Results	Remarks	Reference
rat (Wistar) oral: gavage 75, 150 and 300 mg/kg bw/day (nominal conc.) Vehicle: CMC (carboxymethyl cellulose) Exposure: 1 week (from the 8th to 14th day of gestation) (once a day) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (maternal toxicity): 150 mg/kg bw/day NOAEL (developmental toxicity): 75 mg/kg bw/day LOAEL (developmental toxicity): 150 mg/kg bw/day NOAEL (teratogenicity): 75 mg/kg bw/day NOAEL (fetotoxicity): 75 mg/kg bw/day	2 (reliable with restrictions) key study experimental result **Test material (EC name): salicylic acid**	Tanaka S., Kawashima K., Nakaura S., Nagao S., Kuwamura T., Takanaka (1973a)
rat (Sprague-Dawley) oral: gavage 1- Single dose study: 0, 250, 500 and 625 mg/kg bw on GD9; 0, 500, 625 and 750 mg/kg on GD10; and 500, 750 and 1000 mg/kg on GD11 (nominal conc.) 2- Multiple dose study: 0, 50, 125 or 250 mg/kg bw/day (38, 96, 192 mg/kg as SA) (nominal conc.) Vehicle: 0.5% methyl cellulose Exposure: 1- for Single dose study: GD 9, 10 or 11	NOAEL (maternal toxicity): 50 mg/kg bw/day NOAEL (developmental toxicity): 50 mg/kg bw/day	1 (reliable without restriction) key study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): Acetylsalicylic acid (See endpoint summary for**	Gupta U, Cook JC, Tassinari MS, Hurtt ME. (2003)

Method	Results	Remarks	Reference
2- for multiple dose study: from day 6 to 17 of the gestation (period of organogenesis) (Single daily doses) ICH Topic S 5(R2)		**justification of read-across)**	
rabbit (New Zealand White) oral: gavage For the multiple study: 125, 250 or 350 mg/kg bw/day (96, 192, 268 mg/kg as SA) (nominal conc.) For the single study: 500, 750 and 1000 mg/kg (nominal conc.) Vehicle: methylcellulose Exposure: For the multiple study: from GD7 to GD19 For the single study: individual days, GD 9, 10 or 11 (Single daily doses) ICH Topic S 5(R2)	NOAEL (maternal toxicity): 125 mg/kg bw/day (overall effects mortality; body weight; histopathology NOAEL (developmental toxicity): 250 mg/kg bw/day (overall effects litter size and weights) NOAEL (malformations): 350 mg/kg bw/day (there were no visceral or external anomalies at all doses tested)	1 (reliable without restriction) key study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): Acetylsalicylic acid (See endpoint summary for justification of read-across)**	Cappon GD, Gupta U, Cook JC, Tassinari MS, Hurtt ME (2003)
rat (Wistar) oral: feed 0.06, 0.1, 0.2, 0.4% (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 1 week (from the 8th to 14th day of gestation) (continuously (in the diet)) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (maternal toxicity): 0.2 % NOAEL (fetotoxicity): 0.1 % NOAEL (teratogenicity): 0.1 % NOAEL (developmental toxicity): 0.1 %	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): salicylic acid**	Tanaka S., Kawashima K., Nakaura S., Nagao S., Kuwamura T., Takanaka (1973b) Tanaka S. (1974)

Method	Results	Remarks	Reference
rat (Sprague-Dawley) oral: gavage Aspirin: 50, 100 and 200 mg/kg bw/day (nominal conc.) Vehicle: 0.5% methylcellulose in water Exposure: Gestation days 7 to 17 (Once daily around 9.00 a.m.) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (Aspirin) (maternal toxicity): 100 mg/kg bw/day (nominal) NOAEL (Aspirin) (teratogenicity): 100 mg/kg bw/day (nominal) NOAEL (Aspirin) (fetotoxicity): 50 mg/kg bw/day (nominal)	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (CAS name): Acetylsalicylic acid (See endpoint summary for justification of read-across)**	Nakatsuka T. and Fujii T. (1979)
rat (Sprague-Dawley) oral: gavage 30, 90 or 180 mg/kg (nominal conc.) Vehicle: water Exposure: Day 6 through to day 15 of pregnancy (single daily doses) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (embryotoxicity/ fetotoxicity): 90 mg/kg bw/day NOAEL (teratogenicity): 30 mg/kg bw/day	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): sodium salicylate (See endpoint summary for justification of read-across)**	Fritz H., Giese K. (1990)
rat (Holtzman) oral feed or gavage 99 mg/kg (0.2 % in the diet), 224 mg/kg (0.4 % in the diet), (nominal in diet)	NOAEL (maternal toxicity): < 99 mg/kg bw/day NOAEL (teratogenicity): < 99 mg/kg bw/day	2 (reliable with restrictions) supporting study read-across from supporting substance	Schardein J.L., Blatz A.T., Woosley E.T., Kaump D.H. (1969)

Method	Results	Remarks	Reference
250 mg/kg (gavage) (nominal conc.) Exposure: day 6 through 15 of gestation (daily) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)		(structural analogue or surrogate) **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	
rat (Long-Evans) oral: gavage 500 mg/kg (nominal conc.) Vehicle: water Exposure: from 6 to 15 d of gestation (daily) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (teratogenicity): < 500 mg/kg bw/day	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Mankes R.F., Rosenblum I., Benitz K.F., Lefevre R., Abraham R. (1982)
rat (Sprague-Dawley) subcutaneous 380 mg/kg (nominal conc.) Vehicle: water Exposure: Two administrations at 2 hr interval, on day 9. (One treatment) Biochemical mechanisms of salicylate teratology were investigated: Agents were administered by s.c. injection	no NOAEL identified	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): salicylic acid**	Koshakji R.P., Schulert A.R. (1973)

Method	Results	Remarks	Reference
followed by mineral isotopes on day 9 or 16 of pregnancy in rats. Urinary excretion and fetal uptake of the mineral isotopes were measured and the fetuses (on day 20 of gestation) were removed and inspected noting death, resorption, as well as external congenital malformations.			
rabbit (New Zealand White) oral: gavage 100 mg/kg (actual ingested) Vehicle: water Exposure: 4 days (Once daily on GD 4, 5, 6 and 7) Administration of test substance to rabbits on gestation days 4 to 7.		3 (not reliable) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): sodium salicylate (See endpoint summary for justification of read-across)**	Fabro S, McLachlan JA, Dames NM (1984)
rabbit (Dutch) oral: gavage 200 and 250 mg/kg (nominal conc.) Vehicle: gum acacia Exposure: From day 6 to day 18 (at 200 mg/kg) and day 6 to day 13 (250 mg/kg) (Daily) Teratogenic potential: To assess the teratogenic effect, animals were treated by gavage during the period of organogenesis (day 6 through day 18 of gestation).	NOAEL (maternal toxicity): < 200 mg/kg bw/day NOAEL (fetotoxicity): < 200 mg/kg bw/day	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for**	Schardein J.L., Blatz A.T., Woosley E.T., Kaump D.H., (1969)

Method	Results	Remarks	Reference
		justification of read-across)	
rat and rabbit oral: gavage Oral exposure on specific gestational days during period of organogenesis, with termination just prior to normal delivery		2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Cappon GD, Cook JC, Hurtt ME (2003)

4.11.2.2 Human information

The exposure-related observations in humans are summarised in the following table:

Table 30. Exposure-related observations developmental toxicity in humans

Method	Results	Remarks	Reference
Study type: cohort study (retrospective) Endpoint addressed: toxicity to reproduction / fertility Endpoint addressed: developmental toxicity / teratogenicity	No link in pregancy with aspirin medication.	key study **Test material (EC name): O-acetylsalicylic acid**	Bard, D (2012)
Study type: cohort study (retrospective) Type of population: infants	FINDINGS: Prevalence of any maternal aspirin use was similar for	2 (reliable with restrictions)	Werler MM, Mitchell AA, Shapiro S (1989)

Method	Results	Remarks	Reference
Details on study design: HYPOTHESIS TESTED : Whether the ingestion of aspirin by women during pregnancy increases their infants' risk of certain congenital heart defects METHOD OF DATA COLLECTION - Type: Clinical tests: - Details: STUDY PERIOD: not stated SETTING: STUDY POPULATION Case groups were composed of infants with: Any structural cardiac defect (n = 1381) Aortic stenosis (n = 43) Coarctation of the aorta (n = 123) Hypoplastic left ventricle (n = 98) Transposition of the great arteries (n = 210) Conotruncal defects (n = 791) COMPARISON POPULATION - Type: Control or reference group: - Details: infants with other congenital defects HEALTH EFFECTS STUDIED - Disease(s): Heart defects Endpoint addressed: developmental toxicity / teratogenicity	cases (25 to 33 percent) and controls (27 percent). Relative risks (and 95% C.I.) among infants whose mothers were aspirin users as compared with those whose mothers did not use aspirin, adjusted for potential confounding factors, were: 0.9 (0.8 to 1.1) for any cardiac defect 1.2 (0.6 to 2.3) for aortic stenosis 1.0 (0.6 to 1.4) for coarctation 0.9 (0.6 to 1.4) for hypoplastic left ventricle 0.9 (0.6 to 1.2) for transposition of the great arteries 1.0 (0.8 to 1.2) for conotruncal defects No dose-effect pattern was identified.	supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	

Method	Results	Remarks	Reference
Study type: cohort study (prospective) Type of population: children Details on study design: HYPOTHESIS TESTED : Relationship between maternal use of aspirin during pregnancy and child's IQ at 4 years of age METHOD OF DATA COLLECTION - Type: Clinical tests: - Details: IQ tests STUDY PERIOD: 1959-1966 STUDY POPULATION - Total population : 19226 - Selection criteria: children of mothers identified in the Collaborative Perinatal Project (USA) as having taken aspirin during the first 20 weeks of pregnancy COMPARISON POPULATION - Type: Control or reference group: - Details: Children of unexposed mothers HEALTH EFFECTS STUDIED - Disease(s): Intelligence Quotient (IQ) Endpoint addressed: developmental toxicity / teratogenicity	FINDINGS The mean IQ of children exposed to aspirin was 98.3, which was 2.1 points higher than that of unexposed children (95% C.I. 1.7-2.6; P < 0.0001) This difference was reduced to one point by adjustment for confounding factors but still statistically significant.	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Klebanoff MA, Berendes HW (1988)
Study type: cohort study (prospective) Type of population: pregnant women	FINDINGS There was a statistically significant increase in incidence of only one	2 (reliable with restrictions)	Correy JF, Newman NM, Collins

Method	Results	Remarks	Reference
Details on study design: HYPOTHESIS TESTED: Association of congenital abnormalities with maternal drug use during pregnancy. METHOD OF DATA COLLECTION - Type: Questionnaire completed by physician during early antenatal period STUDY PERIOD: 1982-1989 STUDY POPULATION - Total population: 56037 - Selection criteria: All births in Tasmania, Australia HEALTH EFFECTS STUDIED - Disease(s): Congenital abnormalities Endpoint addressed: developmental toxicity / teratogenicity	specific congenital abnormality related to maternal aspirin use in pregnancy. Number of aspirin users: 1227/56037 Hypospadia: 5/1227 (0.41%) OR: 3.3 (95% C.I. 1.3-8.4) Reference group Hypospadias: 77/56037 (0.19%) All CAs: 1095/56037 (1.85%)	supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	JA et al (1991)
Study type: cohort study (prospective) Type of population: pregnant women Details on study design: HYPOTHESIS TESTED: Relationship of aspirin use to malformations in offspring METHOD OF DATA COLLECTION - Type: Interview - Details: information on drug use , maternal illnesses, pregnancy complications was recorded at each antenatal visit.	INCIDENCE / CASES - Incidence of a variety of malformation categories was not significantly increased in offspring of mothers with either heavy or intermediate aspirin use. STATISTICAL RESULTS - RR (Relative risk): Uniform malformations: heavy use: 0.95; intermediate use: 1.02; all exposed: 1.00 Major malformations: heavy use: 0.94;	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Slone S, Siskind V, Heininen OP, et al (1976)

Method	Results	Remarks	Reference
STUDY PERIOD: 1959-1965 SETTING: The Collaborative Perinatal Project in 12 hospitals in USA STUDY POPULATION - Total population (Total no. of persons in cohort from which the subjects were drawn): 50282 - Selection criteria: use or non-use of aspirin in lunar months 1-4 of pregnancy - Total number of subjects participating in study: 50282 - Sex/age/race: female, race not stated - Smoker/nonsmoker: smoker/non-smoker (status known) - Total number of subjects at end of study: 14864 subjects exposed to aspirin - Matching criteria: 35418 subjects not exposed - Other: COMPARISON POPULATION - Type: Control or reference group - Details: pregnant women attending the same antenatal clinics HEALTH EFFECTS STUDIED - Disease(s): malformations in offspring Endpoint addressed: developmental toxicity / teratogenicity	intermediate use: 1.04; all exposed: 1.01		

Method	Results	Remarks	Reference
Study type: case control study (retrospective) Type of population: pregnant women Details on study design: HYPOTHESIS TESTED: Association of congenital abnormalities with maternal aspirin use during weeks 5 to 12 of gestation. METHOD OF DATA COLLECTION - Type: Statistical analysis of Hungarian Case Control Surveillance of Congenital Abnormalities STUDY PERIOD: 1980-1986 STUDY POPULATION - Total population: 3415 - Selection criteria: children from database with specific congenital abnormalities COMPARISON POPULATION - Type: children from same database with other congenital abnormalities - 19428 children HEALTH EFFECTS STUDIED - Disease(s): Congenital abnormalities: neural tube defects, exomphalos/gastroschisis, cleft lip/palate Endpoint addressed: developmental toxicity / teratogenicity	FINDINGS There were no statistically significant increases in incidence of specific congenital abnormalities related to maternal aspirin use in weeks 5 to 12 of pregnancy. Cases with selected types of CA: maternal aspirin use/total (%): Neural tube defects: 25/1202 (2.1%) Exomphalos/Gastroschisis: 3/238 (1.3%) Cleft lip/palate: 28/1374 (2.0%) Posterior cleft palate: 12/601 (2.0%) Reference group (all other CAs): 272/19428 (4.0%) Unadjusted odds ratio: Neural tube defects: OR: 1.5; 95% C.I. 0.9-2.3) Exomphalos/Gastroschisis: OR: 0.9; 95% C.I. 0.3-2.8 Cleft lip/palate: OR: 1.5; 95% C.I. 0.9-2.2 Posterior cleft palate: OR: 1.4; 95% C.I. 0.8-2.6 Odds ratio adjusted for maternal age, parity, use of folic acid, nausea/vomiting, cold/influenza: Neural tube defects: OR: 1.1; 95% C.I. 0.7-1.6)	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Nørgård B, Puhó E, Czeizel AE et al (2005)

Method	Results	Remarks	Reference
	Exomphalos/Gastroschisis: OR: 0.7; 95% C.I. 0.2-2.2 Cleft lip/palate: OR: 0.9; 95% C.I. 0.6-1.3 Posterior cleft palate: OR: 1.0; 95% C.I. 0.6-1.8		
Study type: cohort study (prospective) Type of population: pregnant women at risk of pre-eclampsia or intrauterine growth retardation Details on study design: HYPOTHESIS TESTED: To determine any benefits or risks, expressed in early childhood, of low dose aspirin treatment in pregnancies at high risk of complications due to pre-eclampsia or intrauterine growth retardation. METHOD OF DATA COLLECTION - Type: Questionnaire - Details: Questionnaire-based follow-up of prticipants in randomised double-blind placebo-controlled trial of low dose aspirin. STUDY PERIOD: not stated SETTING: STUDY POPULATION - Total population: 9364 - Selection criteria: random - Total number of subjects participating in study: 9364 - Sex/age/race: not stated	FINDINGS INCIDENCE / CASES There were no clear differences in any of the main outcome measures (hospital visits during the first 18 months for congenital malformations, motor deficit, developmental delay, respiratory problems, bleeding problems, height or weight below the third centile, delayed acquisition of certain developmental skills.	2 (reliable with restrictions) supporting study **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	CLASP Collaborative Group (1995)

Method	Results	Remarks	Reference
- Smoker/nonsmoker: both - Total number of subjects at end of study: 4365 COMPARISON POPULATION - Type: Control or reference group - Details: children of mothers receiving placebo HEALTH EFFECTS STUDIED - Disease(s): perinatal outcome, congenital malformations Endpoint addressed: developmental toxicity / teratogenicity			
Study type: Meta-analysis of published studies Type of population: pregnant women Details on study design: HYPOTHESIS TESTED: Association of increased risk of congenital malformation with aspirin use in first trimester of pregnancy. METHOD OF DATA COLLECTION - Type: other: Meta-analysis from literature review. - Details:1902 citations identified 180 studies selected for detailed review 22 of these met the inclusion criteria: 15 case-control studies 6 cohort studies 1 randomised control trial.	FINDINGS Overall rate of congenital malformations: All studies, the risk was not significantly higher in the offspring of women exposed to aspirin (OR: 1.33, 95% C.I. 0.94-1.89) Case-control studies alone showed a higher risk of malformations for aspirin exposure (OR: 1.64; 95% C.I. 1.30-2.04) Cohort and randomized control studies (all) indicated no increased risk (OR: 1.03; 95% C.I. 0.94-1.13) Cohort and randomized control studies excluding the results of Slone et al (1976) showed no statistically significant increased risk (OR: 1.72; 95% C.I. 0.69-4.3)	2 (reliable with restrictions) weight of evidence **Test material (EC name): O-acetylsalicylic acid (See endpoint summary for justification of read-across)**	Kozer E, Shekoufeh N, Costei A et al (2002)

Method	Results	Remarks	Reference
HEALTH EFFECTS STUDIED Congenital abnormalities, overall and/or specific defects OTHER DESCRIPTIVE INFORMATION ABOUT STUDY: A search of the literature was carried out for studies that involved the effects of aspirin on the outcome of human pregnancy. Controlled studies of human populations, both prospective and retrospective, were included for data analysis if they examined maternal exposure to aspirin during the first trimester of pregnancy and reported malformations. Only full publications were considered. Endpoint addressed: developmental toxicity / teratogenicity	Specific defects: Gastroschisis (5 case-control studies): higher risk in exposed infants (OR: 2.37; 95% C.I. 1.44-3.88) CNS defects (3 case-control, 1 cohort studies): no significant increase (OR: 1.39; 95% C.I. 0.89-2.16) CNS defects (3 case-control studies only): small but significant increase (OR: 1.68; 95% C.I. 1.23-2.30) Neural tube defects (3 case-control studies): non-significant increase (OR: 2,2; 95% C.I. 0.93-5.17) Congenital heart defects (4 case-control, 2 cohort studies): no increase (OR: 1.01; 95% C.I. 0.91-1.12) Cleft palate (2 case-control studies): significant increase (OR: 2.87; 95% C.I. 2.04-4.02) Hypospadias (2 cohort studies): higher risk from one study, but no increased risk from analysis of both together (OR: 1.82; 95% C.I. 0.58-5.72)		

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

Abbreviations used:
SA: salicylic acid

ASA: o-acetylsalicylic acid (aspirin)
MeS: methyl salicylate
NaS: sodium salicylate

Effects on fertility, animal data

No fertility studies are available on salicylic acid. Assessment of the potential of SA to impair fertility has therefore been based on read-across data from studies on Methyl salicylate (MeS) and Acetylsalicylic acid (ASA). The read-across approach is considered acceptable since the initial step in the metabolism of these salicylate compounds is hydrolysis to free salicylate (see Toxicokinetics, 4.1).

The potential for MeS to affect male and/or female fertility has been assessed in rats and mice in several multi-generation studies, with the study by Collins et al (1971) considered to be the key study for this endpoint.

In a 3-generation study (Collins et al., 1971), MeS was administered to male and female Osborne-Mendel rats in the diet at 500, 1500, 3000 and 5000 ppm (equivalent to 22.5, 67.5, 135, 225 mg/kg bw as SA). Parental generation rats were fed MeS for 100 days prior to mating, then throughout two mating, gestation and lactation periods. F1 and F2 rats received the test compound throughout the study, which terminated with the weaning of the F3 offspring. No statistically significant decrease was reported in fertility index at any dose for any generation. Adverse effects were reported on offspring, representing embryo-foetal toxicity primarily in terms of reduced viability (decreases in litter size, number of live-born progeny, number of survivors to PND4 and PND5 and number of survivors to weaning).

In a 2-generation study (Abbott & Harrisson, 1978), male and female Wistar rats received MeS in the diet at 2500 and 5000 ppm (equivalent to 113 and 225 mg/kg bw/day as SA) for 60 days prior to mating, then throughout the study. Each generation of rats was mated twice. This study reported a non-significant decrease in mating performance for the first generation, and reduced viability of pups.

Abbott and Harrisson also reported data on male and female mice exposed to MeS in the same manner at the same dietary concentration as rats (2500 and 5000 ppm (equivalent to 324 and 648 mg/kg bw/day as SA) from 30 days prior to mating. No adverse effects were reported on any reproductive parameter.

Two 2-generation studies have been conducted on MeS in CD-1 Mice by gavage according to the NTP continuous breeding protocol (NTP, 1984a, 1984b). At dose levels of 25, 50 and 100 mg/kg bw/day MeS (22.5, 45 and 90 mg/kg bw/day as SA) for 7 days prior to mating then for a 98 day cohabitation period, no effects were reported on fertility, number of pups per litter, percentages of live pups or pup weight. Necropsy of F_1 mice revealed no effects on body or organ weights or sperm motility, density or morphology. In a second study at the higher dose levels of 100, 250 and 500 mg/kg bw/day (90, 225 and 450 mg/kg bw/day as SA) there was no effect on fertility index. Reduced pup viability was reported at the high dose, with only a 3% reduction in pup weight at the mid dose level.

As conclusions on above studies, no statistically significant effect on fertility was reported in any study. Reduced embryo-foetal viability was reported at high maternally toxic dose levels, when parental toxicity refers to the systemic NOAELs.

The potential for effects on male and female fertility from ASA was reported by Schardein et al. (1969). This study used only a single dose level of 210 mg/kg ASA (161 mg/kg bw as SA) by oral

gavage as positive control. Male rats were treated for 63 days prior to mating with untreated females. Female rats were treated for 14 days prior to mating with untreated males and up to weaning. ASA did not significantly affect male or female fertility at this dose which caused moderate bodyweight depression in males and severe bodyweight depression in females.

The studies above show a number of deficiencies in relation to current guidelines in terms of parameters studied, however their results are consistent. In addition, 2-year chronic toxicity studies (Webb, 1963) in rats and dogs showed no abnormalities in sexual organs (testes/prostate or ovaries/uterus).

The adverse effects on reduced viability of offspring reported primarily in rats represent developmental toxicity rather than reduction of the fertility of either male or female animals. It can therefore be concluded that SA is not likely to have any significant adverse effect on fertility.

Developmental toxicity, animal data

RAT

The effects of salicylic acid, acetylsalicylic acid or sodium salicylate on organogenesis have been investigated in a large number of studies in several animal species, using a variety of protocols. Many are mechanistic studies, using a single, often high, dose on a restricted number of gestation days. Relatively few are comparable to the prenatal developmental toxicity study OECD guideline 414. For Salicylic acid (SA) itself, two studies in rat (Tanaka et al, 1973a and Tanaka et al 1973b) are acceptable as key studies, although SA was administered only from GD8 to GD14 and there was little information on true maternal toxicity (only effect on growth). To complement these studies and to provide key data on developmental toxicity in the rabbit, two recent developmental toxicity studies on read-across substance Acetylsalicylic acid (ASA, aspirin) in rats (Gupta et al, 2003) and rabbits (Cappon et al, 2003) have been included as key studies. These studies complied with current ICH guidelines for pharmaceuticals.

In a pre-natal developmental toxicity study (Tanaka et al., 1973a), salicylic acid was administered to pregnant Wistar rats at levels of 0.06,0.1, 0.2 and 0.4 % in the diet (30, 50, 100, 200 mg/kg bw/day) on GD 8-14. The high dose of 0.4% caused maternal toxicity, high foetal mortality, growth retardation and a high frequency of complex anomalies including cranioschisis, myeloschisis, pes varus, and oligodactyly. At 0.2%, significant foetal growth retardation and a low frequency of anomalies were observed. No effect levels were NOAEL (maternal): 0.2% (100 mg/kg bw/day) and NOAEL (development): 0.1% (50 mg/kg bw/day). A parallel study by gavage (Tanaka, 1973b) at 75, 150 and 300 mg/kg bw gave similar results, with no effect levels NOAEL (maternal): 150 mg/kg and NOAEL (development): 75 mg/kg bw.

In an experimental segment II study, ASA was administered by oral gavage to pregnant Sprague-Dawley rats at 50, 125 or 250 mg/kg bw/day (equivalent to 38, 96, 192 mg/kg bw as SA) during organogenesis (GD 6 -17) (Gupta & al, 2003). There was a dose-related reduction in maternal bodyweight gain, significant in the mid and high dose groups. At 250 mg/kg bw/day, ASA induced increases in early resorptions, increased post-implantation loss, increased variations and malformations. At 125 mg/kg, foetal viability was reduced.

A number of valid supporting studies in rats report similar results to those described in the key rat studies above. Fritz and Giese (1990), showed a marked increase in embryonic and foetal mortality, delayed ossification and malformations at 180 mg/kg NaS on GD 6-15. Nakatsuka and Fujii (1979), treated SD rats with ASA on GD 7-17. At 200 mg/kg the number of resorptions and

malformed survivors were significantly increased. At 100 and 200 mg/kg the average body weights were significantly reduced in a dose-related manner. Schardein et al. (1969) showed ASA to be embryotoxic to rats fed doses of 250 mg/kg bw/day by gavage, or 0.2 or 0.4% (99 or 240 mg/kg bw/day) in the diet on GD 6-15. These doses caused significant reduction in maternal bodyweight gain. At 240 or 250 mg/kg ASA, all pups were resorbed. There were a number of skeletal malformations in the pups at 99 mg/kg bw/day.

The results of the key and supporting studies in rats demonstrate that SA has an embryofoetotoxic effect in rats at doses causing clear maternal toxicity in systemic assays, with evidence of malformations only at high maternally toxic doses.

Potential for peri- and post-natal developmental toxicity has been reported in IUCLID section 7.8.1 under the multi-generation studies on the read-across substance Methyl salicylate (MeS) and in IUCLID section 7.8.3, under segment III studies on aspirin (ASA). As described in these sections, high doses of salicylate increased perinatal mortality in rats, but did not affect growth or development of survivors.

In summary of developmental toxicity, SCCNFP published an opinion on Salicylic Acid in 2003, giving a threshold of 75 mg/kg/d in rat. In a further opinion on homosalate, SCCP (2005) indicated no teratogenic effect of Salicylic Acid, based on a report (Roberts, 2005, ref. 55).

RABBIT

ASA was administered by oral gavage to pregnant New Zealand White rabbits at 125, 250 or 350 mg/kg bw/day on GD7-19 (Cappon & al, 2003). Maternal body weight gain was significantly reduced in the mid and high dose groups from GD7 to GD13. Food consumption was also reduced in these groups. Three high dose does and one mid dose doe died during the study. There were no treatment-related effects on corpora lutea, implantation sites, pre-implantation losses or embryofoetal mortality. There were no treatment-related visceral or external anomalies. Reduction in mean foetal weight at 350 mg/kg bw/day was the only developmental adverse effect reported at this maternally toxic dose.

In a supporting study (Schardein et al, 1969), rabbits received ASA at 200 or 250 mg/kg on GD 6-13 or GD 6-18. ASA induced maternal toxicity but no skeletal malformations or other effects on offspring.

SPECIES RELEVANCE FOR HUMAN DEVELOPMENTAL TOXICITY ASSESSMENT

It became clear that there are differences in sensitivity between the tested species. Based on developmental toxicity studies equivalent to OECD guideline 414, the rabbit is seen to be considerably less sensitive than the rat to the developmental toxicity of SA and other salicylates. In the multi-generation studies equivalent to OECD guideline 416, it was also seen that the mouse was less sensitive than the rat in this regard..

When analysing the ASA data, it was evident from the metabolism (Rainsford, 2004) that the rabbit is more human-like with high protein binding capacity in contrast to the rat with a low one. In fact, in the rabbit (Cappon, 2003) there is no prenatal loss or teratogenic effect at 350 mg/kg/d, a distinctly maternally toxic dose. This is consistent with the review of epidemiological data in humans by Pr D. Bard who concluded to no potential link of adverse developmental outcome with ASA medication.

Moreover, when comparing human and rat blood levels (for details, see Annex 2), they are comparable at equivalent doses (if the allometric scaling factor is taken in account), while they are

higher in human at the identical dose on a mg/kg bw basis and even higher in human fetuses when comparing fetal blood levels. This further indicates that abnormalities seen in rats are not seen in humans at even higher internal exposure, certainly due to different factors, which were described in the toxicokinetics (e.g. protein binding) and reprotoxicity sections.

There are some obvious differences between species for developmental effects (for details, see Annex 3), the rat being very sensitive and/or not considered relevant for human, based on the data on species differences and limited information on true maternal toxicity. As such the maternal NOAEL found in developmental toxicity reports are not in line with the lower general repeated toxicity NOAEL, nor with the known ulcerogenic activity of ASA in rat and human.

This gives a weight of evidence that the rat is not a relevant species to extrapolate developmental effects to humans. Results showing that the bone effects seen in rats are in contradiction with Human juvenile arthritis treatment (Abbott and Harrisson, 1978) and the epidemiological restrospective evaluation of human data of Pr. D. Bard (2012) support this conclusion.

Key information on effects on both fertility and development from human information

Human information generally does not dissociate information on Fertility and on Developmental Toxicity, and is therefore presented here.

Human experience with salicylic acid is limited to industrial exposure and its use in cosmetology, but as it is a major metabolite of acetylsalicylic acid, a molecule with a long experience in humans ASA can be used in read across, although salicylic acid does not bear the anti-thrombotic property of acetylsalicylic acid.

Apart from its acute oral toxicity, o-acetylsalicylic acid (aspirin) is not classified and the major part is sold as an over the counter (OTC) drug.

Well-designed epidemiological studies (Slone, 1976; Shapiro, 1976; Kozer, 2002) on the use of aspirin at up to the maximum recommended therapeutic dose of 4000 mg/day (equivalent to 66.7 mg/kg bw/day as ASA or 56 mg/kg/day as MeS) have largely demonstrated an absence of increased risk of adverse pregnancy outcome in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay, despite widespread self-administration of aspirin during pregnancy. A meta-analysis of studies on the use of low-dose aspirin at 50-150 mg/day (Kozer, 2003) has demonstrated that this dose range is not associated with any adverse pregnancy outcomes, in terms of perinatal mortality, birth complications, congenital malformations or adverse effect on subsequent development. For pregnancies where there was moderate or high risk of pre-eclampsia and/or premature delivery, adverse pregnancy outcome rate was reduced with low-dose aspirin. There was no increased risk of early miscarriage with this dose regime. These data have been reviewed and completed by an Epidemiologist expert (Pr. D. BARD report to Novacyl, 2012, document attached) with a conclusion of no link between ASA use during pregnancy and deleterious effects at low and high human doses. "Low dose" relates to antithrombotic regular use, while "high dose" refers to antalgic more sporadic use.

Therapeutic doses can be compared with the natural exposure trough food to salicylates: the values for salicylate in foods recorded comprise a range from about 20 mg to 300 mg/day in Western diets. This is of the same order of magnitude as the challenge dose of salicylate used in clinical studies, usually a 300-500 mg aspirin tablet. The usual adult pharmacological dose of aspirin for acute uses is 600 -1000 mg (two tablets) at a time, often several times a day and 60 to 360 mg/day for chronic uses, so that it is difficult to see how the food consumption could have similar effects to salicylate medication in sensitive individuals

There is a large set of publications indicating the benefit of daily o-acetylsalicylic acid low doses to improve cardiovascular diseases and cancers. As such, with more than one-century of use, o-acetylsalicylic acid human experience, with known upper limits, had proven its safety for Human health. This is certainly why salicylic acid, together with other salicylates, is now approved as flavouring ingredient quantum satis (Regulation EU No 872/2012 of 01/10/2012).

Discussion:

As a final conclusion on reprotoxicity data evaluation, no adverse effect of aspirin treatment can be considered as established during pregnancy, either at low (150 mg daily) or higher usual dose. Low-dose aspirin for prevention of pre-eclampsia and associated adverse outcome may be modestly effective, although some uncertainties remain on the time window bringing such benefit with respect to possible adverse effects, e. g. mother or infant bleeding (Benefit in case of thrombosis). Humans are exposed to therapeutic doses (up to 5g /day for 5 days as analgesic or anti-pyretic and up to 360 mg /d for long term use for anti-thrombotic effects), far above potential occupational use or exposures. O-acetylsalicylic acid is not restricted during the 1st trimester of pregnancy when morphogenesis is occurring. The recommendation for non-use in pregnancy relates to the 3^{d} trimester due to a possible risk of bleeding based on the antithrombotic effects, although low-dose aspirin has been shown to have beneficial effects on women who are at risk for pregnancy-induced hypertension and preeclampsia (hypertension plus proteinuria or edema) and on their offspring (Helms, 2009, cited in Bard, 2012).

This absence of any clear evidence of adverse effects from aspirin on human development demonstrated in well-designed epidemiological studies despite widespread prescribed use and self-medication with aspirin at all stages of pregnancy over a period spanning several decades appears to indicate that humans are considerably less sensitive than rats to the developmental toxicity of salicylate, which is confirmed in mouse (NTP, 1984) and rabbit (Cappon, 2003).

Overall, it can be concluded that salicylic acid does not adversely affect fertility and that the developmental toxicity reported in the rat is of very questionable significance/ relevance for humans.

4.11.5 Comparison with criteria

Adverse effects on sexual function and fertility

Results in animal studies

No fertility studies are available on salicylic acid itself. Assessment of the potential of salicylic acid to impair fertility has been based on read-across data from published data on related salicylates. The key study for this endpoint is a 3-generation reproductive toxicity study in Osborne-Mendel rats on Methyl salicylate (Collins et al, 1971). No statistically significant decrease was reported in fertility index at any dose for any generation. Adverse effects were reported on offspring, representing embryo-foetal toxicity primarily in terms of reduced viability.

Reduced embryo-foetal viability was reported at high maternally toxic dose levels, when parental toxicity refers to the systemic NOAELs: the NOAEL fertility = 225 mg/kg bw/day is distinctly higher than the chronic NOAEL of 45.4 mg/kg bw/day and indicates no special sensitivity with respect to reproductive performance.

This means that there is no effect on fertility at doses that show no chronic general toxicity.

Evidence from humans

A weight of evidence was based on above animal studies, and human information, which supports the results in animal studies.

Well-designed epidemiological studies (Slone, 1976; Shapiro, 1976; Kozer, 2002) on the use of aspirin at up to the maximum recommended therapeutic dose of 4000 mg/day (equivalent to 66.7 mg/kg bw/day as Acetylsalicylic acid or 56 mg/kg/day as MeS) have largely demonstrated an absence of increased risk of adverse pregnancy outcome in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay, despite widespread self-administration of aspirin during pregnancy. A meta-analysis of studies on the use of low-dose aspirin at 50-150 mg/day (Kozer, 2003) has demonstrated that this dose range is not associated with any adverse pregnancy outcomes, in terms of perinatal mortality, birth complications, congenital malformations or adverse effect on subsequent development. For pregnancies where there was moderate or high risk of pre-eclampsia and/or premature delivery, adverse pregnancy outcome rate was reduced with low-dose aspirin. There was no increased risk of early miscarriage with this dose regimen.

These data have been reviewed and evaluated by an Epidemiologist (Pr. D. BARD report to Novacyl, 2012, key study) with a conclusion of no link between Acetylsalicylic acid use during pregnancy and reprotoxic effects.

Overall, it can be concluded that Acetylsalicylic acid, and its metabolite, salicylic acid, do not adversely affect fertility. Therefore the substance does not meet criteria for reproductive toxicity category 1 or 2 (i.e. evidence from humans or animal studies for effects on sexual function and fertility).

Adverse effects on development of the offspring.

Results in animal studies

For Salicylic acid (SA) itself, two studies in rat (Tanaka et al, 1973a and Tanaka et al 1973b) are acceptable as key studies, although SA was administered only from GD8 to GD14 and there was little information on true maternal toxicity (only effect on growth reported). To complement these studies and to provide key data on developmental toxicity in the rabbit, two recent developmental toxicity studies on read-across substance Acetylsalicylic acid (ASA, aspirin) in rats (Gupta et al, 2003) and rabbits (Cappon et al, 2003) have been included as key studies.

The effect of ASA on development has been studied in rats, mice and rabbits with results leading to the conclusion that there are considerable species differences in sensitivity, with the rat being a specifically sensitive species. Data on the effect of aspirin (ASA) in human pregnancy (Bard, 2012) has been used to assess the relevance of the animal data for risk assessment. These data indicate that humans are far less sensitive than rats to the effect of ASA and more comparable to rabbits in several points including ADME or protein binding. Results from all studies showed that acetyl salicylic acid is embryotoxic at medium maternally toxic doses and induces malformations at high maternally toxic doses.

This made a weight of evidence that the rat is not a relevant species to extrapolate developmental effect to humans. This is supported by results showing that the bone effects seen in rat are in contradiction with Human juvenile arthritis treatment (Abbott and Harrisson, 1978).

For effects in rabbits, the key study is Cappon et al (2003). There were no adverse effects on development at doses not causing severe maternal toxicity: the NOAEL development = 268 mg/kg bw/day and the maternal of 96 mg/kg/d is higher than the chronic NOAEL of 45.4 mg/kg bw/day.

Thus there is no effect on development of the offspring at doses that show no chronic general toxicity.

Evidence from humans

A weight of evidence was based on above animal studies, and human information, which supports the results in animal studies.

As introduced in chapter « Fertility », well-designed epidemiological studies (Slone, 1976; Shapiro, 1976; Kozer, 2002) on the use of aspirin at up to the maximum recommended therapeutic dose of 4000 mg/day (equivalent to 66.7 mg/kg bw/day as Acetylsalicylic acid or 51 mg/kg/day as SA) have largely demonstrated an absence of increased risk of adverse pregnancy outcome in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay, despite widespread self-administration of aspirin during pregnancy. A meta-analysis of studies on the use of low-dose aspirin at 50-150 mg/day (Kozer, 2003) has demonstrated that this dose range is not associated with any adverse pregnancy outcomes, there was no increased risk of early miscarriage with this dose regime. These data have been reviewed and evaluated by an Epidemiologist (Pr. D. BARD, 2012, key study) with a conclusion of no link between Acetylsalicylic acid use during pregnancy and reprotoxic effects.

This absence of any clear evidence of adverse effects from aspirin on human development demonstrated in well-designed epidemiological studies despite widespread prescribed use and self-medication with aspirin at all stages of pregnancy over a period spanning several decades appears to indicate that humans are considerably less sensitive than rats to the developmental toxicity of salicylate, which is confirmed in mouse (NTP, 1984) and rabbit (Cappon, 2003).

Conclusion

Overall, it can be concluded that Acetylsalicylic acid, and therefore, Salicylic acid, does not adversely affect development of offspring, and that the developmental toxicity reported in the rat is of no relevance for humans. Therefore the substance does not meet the criteria for reproductive toxicity category 1 or 2 (i.e. evidence from humans or animals relevant for toxicity assessment in humans, for effects on development).

4.11.6 Conclusions on classification and labelling

Not classified for effects on reproduction (fertility) according to CLP criteria.

Not classified for effects on reproduction (development) according to CLP criteria.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The animal data on fertility and development presented by the DS was sufficient to evaluate both endpoints. With respect to human data, the information was not separated between the two endpoints and the DS presented them together.

Effects on fertility, animal data

The assessment of salicylic acid is based on read-across data from studies on MeS and ASA. The studies used in the assessment are summarised in the table below:

Summary of the fertility studies taken into assessment

Study design, test material, species	***Doses***	***Conclusions***
3-generation study (Collins *et al*., 1971), MeS, male and female Osborne-Mendel rats	500, 1500, 3000 and 5000 ppm (equivalent to 22.5, 67.5, 135, 225 mg/kg bw/d as salicylic acid) in the diet	No statistically significant decrease in fertility index was reported at any dose for any generation.
2-generation study (Abbott & Harrisson, 1978), MeS, male and female Wistar rats	2500 and 5000 ppm (equivalent to 113 and 225 mg/kg bw/d as salicylic acid) in the diet	Non-significant decrease in mating performance for the first generation.
2-generation study (Abbott & Harrisson, 1978), MeS, male and female mice	2500 and 5000 ppm (equivalent to 324 and 648 mg/kg bw/d as salicylic acid) in the diet	No adverse effects were reported on any reproductive parameter.
2-generation study,(NTP, 1984a) continuous breeding protocol , MeS, CD-1 mice	25, 50 and 100 mg/kg bw/d (22.5, 45 and 90 mg/kg bw/d as salicylic acid) by gavage	No effects on fertility were reported.
1-generation study (NTP, 1984b), continuous breeding protocol , MeS, CD-1 mice	100, 250 and 500 mg/kg bw/d (90, 225 and 450 mg/kg bw/d as salicylic acid)	No effect on fertility index.
Fertility test, (Schardein *et al*., 1969), ASA , male and female rats	A single dose level of 0.4% in the diet (210 mg/kg bw ASA, equivalent to 161 mg/kg bw as salicylic acid)	ASA did not significantly affect male or female fertility. This dose caused moderate bw depression in males and severe bw depression in females.

Note: all the studies in the table above have a Klimisch reliability score of 2

The studies showed a number of deficiencies in relation to current TGs in terms of parameters studied, but the results were consistent. No statistically significant effect on fertility was

reported in any study. In addition, 2-year chronic toxicity studies in rats and dogs (Webb, 1963) showed no abnormalities in sexual organs (testes/prostate or ovaries/uterus).
The adverse effects on reduced viability of offspring reported primarily in rats represent developmental toxicity rather than a reduction in the fertility of either males or females.

Developmental effects: animal data

The developmental effects presented are from studies with salicylic acid, ASA or NaS. Since the interspecies differences are a key element in the discussions of the developmental effects, the studies will be presented according to the species rather than the test material.

Studies in Rats

In a pre-natal developmental toxicity study (Tanaka *et al*., 1973a, *reliability 2*), salicylic acid was administered to pregnant Wistar rats at levels of 0.06%, 0.1%, 0.2% and 0.4% in the diet (50.7 +/- 0.6, 77.4 +/- 1.0, 165 +/- 2.1, 205.9 +/- 18.9 mg/kg bw/d, respectively) on gestation days (GD) 8-14. The two lower doses (*i.e.* 50.7 and 77.4 mg/kg bw/d) caused neither maternal nor foetal effects. A marked body weight loss of dams was observed in the 0.4% group at the beginning of salicylic acid administration, but a gradual increase in bw was then observed after GD 11 day. This decrease in bw was assumed to be due to a decrease in food intake, but no deaths were observed. No marked changes were noticed in other groups. Very low uterine weights of foetuses and significantly lower placental weights were obtained in the 0.4% group, but there were no marked differences in the number of corpora lutea or in the rate of nidation in all groups. The dose of 0.2% (165 mg/kg bw/d) caused foetal effects (foetal anomalies and growth retardation) in the absence of maternal effects. This dose resulted in a maternal serum concentration of about 116 microgram/mL. The highest dose of 0.4% (205.9 mg/kg bw/d) induced maternal effects expressed as temporary body weight loss with toxic symptoms (salivation, piloerection) and the following foetal effects: high fetal mortality (no live foetuses in 9/15 dams examined), high frequency of complex anomalies (cranioschisis, myeloschisis, pes varus, oligodactyly etc.) and dose-related foetal growth retardation. At the dose of 0.2%, the body weight and length and the tail length were statistically significantly decreased. At the dose of 0.4% litter size and body weight and length as well as tail length were statistically significantly decreased. The general conclusion of the authors was: "*It is clear from the results obtained in the present and previous experiments that salicylic acid through oral route has a teratogenic effect on rat*". Also, "*none of the metabolites of salicylic acid produce congenital anomalies. The teratogenic effects of salicylic acid may be attributable to a direct action of the compound on fetal tissues as relatively well distribution was found in foetus and amnionic fluid in the present experiment*".

The derived no adverse effect levels (NOAELs) were: maternal - 0.2% (165 mg/kg bw/d) and developmental - 0.1% (77.4 mg/kg bw/d).
A parallel study by gavage (Tanaka, 1973b) at 75, 150 and 300 mg/kg bw/d gave similar results, with NOAEL (maternal) of 150 mg/kg bw/d and NOAEL (development) of 75 mg/kg bw/d.

In an experimental segment II study (Gupta *et al*., 2003, Klimisch score 1), ASA was administered by oral gavage both in single and multiple dose studies.

In the single dose study the dosage was as follows: GD9 (0, 250, 500 and 625 mg/kg bw), GD10 (0, 500, 625 and 750 mg/kg bw), GD11 (500, 750 and 1000 mg/kg bw). No maternal deaths were noted in any dose group; there were no treatment related clinical signs or

necropsy findings. Dose-dependent decreases in body weight gain and food consumption were observed for dams dosed on the days when treatment was administered. Body weight gains were decreased for the duration of the study, whereas the food consumption remained decreased for 2 days after the administration of ASA. The decrease in body weight gain was only partly due to reductions in food consumption as the magnitude of this change was minimal compared with the body weight gain decrements. The decrease in foetal weight and the number of foetuses contributed to the decrease in body weight gain. A dose-dependent decrease in the number of viable foetuses was observed in dams administrated ASA on each day with the exception of 500 mg/kg dose group on GD11 although the decreases were not always statistically significant. An increase in resorptions with an associated increase in post-implantation loss was observed in the mid- and high-dose groups on GD10 and GD11. The number of viable foetuses decreased in almost all dose groups, with accompanying decreases in uterine weight in all dose groups on GD9 and GD11 and in the mid- and high-dose groups on GD10. Significant increases in malformations were reported only from 500 mg/kg for GD9 administration or from 625 mg/kg for GD10 or GD11.

In the multiple dose study the dosage was of 0, 50 mg/kg bw/d (38 mg/kg bw/d as salicylic acid), 125 mg/kg bw/d (96 mg/kg bw/d as salicylic acid), 250 mg/kg bw/d (192 mg/kg bw/d as salicylic acid). Maternal toxicity was reported as a dose-dependent decrease in maternal body weight that was statistically significant at the mid-dose (85% of control) and high-dose (52% of control). Hence, the decreases in food consumption were partly responsible for the decrease in body weight. Irregular respiration and sporadic salivation were noted in the dams at 250 mg/kg bw/d. One dam given 125 mg/kg bw/d was killed as moribund on GD13 due to severe weight loss and associated inappetence. At necropsy, this dam showed no unusual findings and no other mortality was noted. The foetal toxicity was expressed as a dose-dependent decrease in the number of foetuses. This was due to the dose-dependent decrease in the numbers of viable foetuses across the dose group. The malformations were statistically significantly increased only in the high dose (250 mg/kg) group and included ablepharia, craniorachischisis, bent forepaw, kinked tail, protruding tongue, gastroschisis, ectopic adrenal, ventricular septal defect (VSD), diaphragmatic hernia, hypoplastic kidney and hypoplastic testis.

Fritz and Giese (1990, Klimisch score 2) performed a gavage study on 17-19 females per dose with NaS during GD 6-15. The dosage was 30, 60 and 90 mg/kg bw/d. The maternal toxicity was seen only at the highest dose and was described as some reduction in food consumption. The foetal toxicity could be observed at the mid dose (in the absence of maternal toxicity) as a dose-related delay in growth. At the highest dose the foetal toxicity was described as a dose-related delay in growth and malformations in 30% of the foetuses, the most common malformation being cranio(rachi)schisis (22.7% of the foetuses).

Nakatsuka and Fujii (1979, Klimisch score 2) treated SD rats with ASA on GD 7-17 with 3 doses: 50, 100 and 200 mg/kg bw/d. Neither maternal nor foetal toxicity were present at the lowest dose. At the middle dose, dose-dependently decreased foetal bodyweight was described in the absence of maternal toxicity. At 200 mg/kg bw/d maternal toxicity was described as significantly decreased bodyweight and the foetal toxicity as: increased number of foetal resorptions and malformed survivors, dose-dependently decreased foetal bodyweight, 21 foetuses (8.5%) with gross malformations, significantly delayed ossification and increased frequency of skeletal malformations (mainly absence, fusion, fragmentation or deformation of vertebral and costal bones) or variations.

Schardein *et al*. (1969, Klimisch score 2) treated rats with ASA at doses of 99 mg/kg bw/d (0.2 % in diet), 224 mg/kg bw/d (0.4% in diet) and 250 mg/kg bw/d (by gavage) during GD 6-15. Maternal toxicity was reported at 0.2% in diet (29% food intake depression with a 52% weight gain depression) and 0.4% in diet (17 % food intake depression and a 90% weight gain depression). Fetal toxicity was registered at all doses with skeletal malformations at 99 mg/kg bw/d and 100% resorptions at the two higher doses.

Studies in Rabbits

ASA was administered by oral gavage to pregnant New Zealand White (NZW) rabbits at 125, 250 or 350 mg/kg bw/d on GD 7-19 (Cappon *et al*., 2003, Klimisch score 2). Maternal body weight gain was significantly reduced in the mid and high dose groups from GD 7 to 13. Food consumption was also reduced in these groups. Three does given the high dose and one given the mid dose died during the study. There were no treatment-related effects on corpora lutea, implantation sites, pre-implantation losses or embryofoetal mortality. There were no treatment-related visceral or external anomalies. Reduction in mean foetal weight at 350 mg/kg bw/d was the only developmental adverse effect reported at this maternally toxic dose.

In a supporting study (Schardein *et al*., 1969, Klimisch score 2), rabbits received ASA at 200 or 250 mg/kg bw/d on GD 6-13 or GD 6-18. ASA induced maternal toxicity. A single kit of a dam had hydrocephaly. There were no skeletal malformations among those examined, but the limited number (9) could have precluded finding such defects. There were no significant findings in kits of the control dams. Under the conditions of this test, ASA induced maternal toxicity and foetotoxicity.

Studies in Monkeys

The study of Wilson *et al.* (1977) is not compliant with OECD TGs; its original purpose was to elucidate toxicokinetic aspects, namely the distribution and embryotoxicity of ASA in rats versus monkeys. Since the administration of ASA was performed during the organogenesis period, some conclusions may however be drawn. Unlike other studies, the protocol of administration was twice per day by gavage. For rats the doses were 100, 150, 175 and 200 mg/kg bw (twice daily on GD 9-12) and for monkeys 100 and 150 mg/kg bw (twice daily, for 10 days starting on GD 23). The same doses were given to non-pregnant females of both species for the purpose of determining comparative plasma concentrations.

Maternal toxicity in rats was described as occasional death and weight loss at 200 mg/kg bw twice daily. Foetal toxicity in rats showed significant effects on intrauterine death, growth and malformations rates at 150 mg/kg (twice daily). At this dose, the percentage of dead or resorbed foetuses was 34% and increased to 73% at the highest dose of 200 mg/kg bw twice daily. Also, the percentage of malformed survivors (including those with cardiac, facial, brain, spinal, tail and other skeletal defects) increased from 55% at 150 mg/kg to 100% at 200 mg/kg bw.

In monkeys, the number of aborted or resorbed foetuses was the same (3) at both dosages. At the dose of 100 mg/kg bw twice daily the foetal effects were reported as growth retardation and at 150 mg/kg bw (twice daily), growth retardation and malformations such as gross abnormality, cranioshisis and cystic kidney were reported. At both doses there were also foetuses which were observed to be normal. The conclusion was that 150 mg/kg twice daily is in the teratogenic range.

A comparison of serum concentration of salicylic acid in mothers vs. whole embryo concentration was performed. Unbound salicylate in rat plasma ranged from 30% to 50% of the total plasma concentration and was closely paralleled by the concentration in the rat embryo. Unbound salicylate in monkey plasma was lower, ranging from 17% to 30% of the total plasma concentration and was to some degree paralleled by the concentration in the monkey embryo. The greater embryotoxicity of ASA in the rat compared to the monkey correlated with higher concentrations and longer duration of concentrations in the respective embryos on a day-to-day basis. The general conclusion was that this association only partially explains the difference between species; the mode of action within the embryo must not be neglected.

The study of Wilson *et al.* (1977) has also been considered in the opinion on salicylic acid issued by The Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers (SCCNFP) in 2002.

Conclusion by the dossier submitter

Based on the argumentation presented above, the DS proposed that salicylic acid should not be classified as a reproductive toxicant, either for the fertility or developmental endpoints.

Comments received during public consultation

First public consultation

The German CA agreed with the proposal of the DS for no classification. The French CA commented that based on the level of detail provided, the relevance of the observed effects to humans cannot be concluded. The Belgian CA considered that information provided in two databases -Toxnet (toxicology data network, US) and eMC (electronic Medicines Compendium, UK) - indicates some concerns, mainly related to development and lactation; they recommended that this information should be assessed in depth as this could be supportive evidence for classification. Following detailed argumentation, the Netherlands CA concluded that the developmental effects in rats are not considered secondary to the maternal toxicity. Therefore, based on the teratogenic effects of salicylates observed in rats and the limited evidence in studies with monkeys, but not in rabbits, the Netherlands CA proposed that salicylate should be classified as Repr. 1B; H360D (May damage the unborn child). One comment received during the public consultation period disagreed with the read across from MeS to salicylic acid because it "is not sufficiently justified, only NOAEL/LOAEL are reported for experimental studies."

Second (targeted) public consultation on the reproductive toxicity of salicylic acid

The French CA commented that it is not possible to easily compare the plasma levels between ASA and salicylic acid in animals and humans. Also, there is not enough evidence not to consider the effects seen in animals. Consequently, a classification for salicylic acid is supported.

Additional key elements

There were two attachments provided with the RCOM by the Dossier Submitter:

1. Bard, D (2012). Reproductive and teratogenic risks of low salicylic acid doses in humans. Owner company: NOVACYL. Report date: 2012-10-30.

2. Aspirin and Related Drugs, Ed Rainsford KD, Taylor & Francis, London (2004)

Both publications have been extensively referred to in the argumentation of the repeated dose discussion and further in the reproductive toxicity assessment.

Written consultations with representatives of the European Medicines Agency (EMA) were conducted in the interval between the meetings RAC 34 and RAC 36 (*ie* in the period September 2015 to March 2016).

Citations from the following publications have been included in the RCOM: "Daston P.G., Beyer B.K., Carney E.W., Chapin R.E., Friedman J.M., Piersma A.H., Rogers J.M. and Scialli A.R., Exposure-based validation list for developmental toxicity screening assays, Birth Defects Res B Dev Reprod Toxicol. 2014 Dec;101(6):423-8. doi: 10.1002/bdrb.21132. Epub 2014 Dec 4", "M Imoru, A Emeribe. Changes In Plasma Proteins And Fibrinolytic Activity In Pregnant Women In Calabar, Nigeria. The Internet Journal of Gynecology and Obstetrics. 2009 Volume 12 Number 2".

Assessment and comparison with the classification criteria

Summary of the animal studies

The results of the studies demonstrated that salicylic acid has an embryo-/foetotoxic effect in rats with dose-dependent growth delays, foetal death and malformations. Early developmental effects were clearly seen in the absence of maternal effects. The teratogenicity of salicylic acid may be attributable to a direct action of the compound. This finding is further supported by the mechanistic study of Greenaway (1982) in which teratogenicity of salicylate in rat embryos was shown independent of maternal factors after exposure *in vitro*. However, although there was a general resemblance in terms of skeletal and internal organ abnormalities observed, the pattern of malformations following exposures to salicylic acid and ASA is slightly different, as described in the studies of Tanaka and Gupta. One explanation could be the differences in the experimental protocol, such as the moment of exposure during organogenesis. However, differences in effects following exposure to salicylic acid and ASA were shown in *in vitro* cultured rat embryos (Yokoyama, 1984): the anomalies induced by ASA were systemic (*e.g.* crown-rump length significantly reduced) while those induced by salicylic acid were more localized (*e.g.* facial anomalies).

The study in monkeys also showed teratogenic properties with ASA but with lower magnitude. By contrast, the effects in rabbits were limited to slight growth retardation and were present only at doses much higher than in the rats and monkeys. No skeletal malformations were reported and at the highest dose only one kit of a dam had hydrocephaly.

Overall, salicylic acid was shown to have teratogenic properties but with species differences in potency: strong in rats and lower in monkeys. In contrast, the teratogenic potential in rabbits was practically non-existent.

Developmental effects: human information

Despite its long usage, data regarding human exposure to salicylic acid itself is lacking. To fill the information gap, an assessment was performed using human data on ASA. This approach is appropriate as stated in the read across paragraph above under 'RAC general comments'; however, the fact that ASA is a pharmaceutical product raises a series of limitations to the use of this information due to the range of doses specific to medical usage. ASA is part of a class of medications generically called Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). Most of the therapeutic effects are common within the group (analgesic, antipyretic, anti-inflammatory activity) but ASA also has an antiplatelet effect. In addition, the mechanism of action differs: unlike the other NSAIDs, ASA inhibits cyclooxygenase (COX) in an irreversible manner, affecting the COX-1 variant more than the COX-2 variant of the enzyme.

Epidemiology. Therapeutic doses of acetylsalicylic acid

The dosage of ASA in prophylactic/therapeutic applications vary according to specific prescriptions/recommendations but guidance ranges for adults are presented by the DS in the following table:

Indicative doses of ASA used in therapy

Indication	**Unit dose strength (mg)**	**Dose regimen**	**Duration**	**Daily dose as ASA (mg/kg bw/d) for a 60 kg person**	**Equivalent dose as SAL (mg/kg bw/d; conversion factor of 0.77)**	**SAC designation**
Treatment of rheumatic fever	350-500	Up to 6500 mg/d in divided doses	Short term (1-2 weeks, then 60-70 mg/kg bw/d for 1-6 weeks)	Up to 108	Up to 83	
Treatment of severe inflammatory conditions such as osteo- or rheumatoid arthritis, and SLE-associated arthritis	350-500	3000-5400 mg/d in divided doses	Medium to long-term	50-90	38.5-69	**"High dose"**
Treatment of mild pain or fever	350-500	Up to 4000 mg/d, 1-2 tablets, 2-3 times per day	Short-term (typically 1 to 4 or 5 days)	11.7-66.7	9-51	**"Standard therapeutic dose"**
Prophylaxis for myocardial infarction, angina stroke etc.	75-350	1 tablet per day	Medium- to long-term	1.25-5.8	1-4.5	**"Low dose"**

Prevention of multiple miscarriage, pregnancy-induced hypertension and other complications of pregnancy	50-150	1-2 tablets once per day	Medium-term: 1st trimester or from 2nd and/or 3rd trimester	1-5	0.77-3.85	

The assessment of "Low doses" in pregnancy

The "low doses" in pregnancy are those referred to in the table above as being indicated for "prevention of multiple miscarriage, pregnancy-induced hypertension and other complications of pregnancy". The (retrospective) cohort study performed by Bard (2012) was provided for this dose range. To further extend the analysis, the DS submitted an additional critical review by the same author (Bard 2015).

The aim of the study and the analysis was to address the effects of ASA within this dose range on the following endpoints: maternal bleeding, neonatal haemostatic abnormalities, pregnancy duration and labour, prevention of pre-eclampsia and intra-uterine foetal growth retardation, stillbirths and infant mortality, birth weight, birth defects and early childhood development. Particular aspects that raised concern were also analysed; the premature closure of *Ductus arterious*, the occurrence of *gastroschisis* and congenital *cryptorchidism*.

As a final conclusion of the study it was stated that: "*no adverse effect of aspirin treatment can be considered as established, either at low (<150 mg daily) or higher, usual dose*". To further illustrate the overall conclusion with respect to dosages higher than that mentioned above, three epidemiological studies (Slone, 1976; Shapiro, 1976; Kozer, 2002) were cited; the conclusion was that the use of aspirin at up to the maximum recommended therapeutic dose of 4000 mg/d (equivalent to 66.7 mg/kg bw/d as ASA, or 51 mg/kg bw/d as salicylic acid) have largely demonstrated an absence of increased risk of adverse pregnancy outcome in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay.

The assessment of "High dose" ASA as prescribed in pregnancy

As already stated, ASA is an NSAID which may be prescribed at "high dose" levels for long-term treatment of a number of severe inflammatory conditions. Only limited information is available regarding the effects of such prescribed medicinal usage of ASA during pregnancy.

In a retrospective survey of 103 patients taking high dose ASA (at least 3250 mg per day) for rheumatoid arthritis or other inflammatory conditions, Lewis and Schulman (1973) reported an increased mean gestational length and increased duration of labour. No malformations was reported, however the study covered ASA exposure only throughout "at least" the last six months of pregnancy, so it cannot be established how many of these patients were also exposed to ASA during the first trimester.

The study of Østensen & Østensen (1996) was not included in the analysis since it had no specific information related to ASA.

Overall, the available data regarding therapeutic doses over 3g/d do not show any association between ASA and malformations in humans.

ASA Overdose during Pregnancy

Two publications (Collins & Turner 1975; Turner & Collins, 1975; reported in detail in Annex 2 of the additional document *"Relevance of plasma levels in humans and rats to establish equivalence of exposure levels"* provided by the DS)*,* describe a prospective study on ASA usage during pregnancy in Australia at doses which were excessive and can be considered as the result of abuse of the substance and toxic. A number of 144 exposed pregnancies were described (6.6% of the Australian-born patients attending the clinics) of which 44% reported ingestion of powders containing ASA at 384 mg (associated with 384 mg of Phenacetin) or 510 mg (associated with Phenacetin, but the quantity was not given) per powder. The subjects took between 2 and 12 doses/day every day throughout pregnancy; 56% used the powders at least once per week. Toxicity to the mother was evidenced by anaemia, ante or post-partum haemorrhage, prolonged labour and increased need for Caesarean and forceps/ventouse. The effects on exposed foetuses were lower birth weight compared to controls. This correlated with the duration of maternal ASA consumption for Group 1 (see below) and increased stillbirth. The data are summarised in the table below.

Summary of developmental effects

No	**Gender**	**Gestation (week)**	**Birth weight (g)**	**Maternal age**	**Salicylate consumption (years)**	**Pregnancy complications**
Group 1						
1	F	37	2305	30	14	None
2	M	39	3050 (macerated)	35	17	Anaemia
3	F	36	2570	38	20	APH, PPH
4	F	36	2490 (macerated)	35	10	None
Group 2						
1	M	29	1920	38	12	APH

APH=ante-partum haemorrhage; PPH=post-partum haemorrhage

The authors commented that the still-births among salicylate users were not all clearly related to pregnancy complications, but all occurred in older women who had been taking salicylates for many years. The two effects correlated with treatment duration were probably more a consequence of the general health of the mothers (among others, the known severe kidney effects of the associated drug Phenacetin) than a direct effect of the ASA treatment.

No increased malformation rate was observed. In this study, depending upon which powder was used and the number of powders taken per day, the ASA dose ranged from 0.8 to over 6 g/d (equivalent to 10-79 mg/kg bw/d as salicylic acid). This level of exposure occurred throughout pregnancy, and specifically throughout the first trimester, which is critical for organogenesis.

Maternal and cord blood serum salicylate levels were measured at the time of delivery or as soon as possible after delivery while the mother was still in the labour ward. Blood samples were not taken from all women, and results from Groups 1 & 2 were not distinguished. These serum salicylate levels are summarised in the following table.

Serum salicylate levels in mothers and babies

	Number	Serum salicylate (µg/mL)				
		0-10	11-30	31-50	51-70	71-90
Mothers	81	13	60	7	1	0
Babies	76	21	45	5	2	3

It was not possible to make a precise comparison between maternal and cord blood salicylate levels, but where the maternal level was high, so was the cord blood level. Since the mean duration of labour in women of Groups 1 & 2 was approximately 5.5 hours, it is clear that many hours had elapsed since the last ASA dose and that therefore these serum levels do not represent peak values.

A short report on analgesic overdose in pregnancy (McElhatton, 1991) stated that only one of the 31 women who had taken an ASA overdose gave birth to a malformed baby (with no indication that the malformation was due to ASA). This study was cited by the DS in the CLH report with no further description and therefore the magnitude of the dose was not available.

Summary of medical concerns regarding the usage of ASA during pregnancy

According to a literature search performed and results from a written consultation with representatives from the European Medicines Agency (EMA), ASA doses up to 100 mg/d are generally considered safe during pregnancy *(*FASS.se; 25 September 2015*)*. A dose of 100 mg/d corresponds to 1.6 mg/kg bw/d of ASA for a 60 kg woman. For the dose range of 100-500 mg (equiv. to 1.6-8.3 mg/kg bw/d) it seems that "*there is not enough clinical experience*" for specific recommendations to be given, so a precautionary approach has been taken, giving the same warnings as for higher doses (above 500 mg/d). For doses exceeding 500 mg/d the concern is related to effects caused by prostaglandin synthesis inhibition having a negative impact on pregnancy and/or foetal development. The following information was obtained from FASS.se (25 September 2015):

During the third trimester, all prostaglandin synthesis inhibitors can lead to the following in the foetus:

- Heart/lung toxicity (with a premature closure of the Ductus arteriosus and pulmonary hypertension);

- Renal dysfunction, which can lead to renal failure with oligo-hydroamniosis;

In the mother and the new-born baby, at the end of pregnancy, it can lead to the following:

- Possible prolongation of the bleeding time, an anti-coagulant effect that can occur also at very low doses.

- Inhibition of uterus contractions which can lead to delayed or prolonged delivery.

Therefore, acetylsalicylic acid at doses above 100 mg/d is contraindicated during the third trimester.

It should be underlined that this text is precautionary. Prostaglandin inhibitors include many substances and for instance, there is evidence of premature closure of *Ductus arteriosus* and pulmonary hypertension for the specific substance indomethacin but not for other NSAIDs. Also, it should be noted that according to written responses from EMA representatives

"*miscarriage is an increased risk with preeclampsia; i.e. the reason for using the aspirin, not aspirin itself (other than if caused by haemorrhage following aspirin overdose)*".

Summary of human data

Aspirin is a widely used medicine and has been used for a long time. Depending on the disease and the degree of severity, several dose regimes are usually employed; general guidance values have been listed in the table "Indicative doses of ASA used in therapy" (above). However, it has to be pointed out that aspirin is a medicine dispensed without prescription and the exact characterization of the exposure is very difficult; exposure during pregnancy being no exception.

Apparently, the severity of effects increases with the dose. The first signs of toxicity are present in the range of 3 g/d (41.7 mg/kg bw/d ASA); the effects are not related to direct effects on the foetus but are described as increased mean gestational length and labour duration. Higher doses (up to 6 g/d, or 77 mg/kg bw/d ASA) show maternal toxicity, labour difficulties, lower birth weight and increased stillbirth. No malformations were identified at any dose. However, the conclusions taken from the studies of Turner and Collins have received criticism over time; although the publications are widely cited, the conclusions are mainly presented as having limited reliability due to the relatively small database and due to lack of consistent support from further studies. In addition, the authors themselves underscored a series of confounding factors such as the concurrent maternal exposure to Phenacetin or the low reliability of the serum levels of salicylic acid.

In medical practice there is a strong concern regarding the toxic effects following the administration of NSAIDs during pregnancy. Therefore, the usage of ASA is subject to precautions; however, the analysis of therapeutic implications of ASA administration during pregnancy is beyond the scope of the RAC opinion on the reproductive toxicity of salicylic acid.

In summary, assessment of the reproductive toxicity of ASA from the human data is difficult due to three main reasons: a) low statistical power of the studies, b) confounding factors are difficult to control and c) it is difficult to distinguish between effects of the drug and effects of the disease for which ASA is used as treatment (written responses from EMA representative).

Discussion on species differences in the effects seen on development

In general, it appears that two major reasons account for observed species differences in teratogenic response of substances: (a) intrinsic sensitivities of the developing tissues; (b) differences in exposure of the embryo during the sensitive stages of gestation (Nau, 1986).

The traditional endpoint in assessing teratogenic potential is structural malformation.
The recognition or identification of teratogens in humans is difficult for several reasons; for example, therapeutic dosages or exposure levels are generally several orders of magnitude lower than doses purposefully given to animals in experimental studies (Schardein, 1985).

These generalities apply to the present assessment. Apparently, the developmental toxicological profile differs between species: in rats and monkeys the effects are growth delays, malformations and eventually foetal death. In rabbits and humans, malformations could not be identified. Again, the magnitude of exposure appears to be the main drawback for the scope of the present analysis.

Dosages and serum level concentrations

As stated above, the difference in the dose range between the animal studies and the human epidemiology studies is very high. In the following, a common ground of comparison is attempted.

In the study of Wilson *et al.* (1977), when general embryotoxicity of rats and monkeys to ASA was compared at equivalent dosages, some differences were detected. According to the study author this difference in effects seen can be attributable to the differences in embryonic exposure; since the free (unbound) salicylic acid is responsible for the teratogenic potential and the binding capacity differs between species, the rat embryo is exposed to higher levels and for a longer duration than the monkey embryo.

Also, as the salicylic acid is the prospective teratogen under analysis, the serum concentration of free salicylate appears to be the only metric representative in the present interspecies and inter-compounds comparison. However, this metric is very sensitive to kinetic factors such as decreasing concentration with time and the variation in concentration due to the albumin binding saturation; consequently, the concentration varies depending on the experimental conditions.

To follow a common ground, the data from the Wilson *et al.* (1977) study will be used. This study offers both concentrations of total salicylate in maternal plasma and the corresponding percentage of unbound salicylate associated to the lowest doses at which malformations were induced. The values are measured at intervals of 1, 2, 4, 8 and 17 hours after gavage. In addition, the validity of the comparison was confirmed by the author when performing his own assessment.

When multiplying the total serum concentration with the corresponding percentage the following values of free serum salicylate in pregnant females are obtained:

Serum concentrations of free (unbound) salicylate in the Wilson et al. (1977) study (rounded values)

Species	Dose (mg/kg bw)	Conc. at 1h (µg/mL)	Conc. at 2h (µg/mL)	Conc. at 4h (µg/mL)	Conc. at 8h (µg/mL)	Conc. at 17h (µg/mL)
Rat	150 twice daily	85	84	114	87	15
Monkey	150 twice daily	52	44	41	13	-

When calculating the average values for the interval of measurement, the concentration of free serum salicylate becomes 77 µg/mL in rats and 37.5 µg/mL in monkeys. These values are in line with the conclusions of Wilson *et al.* (1977) and represent the lowest levels of maternal serum concentrations associated with the induction of malformations. The values are in the same order of magnitude and close enough to assume that an average of 50 (57 to be exact) µg/mL represent an indicative serum level for the two species. Moreover, if the serum level in rats is taken from the study of Tanaka (*i.e.* 115 µg/mL total serum salicylate corresponding to a value of 58 µg/mL free serum salicylate) a very similar conclusion is reached.

In humans no malformation could be detected; consequently, there are no comparable associated values of free salicylic acid serum concentrations. However, based on the premises of the read across assessment, there is no plausible reason to assume a different mechanism of action: salicylic acid is the suspected teratogen, it has similar distribution in serum (both free and serum albumin bound), it crosses the placenta and (depending on the degree of albumin binding) may expose the foetus to lower or higher concentrations. Therefore, as a result, toxic potential may be assumed. If a generic value of 25% (Rainsford, 2004) free plasma salicylate in man is assumed (28% in Kucera & Bullock, 1969) then a value around of 200 µg/mL of total salicylate in maternal serum could be expected as a hypothetical human threshold for malformations.

Conclusions

The type and magnitude of the developmental response to exposure to chemicals depends on the intrinsic sensitivities of the developing tissues as well as on the differences in exposure of the embryo during the sensitive stages of gestation. Since the mode of action of the chemicals inside the embryo is not known, the exposure remains the working tool for the assessment.

Acetylsalicylic acid has been used as a medicine for a very long time and consequently, it has been extensively studied. However, older as well as more recent studies have failed to arrive at a strong conclusion on the potential of ASA to induce malformations; the low doses used in therapy and the absence of reliable epidemiological evidence are often invoked as explanations. Still, when it comes to use during pregnancy, a precautionary dose range and duration of medication are recommended, and no robust conclusion can be drawn regarding the developmental potential of salicylates in humans.

In contrast to humans, salicylate teratogenicity has been seen in both rats and monkeys. In these species, a wide range of malformations was induced at higher doses, at which the malformations occurred together with developmental retardation or death. At lower doses, foetal retardation was not associated with malformations and in rats, growth retardation was seen in the absence of the maternal toxicity.

Based on the assumption of a similar teratogenic potency in all species, a hypothetical human threshold for malformations around of 200 µg/mL of total salicylate in maternal serum was calculated. When compared to the toxic levels given in the literature, this value falls below the value of 300 µg/mL given as an indicative concentration associated with clinical manifestations of acute salicylate intoxication (Pearlman 2009). Consequently, it may be assumed that foetal exposure to such a high concentration of salicylate in maternal blood could go undetected since the mother can be asymptomatic. In terms of classification and labelling this would mean that foetal toxicity could occur in the absence of maternal toxicity. However, this assumption needs to be treated with caution since "it is important to emphasize that serum salicylate levels cannot be used strictly to determine the severity of intoxication" (Pearlman, 2009). Medical practice precludes high exposures to ASA and the overdose cases are subject to emergency treatment. In summary, the assumption appears plausible based on a toxicological approach but is not confirmed by the ASA usage even in cases with "high doses".

Overall, the available evidence from studies in rats and monkeys (but not from rabbits) indicate potential for developmental toxicity. The data from humans are considered inconclusive.

Opinions by other bodies

The Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers (SCCNFP) adopted an opinion on Salicylic acid in 2002 (http://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out170_en.pdf). The reproductive toxicity evaluation concluded that: *"A NOAEL of sodium salicylate administered orally to mated rats has been established to 80 mg/kg bw/d corresponding to 69 mg/kg bw/d of salicylic acid. The results also showed that following oral administration salicylic acid is neither teratogenic nor embryotoxic up to 75 mg/kg bw/d in rodents and up to 100 mg/kg bw/d in monkey. Above these dose levels, foetal malformations (skeletal malformations, cleft lip, and growth retardation), resorptions and perinatal death were recorded with the compounds salicylic acid or acetylsalicylic acid."*

The Scientific Committee on Consumer Products (SCCP) in the opinion on homosalate in 2007 (http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_097.pdf stated that: *"based on the suggested metabolic fate of Homosalate as pointed out by Roberts (2005) and following his conclusions, it can be stated that the metabolite salicylic acid is comprehensively investigated in respect to teratogenicity".*

Salicylates which are naturally present in our alimentary tract were approved as flavouring ingredients *quantum satis* (EU Regulation No 872/2012 of 1 October 2012).

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

RAC concludes that there is insufficient evidence that salicylic acid exhibits adverse effects on sexual function and fertility. Consequently, for this endpoint RAC supports the proposal of the DS and concludes that no classification for salicylic acid for adverse effects on sexual function and fertility is justified.

Adverse effects on development

In the assessment and comparison with the criteria for the development of the offspring endpoint, RAC took into consideration the following:

- There is robust evidence of developmental effects in animals which justifies classification. In animals, the developmental toxicity was clearly shown in two out of three species. The pattern and magnitude of the effects shown in rats but also in monkeys are sufficient to presume that salicylic acid is a developmental toxicant and to justify classification in Category 1B;
- According to experts in the field of pharmaceuticals, ASA is not considered as being a major teratogen, but may have some potential for teratogenic effects, and it should be noted that prostaglandin inhibitors in general, including ASA, could have other adverse effects on foetuses, especially on their renal development and during the third trimester on the development of the circulatory system;
- However, neither ASA nor salicylic acid are proven human developmental toxicants. There is a lack of evidence to support an increased risk of birth defects following exposure to ASA. Also, the evidence for other developmental effects has uncertainties. Taking that into account, classification in Category 1A is not justified.

- Although the information on effects of ASA on development in humans at "high doses" is marginal, it should be acknowledged and cannot be discarded when discussing classification in Category 1B versus Category 2.
- It is noted that the available human epidemiological data on ASA was rather contradictory and with only a few reported exposures at higher doses, nevertheless demonstrated no clear evidence of malformations in humans. Hence, the RAC concluded that Category 1B may not be justified.

Taking into account the available data, including pharmacokinetics, *in vitro* tests with ASA and salicylic acid, developmental studies in animals (positive findings in rat and monkey studies and a negative rabbit study), human epidemiology and medical experience, the RAC considered classification of salicylic acid as **Repr. 2; H361d** (Suspected of damaging the unborn child) to be justified.

4.12 Other effects

5 ENVIRONMENTAL HAZARD ASSESSMENT

6 OTHER INFORMATION

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8 ANNEXES

Annexe 1 :
Reproductive and teratogenic risks of low salicylic acid doses in humans
Prof. Denis BARD, EHESP School of Public Health, Rennes (France), unpublished report, October 30th, 2012

Annexe 2 :
Relevance of plasma levels in humans and rats to establish equivalence of exposure levels
NOVACYL S.A.S. unpublished report, April 2013

Annexe 3 :
O-acetylsalicylic acid and salicylic acid, NOVACYL position paper on Reprotoxicity
NOVACYL S.A.S. unpublished report, March 2013