Additional information

For a Substance under Harmonised Classification and Labelling Process

Substance Name: Salicylic Acid

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

This targeted public consultation is open for 14 days. Interested parties are invited to comment on the possible classification of salicylic acid for developmental toxicity, taking into consideration the new information summarised below or in the Annexes and the original CLH report (section 4.11.2) which is available on ECHA website.

ADDITIONAL INFORMATION REPORT FOR SALICYLIC ACID

**Introduction**

The classification and labelling (CLH) proposal on salicylic acid (EC number 200-712-3) was submitted to ECHA by the Dossier Submitter (DS; Novacyl S.A.S.) on 24 September 2014. Salicylic acid has no entry in Annex VI to the CLP Regulation. The DS proposed a harmonised classification of the substance as Acute Tox. 4 (H302) and Eye Dam. 1 (H318) similar to the self-classification included in the REACH registration dossier submitted to ECHA. In addition, the DS also provided an assessment on developmental toxicity of salicylic acid. The CLH report was subject to a public consultation from 28 October 2014 until 12 December 2014. A total of 13 comments were received during public consultation to which the DS responded in a Response to Comments (RCOM) document (Annex 1).

During the Risk Assessment Committee (RAC) 33rd plenary meeting held in June 2015, the RAC supported the harmonised classification proposal by the DS for Acute Tox. 4 (H302) and Eye Dam. 1 (H318). Reproductive toxicity of salicylic acid was assessed by RAC on the basis of read-across data from studies on structural analogues of salicylic acid, including methylsalicylate and acetylsalicylic acid. RAC supported the proposal for no classification on fertility and sexual development. For developmental toxicity, RAC discussed the relevance of the doses of salicylic acid used in animal studies (rat, rabbit) and the available epidemiological studies of acetylsalicylic acid in humans. RAC and the DS noted that a thorough comparison of the plasma levels in humans and rats to establish equivalence of exposure levels is needed. An analysis of the “relevance of plasma levels in humans and rats to establish equivalence of exposure levels” prepared by the DS is added in Annex 2 to this additional information report.

RAC also supported the necessity to examine further epidemiological studies (available in the original CLH report and in the present document), a developmental toxicity study conducted in monkeys (Wilson, 1977) (see Annex 3) and the effects of the substance on ductus arteriosus with a possible relevance to humans (see section 2.3.2). In addition, due to the fact that conclusions on the developmental toxicity of the substance were made by using data from substances other than salicylic acid, RAC supported a summary table on all substances used for the read-across and a review of the opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers concerning salicylic acid (SCCNFP, 2002) (Annex 4).

RAC postponed the discussion on developmental toxicity of salicylic acid until the RAC-34 plenary meeting in September 2015. Based on the discussion in RAC-33 plenary meeting, the ECHA secretariat has compiled the new information in the present additional information report.

---

1. IDENTIFY OF THE SUBSTANCE

Substance name: Salicylic acid
EC number: 200-712-3
CAS number: 69-72-7

2. TOXICITY TO REPRODUCTION (DEVELOPMENTAL EFFECTS)

2.1 BACKGROUND TO THE CLH PROPOSAL

2.1.1. Classification

Salicylic acid has no entry in Annex VI of the CLP Regulation. This applies also to other salicylates used in the present assessment. The self-classification status for reproductive toxicity of some salicylates in the C&L inventory is presented in the Table 1 below.

Table 1. Self-classification status for reproductive toxicity of some salicylates

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Classification according to CLP Regulation*</th>
<th>C&amp;L Inventory notifications (number of notifiers)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl salicylate</td>
<td>Not classified</td>
<td>Repr. 1B (n=3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Repr. 2 (n=55)</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>Not classified</td>
<td>Repr. 2 (n=1)</td>
</tr>
<tr>
<td>o-acetyl salicylic acid</td>
<td>Not classified</td>
<td>Repr. 1A (n=1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Repr. 1B (n=4)</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>Not classified</td>
<td>Repr. 2 (n=68)</td>
</tr>
</tbody>
</table>

* http://apps.echa.europa.eu/registered/data/dossiers/
** http://echa.europa.eu/regulations/clp/cl-inventory, checked on 07/07/2015

2.1.2. Read across between selected salicylates

Some salicylates have been widely studied but limited information is available for salicylic acid itself. To cover the information gap, the dossier submitter (DS) of the CLH report performed a read across between salicylic acid (SA), sodium salicylate (NaS), methyl salicylate (MeS), and o-acetyl salicylic acid (ASA).

In rats, SA, MeS, NaS and ASA are all rapidly absorbed on oral administration even at high concentrations. A publication by Davison (1961) compared the oral absorption and metabolism of MeS and NaS in rats and humans with that of ASA (Davison, 1961). Since then, several other publications demonstrated that SA is the initial metabolite (hydrolysis product) for related salicylates (ASA, methyl acetylsalicylate, NaS, MeS). Plasma analysis in rats showed rapid hydrolysis to free salicylate after administration of MeS, NaS and ASA, resulting in comparable plasma concentrations of salicylate at 60 minutes post dosing. However, hydrolysis of MeS to SA was slower and less complete. The publications of Rainsford et al. (1980) and Tjalve et al. (1973) revealed that SA was found in the stomach, liver, kidney lungs, bone marrow, intestine, inflamed paws and spleen of rats. ASA and the methyl ester of ASA (AME) were distributed in vivo very similarly to that observed with SA. Tjalve et al. (1973) confirmed that there was no difference in the distribution of SA and ASA in mice (following intravenous
ADDITIONAL INFORMATION REPORT FOR SALICYLIC ACID

administration). In mice, SA was found to be transported across the placenta to the foetus.

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

2.2 ANIMAL DATA

The assessment of developmental toxicity of salicylic acid is presented from studies with salicylic acid, acetylsalicylic acid or sodium salicylate. Since interspecies differences are a key element, developmental toxicity studies are presented according to the species rather than the test material.

The available studies revealed a wide range of effects from reduced birth weight to internal organs and skeletal malformations and foetal death. There are differences between the three species considered for assessment. The NOAELs for developmental toxicity are presented in table 2:

Table 2. NOAEL values in animal studies

<table>
<thead>
<tr>
<th>Species</th>
<th>NOAEL maternal (mg/kg bw/day)</th>
<th>NOAEL developmental (mg/kg bw/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat</td>
<td>50-150</td>
<td>general: 50-75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fetotoxicity: 50-90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>teratogenicity: 30-100</td>
</tr>
<tr>
<td>rabbit</td>
<td>125-200</td>
<td>developmental: 250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>malformations: 350</td>
</tr>
</tbody>
</table>

2.2.1. Studies in Rat

In a pre-natal developmental toxicity study (Tanaka et al., 1973a, reliability = 2), salicylic acid was administered to pregnant Wistar rats at 0.06, 0.1, 0.2 and 0.4% in the diet (equivalent to 30, 50, 100 and 200 mg/kg bw/day) during gestation days (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 (reliability = 1), ASA was administered by oral gavage to pregnant Sprague-Dawley rats at 50, 125 or 250 mg/kg bw/day (equivalent to 38, 96 or 192 mg/kg bw as SA) during organogenesis (GD 6-17) (Gupta & al, 2003). There was a dose-related reduction in maternal bodyweight gain, presumably 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.

Fritz and Giese (1990 - reliability = 2), 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 - reliability = 2) 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 - reliability = 2) 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 a significant reduction in maternal bodyweight gain. At 240 or 250 mg/kg bw/day 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 studies in the rat demonstrate that SA has an embryofoetotoxic effect in rats with evidence of malformations at high doses.

2.2.2. Studies in the 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 et al., 2003 - reliability = 2). 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 doses 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 - reliability = 2), rabbits received ASA at 200 or 250 mg/kg bw/day on GD 6-13 or GD 6-18. ASA induced maternal toxicity. A single kit had hydrocephaly. There were no skeletal malformations among those examined, but the limited number (9) could preclude finding such defects. There were no significant findings in kits of the control dams. Under the conditions of this test, aspirin induced maternal toxicity and fetotoxicity.

2.2.3. Studies in monkey

Since there are differences in the outcome between the rat and the rabbit studies, the study of Wilson et al. (1977) was brought into the reprotoxicity assessment. This study 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. This study is not compliant with OECD test guidelines and the 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 be helpful. Unlike other studies, the protocol of administration was twice a day by gavage. For rats, doses were 100 and 200 mg/kg bw and for monkeys, doses were 100 and 150 mg/kg bw. The same doses were given to non-pregnant females of both species for the purpose of determining comparative plasma concentrations. In rats, the results show significant effects on intrauterine death, growth and malformations rates at 150 mg/kg (twice daily). In monkeys,
ADDITIONAL INFORMATION REPORT FOR SALICYLIC ACID

at 150 mg/kg (twice daily), malformations such as gross abnormality, cranioshisis and cystic kidney were registered. The conclusion was that 150 mg/kg twice daily is in the teratogenic range. The study is summarised in Annex I.

2.3 HUMAN DATA

2.3.1. Therapeutic doses of acetylsalicylic acid

The dosage of ASA in prophylactic/therapeutic applications varies according to recommendations or indications (Table 3).

Table 3. Indicative doses of ASA used in therapy

<table>
<thead>
<tr>
<th>Indication</th>
<th>Unit dose strength (mg)</th>
<th>Dose regime</th>
<th>Duration</th>
<th>Daily Dose as ASA (mg/kg/day) for 60 kg person</th>
<th>Equivalent Daily Dose as SAL (mg/kg/day; conversion factor 0.77)</th>
<th>SAC Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of rheumatic fever</td>
<td>350 – 500</td>
<td>Up to 6500 mg per day in divided doses</td>
<td>Short term (1-2 weeks then 60-70 mg/kg/day for 1-6 weeks)</td>
<td>Up to 108</td>
<td>Up to 83</td>
<td></td>
</tr>
<tr>
<td>Treatment of severe inflammatory conditions such as osteo- or rheumatoid arthritis, and SLE-associated arthritis</td>
<td>350 – 500</td>
<td>3000-5400 mg per day in divided doses</td>
<td>Medium to Long-term</td>
<td>50-90</td>
<td>38.5 – 69</td>
<td>“High dose”</td>
</tr>
<tr>
<td>Treatment of mild pain or fever.</td>
<td>350 – 500</td>
<td>Up to 4000 mg/day 1-2 tablets 2-3 times per day</td>
<td>Short-term (typically 1 to 4 or 5 days)</td>
<td>11.7 – 56.7</td>
<td>9 – 51</td>
<td>“Standard therapeutic dose”</td>
</tr>
<tr>
<td>Prophylaxis for myocardial infarction, angina stroke etc</td>
<td>75 – 350</td>
<td>1 tablet per day</td>
<td>Medium to Long-term</td>
<td>1.25 – 5.8</td>
<td>1 – 4.5</td>
<td>“Low dose”</td>
</tr>
<tr>
<td>Prevention of multiple miscarriage, pregnancy-induced hypertension and other complications of pregnancy.</td>
<td>50 – 150</td>
<td>1-2 tablets once per day</td>
<td>Medium-term: 1st trimester or from 2nd and/or 3rd trimester</td>
<td>1 – 5</td>
<td>0.77 – 3.85</td>
<td></td>
</tr>
</tbody>
</table>

2.3.2. The assessment of “Low doses” in pregnancy

For the analysis of the effects of the aspirin in the medical treatment range, the following endpoints have been assessed: maternal bleeding, neonatal hemostatic abnormalities, pregnancy duration and labor, prevention of pre-eclampsia and intra-uterine fetal growth retardation, stillbirths and infant mortality, birth weight, birth defects and early childhood development (Prof. Denis Bard, Reproductive and teratogenic risks of low salicylic acid doses in humans. Owner Company: NOVACYL. Report date: 2012-10-30 (confidential report).

As a final conclusion of the study it is 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 in terms of doses,
three epidemiological studies (Slone, 1976; Shapiro, 1976; Kozer, 2002) are cited: 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 for a 60 kg body weight person) 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.

However, in the conclusion section, the following specific observations were made:

Stillbirths and infant mortality: Randomized Control Trials (and all meta-analyses of those) concluded that there was no increased risk in aspirin-treated women, as did two powerful observational studies. One large-sized study, however, did report such an association, with surprisingly high risk estimates. NOTE. This study (Li DK, Liu L, Odouli R. Exposure to non-steroidal anti-inflammatory drugs during pregnancy and risk of miscarriage: population based cohort study. BMJ 2003; 327(7411):368) does not provide the doses of aspirin taken by the subjects.

Regarding Ductus arteriosus, no association was seen between aspirin use and the risk of pre-term constriction of the ductus arteriosus. The conclusion was drawn based on the following studies:

- CLASP follow-up study: Pregnant women (gestational age at inclusion 12-13 weeks) received either 60 mg/day aspirin or a placebo, during the second and third trimester of pregnancy, to prevent or treat pre-eclampsia or intra-uterine growth retardation. Subjects: 4168 children at 12 months of age (questionnaire to general practitioners) and 4365 assessed at 18 months (questionnaire to parents). No evidence was found of antenatal closure of ductus arteriosus.
- Di Sessa et al. (1994) carried out in Tennessee a 60mg/day aspirin (n=56) vs. placebo (n=57) randomised control trial, during the 2nd and 3rd trimester of pregnancy for assessing aspirin efficacy in preventing pre-eclampsia. There was no association between aspirin treatment and the risk of an alteration of cardiac function.
- Grab et al. (2000) carried out a randomised control trial where 22 pregnant women were randomly allocated to daily treatment with 100 mg aspirin or to a placebo (n=21). The investigators performed pulsed Doppler measurements of the ductus arteriosus at 14 day intervals starting from 18 gestational weeks until delivery. No difference between groups appeared for ductus arteriosus blood flow.

2.3.3. The assessment of “High dose” ASA as prescribed in pregnancy

ASA belongs to the non-steroidal anti-inflammatory drugs (NSAIDs) which may be prescribed at high dose levels for the long-term treatment of a number of severe inflammatory conditions. Only limited information is available on the effects of such prescribed drug use in 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 incidence of 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 during the first trimester.

A small prospective study (Østensen & Østensen, 1996) of long-term intake of NSAIDs for anti-rheumatic therapy in patients with autoimmune rheumatic diseases reported no association of NSAID use with any teratogenic effects. Since
this study has no specific information related to ASA it will be not included in the analysis.

2.3.4. ASA Overdose in Pregnancy

There are few data available on the effects of aspirin overdose in 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 use during pregnancy in Australia at doses which were excessive and can be considered as abusive and toxic. 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 or 510 mg per powder, taking between 2 and 12 doses/day every day throughout pregnancy; 56% used 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 consequences in the foetuses were lower birth weight and increased stillbirth. The data are summarised in Table 4.

Table 4. Summary of developmental effects

<table>
<thead>
<tr>
<th>No</th>
<th>Gender</th>
<th>Gestation (week)</th>
<th>Birth-weight (g)</th>
<th>Maternal age</th>
<th>Salicylate consumption (years)</th>
<th>Pregnancy complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>37</td>
<td>2305</td>
<td>30</td>
<td>14</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>39</td>
<td>3050 (macerated)</td>
<td>35</td>
<td>17</td>
<td>Anaemia</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>36</td>
<td>2570</td>
<td>38</td>
<td>20</td>
<td>APH, PPH</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>36</td>
<td>2490 (macerated)</td>
<td>35</td>
<td>10</td>
<td>None</td>
</tr>
</tbody>
</table>

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

The comments upon these findings are that the still-births in salicylate users were not all clearly related to pregnancy complications, but all occurred in older women who had been taking salicylates for many years. These 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.

However no increase in the 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/day (equivalent to 10-79 mg/kg bw/day as SAL). This level of exposure occurred throughout pregnancy, and specifically throughout the first trimester 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.
It appears difficult 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 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 is cited by the DS with no description and therefore no information on the magnitude of the dose is available.

2.3.5. Salicylate poisoning during pregnancy

For the present analysis, two case reports regarding extremely high doses ingested during pregnancy were chosen from the TOXNET toxicology data network maintained by U.S. National Library of Medicine (http://toxnet.nlm.nih.gov/):

CASE REPORT 1 (Velez et al. 2001)
A 19-year-old woman in her 38th week of pregnancy ingested a total of 16.25 g of aspirin in a suicide attempt. On arrival to the emergency department, the maternal salicylate level was 31.7 mg/dL. Physical examination revealed stable vital signs with mild tachypnea. The patient, however, denied tinnitus, gastrointestinal, or neurological symptoms. Fetal monitoring applied in labor and delivery revealed fetal distress with bradycardia (HR-60) and late decelerations. For this reason an emergency Cesarean section was performed and APGAR scores were noted to be 5 and 7 at 1 and 5 minutes respectively. Bag-valve-mask ventilations were required for a brief period after delivery. Maternal salicylate level drawn just prior to Cesarean section was 14 mg/dL. The baby’s salicylate level, drawn immediately after delivery, was 35.2 mg/dL. Newborn vital signs soon after delivery were BP 65/46, HR 142, RR 58, pulse oximetry 100% on room air. Laboratory analyses showed a pH of 7.49 with a pCO2 of 27 mmHg; electrolytes were normal except for a bicarbonate of 18 mEq/L. Serial aspirin levels showed a value of 26.4 mg/dL at 28 hours, and 8.1 mg/dL at 101 hours post-delivery. The baby was discharged without any obvious problems. This is a case of perinatal aspirin poisoning, where the baby had higher levels than those of the mother. The baby had significant foetal distress with only minor symptoms reported in the mother.

CASE REPORT 2 (Palatnick et al., 2001)
A 17-year-old, 37-week pregnant woman presented to the hospital stating that she had ingested 50 aspirin tablets per day for 1 month in an attempt to harm her baby and herself. Ultrasound showed fetal demise. Serum salicylate was 620 mg/L with an anion gap of 22.6 and the following blood gases: pO2 108 mm Hg, pCO2 15mm Hg, pH 7.34, and HCO3 8.8 mmol/L. She was successfully treated with alkaline diuresis followed by hemodialysis. She spontaneously delivered a macerated stillborn 2380-g fetus. Autopsy revealed diffuse petechiae in the lungs, heart, thymus, and kidneys. Salicylic acid was found in the cord blood, but quantification was not possible due to the small volume of the blood sample. Our patient supports the hypothesis that the fetus is at greater risk than the mother.
in salicylate poisoning during pregnancy. Consideration should be given to emergent delivery of term or near-term, aspirin-poisoned fetuses. Both cases refer to the 3rd trimester of pregnancy. A summary of the doses and effects in human studies is presented in Table 6.

**Table 6.** Summary of doses vs. effect in the human studies

<table>
<thead>
<tr>
<th>Total ASA per day (mg)</th>
<th>Dose as ASA (mg/kg bw/day)*</th>
<th>Dose as SA (mg/kg bw/day)**</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>2.5</td>
<td>1.92</td>
<td>No effect</td>
</tr>
<tr>
<td>3250</td>
<td>54</td>
<td>41.7</td>
<td>Increased gestational length and labour duration</td>
</tr>
<tr>
<td>800 – 6000 (worst case)</td>
<td>13- 100</td>
<td>10 - 77</td>
<td>stillbirths</td>
</tr>
<tr>
<td>16250</td>
<td>270</td>
<td>208</td>
<td>Newborn in high distress</td>
</tr>
<tr>
<td>10000 (calculated for a 200 mg ASA tablet)***</td>
<td>166</td>
<td>128</td>
<td>Fetal death</td>
</tr>
</tbody>
</table>

*Calculated for a woman of 60 kg  
** Calculated with a conversion factor of 0.77  
*** The dose might be much higher depending on the dosage of the ingested tablets (not reported)

A summary of salicylate plasma levels is presented in Table 7.

**Table 7.** Plasma levels in the human studies

<table>
<thead>
<tr>
<th>Plasma level mother (microgram/ml)</th>
<th>Plasma level baby (microgram/ml)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 70</td>
<td>Up to 90</td>
<td>stillbirths</td>
</tr>
<tr>
<td>317 on arrival 140 at cesarean</td>
<td>-</td>
<td>Newborn in high distress</td>
</tr>
<tr>
<td>620</td>
<td></td>
<td>Foetal death</td>
</tr>
</tbody>
</table>

2.3.6. Comparative plasma levels of salicylic acid in humans versus rats

The analysis presented by the DS is summarised below (see also Annex 3).

- **SAL plasma levels achieved in human epidemiology studies**

The DS presented two publications where SAL plasma levels have been measured after ASA administration at several high dosages in patients treated for rheumatoid arthritis or non-inflammatory back pain.

In the first publication (Bochner et al., 1987), 8 patients (4 males and 4 females) received 1, 2 and 4 g enteric coated aspirin (ASA) daily in ascending order. Initially, patients received one capsule containing 500 mg ASA twice per day for 2 weeks. For the second two week period this dose was doubled and for the final two week period it was quadrupled. At the end of each 2-week dosage period, plasma and urine were collected over a dosage interval for the estimation of various pharmacokinetic parameters. Results clearly demonstrated the non-linearity of the dose/concentration relationship with an increase of plasma level much greater than dose increase. This was confirmed in the study of Gibson et al. (1975) where 9 patients received 65 mg/kg bw/day ASA daily, divided in four doses. Peak plasma salicylate was measured after three days and then, for four
patients, investigation was prolonged over 11 days with a dose of 100 mg/kg bw/day according to the same protocol. Mean plasma concentration at 100 mg/kg bw/day was almost double that obtained from dosing at 65 mg/kg bw/day. The data are summarised in Table 8.

**Table 8.** Plasma concentrations of SAL in humans

<table>
<thead>
<tr>
<th>Mean Dose ASA (mg/kg bw/day)</th>
<th>Mean Dose calculated as SAL (mg/kg bw/day)*</th>
<th>Mean peak plasma SAL (μg/mL)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.5</td>
<td>11.93</td>
<td>22±2</td>
<td>Bochner <em>et al.</em>, 1987</td>
</tr>
<tr>
<td>31</td>
<td>23.87</td>
<td>64±6</td>
<td>Bochner <em>et al.</em>, 1987</td>
</tr>
<tr>
<td>61.9</td>
<td>47.43</td>
<td>181±19</td>
<td>Bochner <em>et al.</em>, 1987</td>
</tr>
<tr>
<td>65</td>
<td>50.05</td>
<td>180</td>
<td>Gibson <em>et al.</em>, 1975</td>
</tr>
<tr>
<td>100</td>
<td>77</td>
<td>343.75</td>
<td>Gibson <em>et al.</em>, 1975</td>
</tr>
</tbody>
</table>

*Conversion factor: 0.77

**- SAL plasma levels achieved in the rat**

The plasma levels in rats were taken from two publications already presented in the section dedicated to animal studies. The values used for the present analysis are presented in Table 9.

**Table 9.** Plasma concentrations of SAL in rat

<table>
<thead>
<tr>
<th>Dose SAL (mg/kg bw/day)</th>
<th>Plasma SAL (μg/mL)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>221, at 3 h after the last administration, mean plasma level following 7 days exposure</td>
<td>Tanaka (1973), gavage</td>
</tr>
<tr>
<td>167 (estimated)</td>
<td>116, mean plasma level following 7 days exposure</td>
<td>Tanaka (1973), diet</td>
</tr>
</tbody>
</table>

**- Comparative analysis**

Using the data from the studies of Bochner & Gibson, a graph was plotted giving a “reference curve” of SAL dose versus plasma SAL level in humans.
Then, on the same graph the data from the rat studies are plotted. The next step was to deduce the equivalent doses in humans corresponding to the plasma levels in rats by interpolation. Consequently, it was determined that the level of SAL plasma achieved in the rat gavage study (221 micrograms/mL) corresponds to a dose of 56 mg/kg bw/day as SAL in humans and the level of 116 micrograms/mL in the rat diet study corresponds to a dose of 32 mg/kg bw/day as SAL in humans.

The dose levels of 150 and 167 mg SAL/kg bw/day, delivering serum SAL concentrations of 221 and 116 µg/mL respectively in the studies of Tanaka (1973a & 1973b) represent the threshold for developmental toxicity of SAL in the rat. For a 60 kg woman, the serum concentration of 221 µg/mL was shown by graphical interpolation to be achieved from a dose of about 73 mg ASA/kg bw/day (56 mg SAL/kg bw/day). This is equivalent to a daily consumption of approximately 2.7 g ASA/day (i.e. slightly more than five 500 mg tablets per day).

Based upon the above comparison, the absence of developmental toxicity in pregnant women at dose level toxic to the mothers is observed at SAL serum concentrations at least equal to those where rat studies demonstrate clear teratogenic effects.

**Table 10. Corresponding doses to percentages used in the diet study**

<table>
<thead>
<tr>
<th>Document</th>
<th>Equivalent value for 0.06%</th>
<th>Equivalent value for 0.1%</th>
<th>Equivalent value for 0.2%</th>
<th>Equivalent value for 0.4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLH Report</td>
<td>30 mg/kg bw/day</td>
<td>50 mg/kg bw/day</td>
<td>100 mg/kg bw/day</td>
<td>200 mg/kg bw/day</td>
</tr>
<tr>
<td>Additional info</td>
<td>50 mg/kg bw/day</td>
<td>83 mg/kg bw/day</td>
<td>167 mg/kg bw/day</td>
<td>333 mg/kg bw/day</td>
</tr>
</tbody>
</table>
2.4 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 (Annex 4). The reprotoxicity evaluation concluded that: “A NOAEL of sodium salicylate administered orally to mated rats has been established to 80 mg/kg/day corresponding to 69 mg/kg/day of salicylic acid. The results also showed that following oral administration, salicylic acid is not teratogenic or embryotoxic up to 75 mg/kg/day in rodents and up to 100 mg/kg/day in Monkeys. 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\(^3\) 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 alimentation were approved as flavouring ingredients quantum satis (Regulation EU No 872/2012 of 1 October 2012).

2.5 ADDITIONAL REFERENCE TO THE CLH REPORT


\(^3\) http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_097.pdf
COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: Salicylic acid
CAS number: 69-72-7
EC number: 200-712-3
Dossier submitter: Industry (NOVACYL S.A.S.)

GENERAL COMMENTS

<table>
<thead>
<tr>
<th>Date</th>
<th>Country</th>
<th>Organisation</th>
<th>Type of Organisation</th>
<th>Comment number</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.12.2014</td>
<td>France</td>
<td>MemberState</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Comment received

MS-FR agrees with the proposed classifications for acute toxicity and eye damage. Nevertheless, we have specific comments regarding reproductive toxicity endpoint and environmental hazards (see below).

**ECHA note: Please refer to comments 4 and 13.**

Dossier Submitter’s Response

Environmental hazards were not documented because not requested in the CLH dossier. And, as salicylic acid is a natural substance, environmental assessment is particular, See comment 13.

<table>
<thead>
<tr>
<th>Date</th>
<th>Country</th>
<th>Organisation</th>
<th>Type of Organisation</th>
<th>Comment number</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.12.2014</td>
<td>Germany</td>
<td>MemberState</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

Comment received

The German CA supports the CLH proposal of Salicylic acid. There were differences in self-classification between different notifiers in the C&L Inventory and the registration dossier. The CLH proposal aims to harmonise these endpoints where there was no agreement. In particular, deeper analysis of the reproductive toxicity endpoint, including an epidemiology literature analysis was performed.

Dossier Submitter’s Response

This summarises very concisely our position.
The Netherlands was the rapporteur member state (RMS) for the biocide application of salicylic acid. During the assessment of the provide data a concern regarding the classification for developmental toxicity was identified and discussed with the applicant in several meetings. However, the CAR has not yet been finalised. Therefore, no CLH proposal from the RMS is available.

The scope of the CLH proposal submitted by industry was limited to three endpoints (Acute toxicity, serious eye damage/eye irritation and reproductive toxicity). We can support the conclusions on acute toxicity and serious eye damage/eye irritation. However, we disagree with the absence of a classification proposal for developmental toxicity. Our comments are focused to developmental toxicity.

Dossier Submitter’s Response

The submitter was not involved in the Biocide submission. The Biocide dossier was prepared in 2007 by a downstream users consortium, on the basis of a previous application in NL by one user. The salicylic acid (SA) REACH registration dossier submitted in 2010 was updated in 2013, then improved in the CLH IUCLID file, by the Submitter, according to other salicylic compounds and particularly acetylsalicylic acid, with new information found in Rainsford book (Aspirin and related drugs, 2004) and the epidemiological analysis done by an expert (Pr Denis Bard, EHESP, France, 2012, report provided as Annex 1 of CLH report, in IUCLID file chapter 13). These documents are provided with the RCOM.
**ANNEX 1 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON SALICYLIC ACID**

**TOXICITY TO REPRODUCTION**

<table>
<thead>
<tr>
<th>Date</th>
<th>Country</th>
<th>Organisation</th>
<th>Type of Organisation</th>
<th>Comment number</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.12.2014</td>
<td>France</td>
<td>MemberState</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

Comment received

The reproductive endpoint for which the dossier submitter proposes no classification is partially based on data with methylsalicylate. Methylsalicylate has been identified because of concern related to embryofoetotoxicity. Indeed, methylsalicylate is included in the Corap list (FR as e-MSCA; 2015) based on the following concern: Available data with MeS give hint that it could be embryofoetotoxic. From the tremendous amount of data presented for read-across from aspirin and salicylic acid, the results of the key and supporting studies suggests that salicylic acid has embryofoetotoxic effect in rats at doses not causing clear maternal toxicity, with evidence of malformations at maternally toxic doses (registration data). Therefore this point deserves to be evaluated. Therefore, after evaluation, FR will gain confidence on this endpoint and might have additional information than those presented in this proposal.

We consider that the level of details of each study presented in the CLH report is not sufficient to assess this concern for salicylic acid and its subsequent classification. For example, the read-across between salicylic acid and methylsalicylate or acetylsalicylic acid is not sufficiently justified, only NOAEL/LOAEL are reported for experimental studies. Indeed, some effects were observed in experimental studies such as reduced pup viability, resorptions and malformations reported in several studies. The CLH report concluded that these effects were not applicable to humans. Some human data with acetylsalicylic acid have shown adverse effects such as increased miscarriage but their relevance has not been discussed in the dossier so far.

However, based on the level of details provided, we cannot conclude on the relevance of these effects in humans and we believe that RAC cannot conclude either. Furthermore, if further justification will be provided during the RCOM period, we cannot judge the reliability of the given information and we consider that this point should not warrant any RAC discussion unless any relevant information has gone through public consultation.

**Dossier Submitter’s Response**

“this point (developmental toxicity endpoint) deserves to be evaluated”

The Submitter has registered as Lead Registrant in 2013, acetylsalicylic acid (ASA, initial registration) then has submitted updates of salicylic acid (SA) and methyl salicylate (MeS) initial dossiers on the basis of ASA dossier data analysis. These updates have been made because in the ASA registration dossier, an additional review book (Rainsford, 2004), and a new expert report on epidemiology (Bard, 2012, report provided as Annex 1 of CLH report, in IUCLID file chapter 13).) have been considered, leading to conclusions for ASA that could be applied to salicylic acid and methyl salicylate as well, due to the demonstration of a common metabolic pathway, and therefore a common mechanistic mode of action. The practical consequence is that the “developmental toxicity” chapters in the IUCLID files of all 3 substances are identical.

In the MeS dossier, the endpoint discussion begins with the sentence: “No developmental toxicity studies according to current guidelines are available for MeS itself. Assessment has
been made by read-across primarily from studies on SA and ASA”. See below the read-across rationale. After this statement, the chapters in the CLH report from page 89 to 92 (“Developmental toxicity, animal data” and “Key information on effects on both fertility and development from human information”) were written.

“the read-across between salicylic acid and methylsalicylate or acetylsalicylic acid is not sufficiently justified”

The rationale for this read-across is as follows:
Salicylic acid is the common metabolite of salicylates, and all salicylates are “active” through this metabolite, therefore the mode of action is common (except for antithrombogenic effects of ASA). In the organism within a few minutes, methyl salicylate (MeS) and acetyl salicylate are transformed in SA (see for more details Rainsford, 2004, Chapter 4)

Davison et al. (1961) reported that gavage of Methyl salicylate, Sodium salicylate and Acetyl salicylate to Wistar rats at doses equivalent to 500 mg/kg Salicylic acid resulted in the appearance of hydrolyzed free salicylate in both the plasma and brain tissue within 20 min. This study showed rapid hydrolysis to free salicylate from Methyl salicylate, Sodium salicylate and Acetyl salicylate, with comparable plasma concentrations of salicylate at 60 minutes post dosing, with no measurable parent compound. In the same study, the authors demonstrated that the major site of hydrolysis of methyl salicylate in the rat, rabbit, dog and monkey is the liver. These results indicated that following absorption, the initial metabolic step for all these salicylates (MeS, NaS and ASA) is hydrolysis to free salicylate.

Rainsford and his colleagues (1980) compared the distribution of acetylsalicylic acid (ASA), salicylic acid (SA) and the methyl ester of ASA in rats. Salicylic acid was found in the stomach, liver, kidney, lungs, bone marrow, intestine, inflamed paws and spleen. The methyl ester of ASA 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 salicylic acid versus acetylsalicylic acid in mice after injection of these compounds.

The pathways of biotransformation of ASA, SA, MeS, NaS and other salicylate esters are considered to be the same following initial hydrolysis to free salicylate. In qualitative terms, types of adverse effects reported from all of these salicylates is predicted to be similar, (except for antithrombogenic effects of ASA) supporting a read-across approach of toxicological data between these substances (see Rainsford, 2004).

A very complete analysis of metabolism data is done in the Rainsford Book (ASPIRIN and related drugs, Chap 4, 2004), used as reference in the IUCLID registration file and provided as attached document.

“Some human data with acetylsalicylic acid have shown adverse effects such as increased miscarriage but their relevance has not been discussed in the dossier so far. However, based on the level of details provided, we cannot conclude on the relevance of these effects in humans”

Relevance of human data with acetylsalicylic acid has extensively been reviewed in the report by Pr Denis Bard attached as Annex 1 in the CLH dossier as well as in IUCLID chapter 13 “Assessment reports” and in IUCLID chapter 7.8 “Reproductive toxicity Endpoint summary”). Details have not all been reported as IUCLID summaries, as 90 references have been analysed, among them some meta-analyses, but are discussed in depth in the report of Pr. D. Bard (provided with the RCOM document)

Hereafter, conditions, conclusions, and general comments of the report by Pr. D. Bard are cited (please note it is an extract of a whole document an some parts may not be self-explaining and need to refer to the whole text) :
Conditions
In this review, it is considered that salicylic acid (SA) risk assessment for reproductive outcomes is best approached by studies on the same outcomes associated with acetylsalicylic acid (ASA), since SA is the initial metabolite of ASA. In addition, no epidemiological data seem to be available for exposure to SA. This work is not intended as an exhaustive review on reproductive and teratogenic risks of SA in humans, since i) the literature available on these topics is very large; ii) some investigations date back to the 1950s, raising questions about the relevance of observations made in the population in these times for the present populations; iii) the literature from this early period up to the year 1989 was reviewed in-depth by Hertz-Picciotto et al. (1990)\(^1\).

Rather, a reasoned approach was adopted, considering the most important-or most cited-papers from the pre-1989 period and analyzing comprehensively the literature from 1989 up to the current times. Papers of similar quality leading to discrepant conclusions are specifically discussed. Also, when available, meta-analyses were preferred to the discussion of each paper included in these analyses. Finally, studies considering only non-steroidal anti-inflammatory drugs (NSAIDs) other than aspirin (e.g.\(^2\)) were not discussed in this aspirin-targeted review, although this group is considered to share a common mechanism of action, that is, the inactivation of the cyclooxygenase (COX) enzyme, which is required for prostaglandin synthesis.

Conclusions
Considering the various outcomes, the results of this review are in summary the following:

For maternal bleeding, 3 observational studies found an increased risk, whereas only one out of 10 randomized control trials (RCTs) found such an effect.

For neonatal hemostatic abnormalities, 3 observational studies found an increased risk; one (the most powerful) did not, whereas two out of 14 RCTs found such an effect.

Pregnancy duration and labor: One observational study and one RCT found an increase in gestation duration, other RCTs showing no such association. For labor duration, only one observational study concluded to such association. The most powerful meta-analysis of available RCTs, as of 2007, did not show any effect of aspirin treatment on both outcomes.

Prevention of pre-eclampsia and intra-uterine fetal growth retardation: All the RCTs figured out in this review were conducted to study the effect of aspirin in the prevention of pre-eclampsia. In spite of some inconclusive studies, most concluded to a modest, positive effect of aspirin in the prevention of pre-eclampsia, which was also the conclusion of meta-analyses. However, it appears difficult to conclude on whether this positive effect applies only to high-risk women or are more generally valid. The same statement applies to the optimal timing of treatment, since published results do not allow to concluding.

Stillbirths and infant mortality: RCTs (and all meta-analyses of those) concluded to no increased risk in aspirin-treated women, as did two powerful observational studies. One large-sized study\(^89\), however, did report such an association, with surprisingly high risk estimates.

Birth weight: Most RCTs concluded to no birth weight differences between infants whose mother was either aspirin-treated or placebo-treated during pregnancy. However, the Cochrane review\(^16\) of these RCTs concluded to a small but significant increase in birth weight in infants born from aspirin-treated women whereas other meta-analyses, less powerful, did not.
**Birth defects:** Taking into account only the prospective studies of sufficient size, it is concluded to no birth defect excess in women having taken aspirin during the 1st trimester of pregnancy, whereas 2 studies found a significant association. Case-control studies found more frequently an increased risk in exposed women (3/5).

No association was seen between aspirin use and the risk of pre-term constriction of the ductus arteriosus.

As regards gastroschisis, the results of case-control studies were inconsistent, the same groups reporting different results from the same database across different papers and time periods. It should be mentioned that a meta-analysis considering the case-control studies conducted before 2005 found an elevated risk. Prospective studies do not report an elevated risk of gastroschisis. Thus, it is not warranted to conclude to an association between aspirin use during the 1st trimester of pregnancy and an increased risk of gastroschisis, although it cannot be completely ruled out, see also below 'general comments'.

Conversely, available good-quality studies essentially do not show an increased risk of cardiac defects in aspirin users, as it appears from a meta-analysis of studies published prior to 2001 and in a more recent one.

For cleft palate, most studies, in particular prospective ones, did not show an association with aspirin taking in pregnancy. Only 2 prospective studies out of 8 and one case-control study out of 6, found such an association.

For central nervous system and neural tube defects, most studies did not found such association.

Some excess risk of specific birth defects were occasionally reported, e.g., pyloric stenosis or hypospadias.

**Early childhood development:** Children neurodevelopment and intelligence are the results of an extremely complex array of influences, be they proximal (such as *in utero* exposure to aspirin, nature of food, pollutants such as lead) or more distal ("the causes of the causes"). Thus, in the absence of a convincing argument on a mechanism of action, positive associations such as that found by Streissguth et al. should be considered with an extreme caution.

**General comments**

The case-control studies yielded overall inconsistent results, but tended to be more frequently positive than the cohort studies. The possibility of a recall bias, in particular in those studies where exposure was assessed retrospectively, is not supported by all such studies that conclude to no association between aspirin taking and the outcome. The reasons for such discrepancy are all but clear. Publication bias doesn't appear to play a major role in case-control studies, as it appears from meta-analyses. The meta-analyses results are not consistent overall.

Bradford-Hill considerations for causation, that is, the strength of associations, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment and analogy are to be put forward here.

The above results show no consistency overall, although some associations are found high in several studies for a given endpoint.

Findings are by no means specific: to address this point, it can be said that not only medications studied often included specialties mixing different molecules (e.g., aspirin and
dipyridamole) but in many studies the quality of the data doesn't allow a separate analysis of the effects of aspirin alone, i.e. conversely, the possible effects of aspirin may be caused by other agents.

As regards biological gradient, observational studies never provided accurate dose estimates. The pattern of aspirin taking by the subjects studied (doses and time course) is often self-reported, sometimes a long time after pregnancies, notwithstanding with recall bias, e.g., in case-control studies. In addition, even when drug consumption is recorded during antenatal visits or characterized from prescription data, there is little means to check whether over-the-counter medications were accurately recorded. If such unrecorded consumption is greater in pregnant women who had an abnormal outcome, this may lead to underestimate the true effect of SAL, if any. Low dose testing was in fact addressed only in randomized control trials, e.g., for assessing the efficacy of aspirin low doses in the prevention of pre-eclampsia and IUGR, except in the study by Czeizel et al. (2000) where low-dose aspirin was explicitly investigated.

As regards plausibility, much is known on the basic mechanism of action (see introduction). However, addressing coherence (a causal conclusion should not fundamentally contradict present substantive knowledge), either conclusion of the existence or absence of an association between aspirin and a specific outcome would not fundamentally contradict the present state of knowledge. Experimental evidence, in particular in the light of effects observed in the laboratory animal, raises difficult questions, in particular for teratogenicity, although this point is not addressed in the present review. Analogy is a very general viewpoint, that is, some drugs are teratogenic in man, so other drugs may be, too. Nevertheless, considerations on plausibility, coherence, experimental evidence and analogy are most useful when an association is convincingly assessed, including consistency across studies.

Some additional considerations should be discussed: It should be kept in mind that the subjects tested in a RCT are usually highly selected through stringent inclusion/exclusion criteria. As a result, a simple extension of RCT conclusions to the general population cannot be straightforward. In addition, these studies were designed to test treatments for specific conditions (e.g., pre-eclampsia), often for high-risk pregnancies. Thus, inferring results for the general population as regards outcomes such as pregnancy duration, not specifically targeted by the study design, is questionable.

For birth defects, not all studies considered mothers' medical conditions during the 1st trimester, that is, viral fever that could lead to birth defects. In such a case, aspirin taken for treating fever may be seen wrongly as a risk factor. In addition, symptoms produced in the mother by a congenitally abnormal fetus might result in a significant association between any drug used for the treatment of these symptoms and the congenital abnormality. Finally, birth defect studies considered only live births, so birth defects risk may have been underestimated since dead-born infants were not accounted for. Furthermore, a drug inhibiting the spontaneous abortion of already malformed embryos might wrongly appear to be responsible of birth defects.

As a final conclusion, no adverse effect of aspirin treatment can be considered as established, either at low (150 mg daily) or higher, usual dose. Low-dose aspirin 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.

References cited in above extract:
1. Hertz-Picciotto I, Hopenhayn-Rich C, Golub M, Hooper K. The risks and benefits of taking...

Complete reference of Rainsford Book:
A quick search on the websites Toxnet (toxicology data network, US) and eMC (electronic Medicines Compendium, UK) clearly indicate some concerns when using salicylic acid/aspire during the pregnancy and lactation. Here is an example of information found on aspirin and salicylic acid in those database:

- Aspirin 300mg Gastro-resistant Tablets:
  **Pregnancy**
  Although clinical and epidemiological evidence suggests the safety of aspirin for use in pregnancy, caution should be exercised when administered to pregnant patients. Aspirin has the ability to alter platelet function and, therefore, there may be a risk of haemorrhage in infants whose mothers have consumed aspirin during pregnancy. The onset of labour may be delayed and the duration increased, with an increase in maternal blood loss. Therefore, analgesic doses should be avoided during the last trimester of pregnancy. High doses of aspirin may result in closure of foetal ductus arteriosus in utero and possibly persistent pulmonary hypertension in the new born. Kenicterus may be a consequence of jaundice in neonates. Administration of aspirin at doses greater than 300 mg/day, shortly before birth, can lead to intra-cranial haemorrhage, particularly in premature babies.
  **Lactation**
  The intake of aspirin by breast-feeding patients is contraindicated as there is a risk of Reye's syndrome. Regular use of high doses could impair platelet function and produce hypoprothrombinaemia in the infant if neonatal vitamin K stores are low. [https://www.medicines.org.uk/emc/medicine/29215#CONTRAINDICATIONS](https://www.medicines.org.uk/emc/medicine/29215#CONTRAINDICATIONS)

- Salicylic Acid
  **Teratogenicity:** There is no evidence that moderate therapeutic doses of salicylates cause fetal damage in human beings; however, babies born to women who ingest salicylates for long periods may have a significantly reduced mass at birth. In addition, there is an increase in prenatal mortality, anemia, antepartum and postpartum haemorrhage, prolonged gestation and complicated deliveries. These effects occur when salicylates are administered during the third trimester, and thus its use during this period of pregnancy should be avoided. [http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~Om5Vgz:3](http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~Om5Vgz:3)

Also in the eMC database where salicylic acid is used in some medicines, it is reported that even if there are no known contra-indications to use of this medicine during pregnancy and lactation, the safety has not been established yet. This medicine should therefore be used with caution or following professional advice.

This quick screening in those databases indicates some concerns mainly related to development and lactation. We consider that this information should be deeply assessed as this could be supportive evidence for classification.

**Dossier Submitter’s Response**

This type of statements is at first a general precaution for drugs during pregnancy. For Acetyl Salicylic Acid (ASA) it is said "to avoid during the 3d trimester" due to effects on coagulation (except when mothers have such problems and are followed by physician) and due to the action on prostaglandins (i.e. the mode of action) like other Non Steroidal Anti Inflammatory drugs (NSAID). The recommended subacute high dose is 3000 mg/d (60...
mg/kg). The usual dose for cardiovascular-antithrombotic effects is 100-200 mg/day. Teratogenic effects of salicylic acid were analysed in the Pr Denis Bard Epidemiology report provided as Annex 1 of CLH report, in IUCLID file chapter 13). (Bard, 2012) and “no evidence of effects due to aspirin in pregnancy” was concluded (see comment 4 and report provided with the RCOM document).

The following reference has been analysed in (Bard, 2012):

THE RISKS AND BENEFITS OF TAKING ASPIRIN DURING PREGNANCY

IRVA HERTZ-PICCIOTTO,1 CLAUDIA HOPENHAYN-RICH,2 MARI GOLUB,3 AND KIM HOOPER4

In nancy (21). In 1988, the US Food and Drug Administration proposed the following warning label for products containing aspirin: “IMPORTANT: Do not take this product during the last three months of pregnancy unless directed by a doctor. Aspirin taken near the time of delivery may cause bleeding problems in both mother and child” (123). On April 6, 1990, the


RAC’s response

The quantitative ranges of the doses for the onset of the emphasized effects are not mentioned; therefore, it is difficult to use the information for regulatory purposes. RAC agrees that the teratogenicity aspects have been analysed according to the DS’s response.
The conclusion that salicylic acid does not present a developmental toxicity hazard for humans and that classification as such is therefore not appropriate is supported.

Results from developmental toxicity studies with salicylic acid in rats have shown reduced fetal viability and delayed development at doses below those causing evident maternal toxicity and with malformations at maternally toxic dose levels. Results from developmental toxicity studies with acetylsalicylic acid (aspirin, its metabolite being salicylic acid) in rats, mice and rabbits have led to the conclusion that there are considerable species differences in sensitivity, with the rat being a particularly sensitive species. Human epidemiological data on aspirin have clearly demonstrated the absence of developmental toxicity in pregnant women at dose levels toxic to the mothers and delivering salicylic acid serum concentrations at least equal to those where rat studies demonstrate clear teratogenic effects. The human epidemiological studies are therefore considered fully representative of human exposure.

Dossier Submitter’s Response
In accordance with the Submitters assessment.

The Netherlands notes the following:
Salicylates have a long history of use and are still used to date in human medicine, food products and cosmetics. Epidemiological studies on the effects of acetylsalicylic acid on the development of the unborn child do not show consistent results. It seems reasonable to conclude that at low exposure levels of humans salicylates are likely to be safe. However, a number of guideline or similar studies on salicylates including salicylic acid (SAL) (Tanaka, 1973a, 1973b, 1974), sodium salicylate (Fritz, 1990) and acetylsalicylic acid (ASA) (Gupta, 2003) report developmental toxicity in rats. Dose levels not clearly maternally toxic have shown reduced pup viability, while studies at higher, maternally toxic, dose levels have shown delayed development, variants and/or malformations. In addition in a limited study with ASA in rats and rhesus monkeys malformations were reported (Wilson et al., 1977). In the monkeys, 3 out of 15 fetuses at 300 mg/kg bw/d had malformations. At 200 mg/kg bw/d no malformations were observed. The results indicate that ASA may induce teratogenic effects in monkeys. This is in line with the findings in rats, although malformations occur in rats at lower doses. Although the study in monkeys has its limitations, it can be considered supportive of the classification of salicylic acid for developmental effects. It is noted that the fact that treatment of the monkeys covered only
a part of the organogenesis does not disqualify the findings. No developmental toxicity was observed in studies with ASA in rabbits. No developmental toxicity studies in mice were available. The reproductive toxicity studies in mice that are referenced in the CLH proposal are not appropriate to conclude on the teratogenic effects of ASA and SAL in mice, although the studies do show developmental toxicity (e.g. decreased number of live pups, decreased pup weight. It is further noted that in the NOVACYL position paper it is stated that ASA and SAL do not induce developmental toxicity in the mouse. However, in the referenced study (Takahashi, 1985) the developmental toxicity of ASA and SAL was not investigated. In the C&L proposal a number of arguments are put forward in favour of the applicant’s conclusion that no classification for developmental toxicity is required.

- In the NOVACYL position paper it is suggested that rats may be not an appropriate species to study the effects of salicylates on the developing embryo since the placental disposition in the rat may lead to higher fetal salicilate levels in the rat. Although this may be the case, no data are provided to support this suggestion. It is also not indicated how much higher the fetal salicylate levels would be as compared to the human fetus at equimolar maternal plasma concentrations. Furthermore, the Netherlands recognizes that rats may be more susceptible than humans to developmental toxicity induced by salicylates, although the experimental evidence for this supposition is limited. But even if rats are more susceptible than humans, effects observed in this species can still be considered relevant for humans.

- It is also suggested that lower doses of salicylates in humans (on a mg/kg bw basis) lead to similar plasma levels as in rats treated with higher doses. This assumption is based on kinetic studies in males and non-pregnant female patients (Bochner et al., 1987 and Gibson et al., 1975). These studies indicate a peak plasma concentration of around 180 ug/ml at an external ASA dose of 60 – 65 mg /kg bw/day and around 340 ug/ml at 100 mg ASA/kg bw/day. The peak plasma levels did not vary strongly between exposures (twice daily) especially for the high exposure level (Bochner et al, 1987). The lower plasma concentration is within the therapeutic concentration range of 150 – 300 ug/ml required for optimal anti-inflammatory activity and the higher is above the concentration of 300 ug/ml where more serious adverse effects occur (Martindale, The complete drug reference on line, third quarter 2012). There are only two rat studies in which SA levels were determined at the LOAEL for developmental effects. In the dietary developmental study by Tanaka et al (1973), serum levels were determined after 7 days of feeding 0.2% SA (equivalent with approximately 165 mg/kg bw/day) at an unknown point in time during feeding. The serum SA level was 116±9 ug/ml. However, it is unclear whether this value is representative of the steady state level of SA in the serum of rats. Rats normally eat in the evening and morning and when the blood is sampled during the day, there is a period over which the SA concentration may decline. This decline depends on the time between feeding and blood sampling and the kinetics in rats. In the gavage developmental study by Tanaka et al (1973), the blood was sampled at 3 hours after the last of 7 daily gavage treatments with 150 mg/kg bw/day. The average SA serum value was 247±21 ug/ml. However, it is unclear whether the value after 3 hours is a value that can be compared to the values observed for humans.

As this comparison is based on pregnant rats versus non-pregnant humans and it is known that pregnancy can affect the plasma protein concentration, the relevancy of this comparison can be doubted. Therefore, as there is no information provided on the kinetics of SA in rats, it cannot be excluded that the measurement in rats was performed well before or after the Tmax and that the peak value is much higher. Also there is no information from pregnant women. Thus, it cannot be concluded on the basis of the available data that therapeutic doses in humans lead to plasma levels similar to those in rat studies in which teratogenic effects of ASA and SAL are observed.

- Further, it is argued that, although no maternal toxicity was observed in the developmental study in rats at dose levels inducing developmental toxicity, maternal
toxicity should be expected at these dose levels. The absence of maternal toxicity was argued to be due to the limited number of parameters that was measured. Assessment of the possible effect of maternal toxicity on the developmental toxicity should focus on SA and should not use ASA as ASA has additional toxicodynamic effects via acetylation of COX. Also the comparison as provided in Annex 5 of the Annex on plasma levels is inconsistent as the available study on MeAS with a high dose of 2% (=1000 mg/kg bw/day) showed no haemorrhagic effects whereas a reduced prothrombin index was observed with SAL at 204 mg/kg bw (Takahashi, 1985). Therefore, it cannot be concluded that ASA and SA have similar haemorrhagic potential. In addition it is more relevant to compare the 7-day exposure study than the 28-day exposure study with the developmental study with exposure from day 8 to 14 (Tanaka, 1973 and Tanaka, 1974). In the 7-day study with ASA by Takahashi (1985), the NOAEL for haemorrhagic effects was 150 mg/kg bw/day and the LOAEL of 300 mg/kg bw/day.

The Netherlands recognizes that it is possible that developmental toxicity may have occurred in concomitant with maternal toxicity. However, the Netherlands notes that the presence of maternal toxicity is not determinative for the classification for reproductive toxicity. The relevant question is whether the developmental effects are secondary to maternal toxicity. It cannot be concluded from the limited available data that there was marked maternal toxicity at the relevant dose levels in the developmental rat study and furthermore there is no evidence that the developmental effects were secondary to those maternal toxic effects (if present) that, even if developmental effects are induced at high doses only, i.e. doses that would be considerably higher than those to which humans are likely to be exposed to, classification of salicylic acid is warranted, since classification of substances is based on hazard rather than potency/risk.

The dossier submitter states that there is a difference in plasma binding between humans and rats however this is based on information from non-pregnant rats and humans. According to Rainsford (2004, page 126)

Conclusion

In conclusion, there are clear developmental effects in the rat. These effects are not considered secondary to the maternal toxicity. Systemic exposure of humans to SA due to intake of ASA seems not to clearly result in comparable developmental effects but does result in comparable effects during parturition. Based on all available information it is considered that the use of low doses of salicylates during pregnancy is safe. However, the epidemiologic database on highly exposed pregnant females is too small to conclude on the absence of developmental effects in humans. In addition, too little information is available on kinetic or dynamic differences in rats and humans that could justify why the effect in rats would not be relevant to humans. In view of this, based on the teratogenic effects of salicylates in rats and limited evidence in monkeys, but not in rabbits, the Netherlands proposes that salicylate should be classified with Repr 1B, H360D/May damage the unborn child.

Dossier Submitter’s Response

“Epidemiological studies on the effects of acetylsalicylic acid on the development of the unborn child do not show consistent results. It seems reasonable to conclude that at low exposure levels of humans salicylates are likely to be safe.” This has been analysed in D. Bard, 2012 (Annex 1 in chapters 13 and attached in 7.8 in the IUCLID file and provided here with the RCOM document). The overall conclusion (see comment 4 for detailed conclusions) is:

As a final conclusion, no adverse effect of aspirin treatment can be considered as established, either at low (150 mg daily) or higher, usual dose. Low-dose aspirin 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.

“Although the study in monkeys has its limitations, it can be considered supportive of the classification of salicylic acid for developmental effects”

The monkey study had been assessed but rated as reliability 3 (invalid) e.g. due to lack of controls. Considering the large human database, and its analysis (Bard, 2012), it seems inappropriate to use animal study results of low reliability.

“The reproductive toxicity studies in mice that are referenced in the CLH proposal are not appropriate to conclude on the teratogenic effects of ASA and SAL in mice”

The submitters agrees with this comment. The NTP (1984a, b) studies in mice have indeed been referenced for effects in fertility, but not for developmental effects. However, some parameters of development have been observed, and they were negative, while the same parameters were positive in studies in rats.

“in the referenced study (Takahashi, 1985) the developmental toxicity of ASA and SAL was not investigated”

The submitter agrees with this comment. Takahashi (1985) reports on bleeding effects in rats and mice, not directly on developmental effect as was erroneously written in the document. It was intended to push forward that the bleeding effects induced by ASA (and SA) are at lower doses in rats than mice, and associated with gut ulcerogenic activity, typical toxicological effects in rats not reported in reprotoxicological studies, and below the official criterion of maternal toxicity “Body weight gain” (see Rainsford, 2004, Chap. 8, Table 8.12 and 8.14). Therefore one should read in NOVACYL ASA reprotox position paper provided as Annex 2 in IUCLID chapter 13, page 2 the following amended (underlined) text:

“Species differences
  - The mouse did not showed the bleeding effects/gut ulcerogenic activity seen in the rat for ASA and SA (ulcerogenic), even at higher doses (Takahashi 1985).
  - Similarly the NTP for Mouse / MeSal (NTP, 1984 a, b): the developmental effects seen in rats are not retrieved in parameters observed in mice.
  - For ASA, the Rat (Gupta, 2003) showed effects at high doses (NOAEL maternal and fetal : 50 mg/kg), not seen in rabbits (Cappon, 2003) at 350 mg/kg with a lower maternal toxicity.

…”

Comments on rationale used for concluding that the Rat is not an appropriate species for assessing Human developmental toxicity

As developed in the CLH report p. 90, and more in-depth in the IUCLID file, the arguments are the following ones:

1. In reliable studies on developmental effects of salicylates performed in different species (rat (Tanaka 1973a, 1973b, 1074, Gupta 2003), rabbit (Cappon, 2003), mice (MeSA, NTP, 1984), there are differences in sensitivity in these species.

2. Several mechanistic possibilities as the underlying reason for species specific sensitivity were assessed and the metabolic one could explain simply the situation (see Rainsford Chapter 4 summary). In man, like in rabbit, there is a strong binding of SA to plasma proteins. When comparing SA binding in rabbits and rats, it is observed that it is significantly lower in rat. So, as only free SA could be transfered through placenta to embryos, this explains the effects in rats, which were “illustrated” by different blood levels in human and rat adults and embryos. Note also that the visceral yolk sac placenta in rats as an ion trapping environment for weak acids (another difference between rats and rabbits) resulting in higher embryonal levels of SA in rat foetuses than in rabbit foetuses.
Mechanisms Regulating Toxicant Disposition to the Embryo during Early Pregnancy: An Interspecies Comparison

Edward W. Carney,* Anthony R. Scialli, Rebecca E. Watson, and John M. DeSesso

The dose of toxicant reaching the embryo is a critical determinant of developmental toxicity, and is likely to be a key factor responsible for interspecies variability in response to many test agents. This review compares the mechanisms regulating disposition of toxicants from the maternal circulation to the embryo during organogenesis in humans and the two species used predominantly in regulatory developmental toxicity testing, rats and rabbits. These three species utilize fundamentally different strategies for maternal-embryonic exchange during early pregnancy. Early postimplantation rat embryos rely on the inverted visceral yolk sac placenta, which is in intimate contact with the uterine epithelium and is equipped with an extensive repertoire of transport mechanisms, such as pinocytosis, endocytosis, and specific transporter proteins. Also, the rat yolk sac completely surrounds the embryo, such that the fluid-filled excocelom survives through most of the period of organogenesis, and can concentrate compounds such as certain weak acids due to pH differences between maternal blood and excocelomic fluid. The early postimplantation rabbit conceptus differs from the rat in that the yolk sac is not closely apposed to the uterus during early organogenesis and does not completely enclose the embryo until relatively later in development (~GD13). This suggests that the early rabbit yolk sac might be a relatively inefficient transporter, a conclusion supported by limited data with ethylene glycol and one of its predominant metabolites, glycolic acid, given to GD9 rabbits. In humans, maternal-embryo exchange is thought to occur via the chorioallantoic placenta, although it has recently been conjectured that a supplemental route of transfer could occur via absorption into the yolk sac. Knowledge of the mechanisms underlying species-specific embryonic disposition, factored together with other pharmacokinetic characteristics of the test compound and knowledge of critical periods of susceptibility, can be used on a case-by-case basis to make more accurate extrapolations of test animal data to the human. Birth Defects Research (Part C) 72:345-360, 2004. © 2005 Wiley-Liss, Inc.

See hereafter:

### TABLE 2. Summary of Case Study Data

<table>
<thead>
<tr>
<th>Compound</th>
<th>pKₐ</th>
<th>Species</th>
<th>Gestational Age</th>
<th>Fetal/maternal Protein Binding</th>
<th>Fetal/maternal Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLU-11</td>
<td></td>
<td>H. CD0</td>
<td>60-2.20</td>
<td>40.2</td>
<td>23.8</td>
<td>LC H (200)</td>
</tr>
</tbody>
</table>
Furthermore it has been observed in developmental toxicities studies with SA esters in rats, that, according to the length of ester chain, the SA is released less quickly in longer chain salicylates. For example, SA release in blood from hexyl salicylate (IUCLID registration dossier, 2010) is slower than from methylsalicylate (see Rainsford, 2004). Release being slower, there is a balance between this SA release in blood and its degradation, leading to negative results on development even at high doses of hexyl salicylate (up to 350 mg/kg/ d oral). The fraction unbound in plasma (which exerts the pharmacological effect) is lower and does not reach embryotoxic concentrations . This supports the essential role of free salicylate in blood in salicylates, including salicylic acid, mode of action. The following scheme is a simplified explanatory one:
Finally a recent paper was published (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) to define doses which could lead to developmental effects in vitro. They concluded that a minimum of 494 mg/L (3 mM) salicylic acid level was necessary to get some developmental effects in vitro. This is even more (if we use the approximation $L = kg$) than doses able to have these effects in vivo, with clear maternal toxicity.

This author (Daston et al., cited in the IUCLID file, chapter 7.8.3 were referred to a figure provided in conclusion of the report provided as Annex 2 in the CLH IUCLID file chapter 13 (Relevance of plasma levels in humans and rats to establish equivalence of exposure levels).

"The dossier submitter states that there is a difference in plasma binding between humans and rats"

It was effectively written in Rainsford (2004, page 126) “while the lower binding of salicylate in pregnancy is associated with a lower concentration of plasma albumin (Yoshikawa et al., 1984a) ».

Plasma levels were indeed assessed in pregnant rats (It is easier to compare foetus and mother plama level) in GEORGE P. DASTON, Embryonal Disposition of Salicylate: In Vivo-In Vitro Comparisons, TERATOLOGY 42:225-232 (1990) :

“Salicylate exposure in vivo : Pregnant animals were exposed to salicylic acid via i.v. infusion for 24 hr, from gestation day 11.5 to 12.5. »

The observation that the free fraction of salicylate increases in pregnant woman due to a
Annex 1 - Comments and Response to Comments on CLH Proposal on Salicylic Acid

Reduction of plasma proteins was not confirmed in a more recent publication, and variability is such high that there is no significant variation between pregnant and non pregnant women (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.)

Hereafter a Table extracted from this publication:

Plasma Proteins and Fibrinolytic Parameters of Pregnant and Nonpregnant Women with Regard to Age

<table>
<thead>
<tr>
<th>Parameter</th>
<th>19-25 years</th>
<th>26-32 years</th>
<th>33-39 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant</td>
<td>Non-pregnant</td>
<td>Pregnant</td>
</tr>
<tr>
<td>Number</td>
<td>27</td>
<td>60</td>
<td>27</td>
</tr>
<tr>
<td>PFC (g/l)</td>
<td>3.22 ± 0.73</td>
<td>2.48 ± 0.67*</td>
<td>3.18 ± 0.89</td>
</tr>
<tr>
<td>ELT (min)</td>
<td>351.9 ± 99.6</td>
<td>261.5 ± 92.6*</td>
<td>358.3 ± 81.7</td>
</tr>
<tr>
<td>Total proteins (g/l)</td>
<td>61.2 ± 10.9</td>
<td>61.7 ± 11.9</td>
<td>61.6 ± 8.9</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>37.6 ± 6.9</td>
<td>37.5 ± 9.0</td>
<td>38.1 ± 7.4</td>
</tr>
<tr>
<td>Globulin (g/l)</td>
<td>20.4 ± 8.4</td>
<td>21.8 ± 8.8</td>
<td>20.3 ± 9.1</td>
</tr>
</tbody>
</table>

This table shows changes in plasma proteins and fibrinolytic parameters with age during pregnancy. The differences in the values of PFC (Plasma Fibrinogen Concentration), ELT (Euglobulins Lysis Time), total proteins, albumin and globulin in the three age groups (19-25 years, 26-32 years and 33-39 years) were not statistically significant (P>0.05).

So, the final comment “Therefore, a comparison between non-pregnant rats and humans is not relevant for developmental effects.” does not apply.

General comment on use of animal vs Human data

As developmental effects of salicylic acid (SA), acetylsalicylic acid (ASA) or methyl salicylate (MeS) were only seen in rats but not in other species (rabbit, mouse) which implies a rat specific high sensitivity for salicylates, and findings - at high maternally toxic doses in low reliability studies - in monkeys, are overruled by human epidemiological data, classification for reproductive toxicity isn’t warranted.

The CLP regulation has the aim of assigning Hazard categories for Human reproductive toxicants.

Therefore, when doses applied in Humans are representative of actual Human exposure (medical treatment at least, at doses higher than the rat NOAELs) and lead to no classification, no classification has to apply.

In Summary, There is no effect on fertility in the animal studies with salicylates and particularly ASA, the main metabolite of which is SA.

When analysing the ASA data, it was evident from the metabolism (Rainsford, 2004) that the rabbit is more like humans with high protein binding capacity on the contrary to rats with a low one. In fact, in the rabbit (Cappon, 2003), there is no teratogenic effect at 350 mg/kg/d, a maternal toxic dose. In humans, an epidemiologist expert reviewed the data...
(Bard, 2012), and concluded to no link with ASA medication. This made our weight of evidence that the rat is not a relevant species to extrapolate developmental effects to humans.

As further example, as reported in IUCLID, bone effects were observed in rat, while ASA was used for juvenile arthritis treatment in Human without such effects (Abbott and Harrisson, 1978).

Even the human subacute high dose of ASA (3000 mg/d or 60mg/kg for 50 kg) which corresponds to an allometric rat dose of 240 mg/kg, is higher than the rat NOAELS and far higher than DNELs. Note than in other regions the subacute human dose could be higher.

Several Competent Authorities had similar conclusions:
- With respect to developmental toxicity, SCCNFP published an opinion on SA in 2003, after the approval of SA as biocide by NL, giving a threshold of 75 mg/kg/d in rats.
- In a further opinion on homosalate (a salicylic acid ester), SCCP (2005) indicated no teratogenic effect of SA, based on a report (Roberts, 2005, ref. 55).
- Salicylates which are naturally present in our alimentation, were approved as flavouring ingredients quantum satis (Regulation EU No 872/2012 of 1 October 2012).
Relevance of plasma levels in humans and rats to establish equivalence of exposure levels.

(Human epidemiological studies on acetylsalicylic acid can take precedence over experimental studies in rats for determination of developmental toxicity classification of salicylic acid)

Summary & Conclusion

Developmental toxicity studies with salicylic acid (SAL) in rats have shown reduced foetal viability and delayed development at doses below those causing evident maternal toxicity and with malformations at maternally toxic dose levels. Although not confirmed with other animal species, these effects suggest that classification of SAL for developmental toxicity could be required (?).

In contrast, well-designed epidemiological studies on pregnant women having consumed acetylsalicylic acid (ASA) at different dosage from low dose for miscarriage prevention to excessive dose with clear toxicity to the mother have not demonstrated any clear increased risk of adverse pregnancy outcome in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay.

Although administered SAL dose levels in pregnant women were somewhat lower than the doses administered in the developmental toxicity studies on rats (without allometric scaling factor), a comparison of serum SAL levels in experimental studies in humans and rats has shown that equivalent serum concentrations are obtained with considerably lower administered doses in humans than in rats. Exposure data from reliable epidemiological studies demonstrates that many of the women having taken ASA as a classical pain/fever release treatment or due to addictive behaviour during pregnancy have reached equivalent or higher plasma level than those achieved in rats, which were associated in this species with congenital abnormalities.

It can therefore be considered that the human epidemiological data on ASA are also relevant for classification of SAL as it clearly demonstrates the absence of developmental toxicity in pregnant women at dose levels toxic to the mothers and delivering SAL serum concentrations at least equal to those where rat studies demonstrate clear teratogenic effects. The human epidemiological studies are therefore considered fully representative of human exposure.

The Consortium believe that these data support the proposition that SAL does not present a developmental toxicity hazard for humans and that classification as such is therefore not appropriate.
Introduction:

Developmental toxicity studies with salicylic acid (SAL) in rats have shown reduced foetal viability and delayed development at doses below those causing clear maternal toxicity and with malformations at maternally toxic dose levels. Although not confirmed with other animal species particularly rabbit, these effects suggest that classification of SAL for developmental toxicity could be required (?).

In contrast, an extensive literature on the effects of acetylsalicylic acid (ASA, aspirin) covering individual case reports, prospective and retrospective cohort and case-control studies on adverse effects in pregnancy and on offspring, as well as meta-analyses of some of these studies demonstrates the absence of ASA developmental toxicity during pregnancy even at very high dose.

Regulation (EC) No 1272/2008 (1.1.1.4) states that “Where evidence is available from both humans and animals and there is a conflict between the findings, the quality and reliability of the evidence from both sources shall be evaluated in order to resolve the question of classification. Generally, adequate, reliable and representative data on humans (including epidemiological studies, scientifically valid case studies as specified in this Annex or statistically backed experience) shall have precedence over other data.”

The term “representative” is not defined in the regulation, but would generally be considered as data covering all the reasonably predictable human exposure levels. For ASA, the range of doses used in therapeutic and prophylactic applications is shown in Annex 1.

The principal question to be answered is whether the absence of developmental effects observed in human epidemiology studies is due only to lower exposure levels in these studies than those achieved in experimental studies in rats. The best procedure to answer this question appears to be to compare plasma levels, which represent internal exposure levels, in the human and rat studies. If the human studies demonstrate absence of developmental toxicity at maternal plasma levels comparable to those achieved in the rat studies where foetal abnormalities were reported, the human data should be considered fully representative and could therefore take precedence over the results of the animal studies.

Developmental toxicity is of particular concern where developmental effects occur in the absence of maternal toxicity. A supplementary question is therefore whether repeated dose toxicity studies on salicylates in rats demonstrate systemic toxicity which was not detected from the limited toxicity endpoints examined in the studies of Tanaka et al.

ASA epidemiological studies at high dose in human pregnancy:

The use of ASA in pregnant women for treatment for mild pain/fever (standard dose) and for prevention of pregnancy complications (low dose) was discussed in 2011. It was concluded that large, well-designed epidemiological studies, both prospective and retrospective on ASA in human pregnancy have not demonstrated any clear evidence of developmental toxicity from widespread use of ASA at either low or standard therapeutic dose levels.
Two publications (Collins & Turner 1975; Turner & Collins, 1975) reported in detail in Annex 2 describe a prospective study on ASA use during pregnancy in Australia at doses which were excessive and can be considered as abusive and toxic. 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 or 510 mg per powder, taking between 2 and 12 doses/day every day throughout pregnancy; 56% used 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 consequences in the foetuses were lower birth weight and increased stillbirth. However no increased malformation rate was observed.

In this study, depending upon which powder was used and the number of powders taken per day, ASA dose ranged from 0.8 to over 6 g/day (equivalent to 10-79 mg/kg bw/day as SAL). This level of exposure occurred throughout pregnancy, and specifically throughout the first trimester critical for organogenesis. Mothers self-administering ASA at the higher end of this range therefore received doses of SAL at the critical stage in pregnancy for malformations at close to the threshold for such effects as reported in studies on SAL, (Tanaka, 1973a, 1973b) and ASA (Gupta, 2003). These studies are described in Annex 3.

Limited information is available on high dose ASA as prescribed in pregnancy for treatment of severe inflammatory diseases and from reports on ASA overdose in pregnancy and are described in Annex 4. There is no indication from these studies of adverse developmental effects from ASA under these extreme conditions.

**ASA / SA pharmacokinetics in Humans:**

According to Needs CJ, Brooks PM (1985), after oral administration ASA is rapidly absorbed at the low pH of the stomach. ASA absorption follows first-order kinetics with an absorption half-life ranging from 5 to 16 minutes. Hydrolysis of ASA to salicylate by non-specific esterases occurs in the liver and, to a lesser extent, the stomach so that most of the ASA dose reaches the systemic circulation as SAL. Both ASA and SAL are bound to serum albumin. The serum half-life of ASA is approximately 20 minutes. The fall in ASA concentration is associated with a rapid rise in SAL concentration. This rapid hydrolysis of ASA to salicylate allows ASA to be used as a surrogate for SAL, for which no direct human data in pregnancy are available.

SAL is renally excreted in part unchanged and the rate of elimination is influenced by urinary pH, the presence of organic acids (including SAL itself), and the urinary flow rate. Metabolism of SAL occurs through glucuronide formation (to produce salicylic acid), and salicyl phenolic glucuronide), conjugation with glycine (to produce salicylic acid), and oxidation to gentisic acid. The rate of formation of salicyl phenolic glucuronide and salicylic acid are easily saturated at low SAL concentration. The other metabolic products follow first-order kinetics. For these reasons, serum half-life of SAL increases with dose and a doubling of the dose intakes produce more than a doubling in plasma level. No significant differences exist between the pharmacokinetics of the salicylates in the elderly or in children when compared with young adults. However, the clearance of SAL appears to be greater in male than in female patients. The difference appeared to be of clinical significance in the study of Graham et al (1977) since fewer men than women achieved therapeutic plasma concentrations of SAL. This is also confirmed in a paper by Gibson et al (1975) where average achieved SAL plasma level after 65 mg/kg bw was 206.25 and 149.75 µg/mL for females and males respectively.
SAL plasma levels achieved in human epidemiology studies:

Two publications have been identified where SAL plasma levels have been measured after ASA administration at several high dosages in patients treated for rheumatoid arthritis or non-inflammatory back pain.

In the first publication (Bochner et al, 1987), 8 patients (4 males and 4 females) received 1, 2 and 4 g enteric coated aspirin (ASA) daily in ascending order. Initially, patients received one capsule containing 500 mg ASA twice per day for 2 weeks. For the second two week period this dose was doubled and for the final two week period it was quadrupled. At the end of each 2-week dosage period, plasma and urine were collected over a dosage interval for the estimation of various pharmacokinetic parameters. Results clearly demonstrated the non-linearity of the dose/concentration relationship with an increase of plasma level much greater than dose increase (Table 1).

Table 1: Doses of ASA in mg/kg for each individual and mean peak plasma SAL concentration

<table>
<thead>
<tr>
<th>BW in kg</th>
<th>Dose in mg/kg For 1 gram/ day</th>
<th>Dose in mg/kg For 2 grams/day</th>
<th>Dose in mg/kg For 4 gram/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>20,8</td>
<td>41,7</td>
<td>83,3</td>
</tr>
<tr>
<td>75</td>
<td>13,3</td>
<td>26,7</td>
<td>53,3</td>
</tr>
<tr>
<td>74</td>
<td>13,5</td>
<td>27,0</td>
<td>54,1</td>
</tr>
<tr>
<td>64</td>
<td>15,6</td>
<td>31,3</td>
<td>62,5</td>
</tr>
<tr>
<td>52</td>
<td>19,2</td>
<td>38,5</td>
<td>76,9</td>
</tr>
<tr>
<td>77</td>
<td>13,0</td>
<td>26,0</td>
<td>51,9</td>
</tr>
<tr>
<td>63</td>
<td>15,9</td>
<td>31,7</td>
<td>63,5</td>
</tr>
<tr>
<td>80</td>
<td>12,5</td>
<td>25,0</td>
<td>50,0</td>
</tr>
<tr>
<td>Mean</td>
<td>15,5</td>
<td>31,0</td>
<td>61,9</td>
</tr>
</tbody>
</table>

SAL concentration in mg/L (µg/mL) predicted from linear dose increase:

- 22±2
- 64±6
- 181±19

This was confirmed in the study of Gibson et al (1975) where 9 patients received 65 mg/kg bw/day ASA daily, divided in four doses. Peak plasma salicylate was measured after three days and then, for four patients, investigation was prolonged over 11 days with a dose of 100 mg/kg bw/day according to the same protocol (Table 2). Mean plasma concentration at 100 mg/kg bw/day was almost double that obtained from dosing at 65 mg/kg bw/day.

Table 2: Individual SAL plasma concentration for individuals in µg/mL

<table>
<thead>
<tr>
<th>Sex</th>
<th>SAL concentration (65 mg/kg group)</th>
<th>SAL concentration (100 mg/kg group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>196</td>
<td>455</td>
</tr>
<tr>
<td>F</td>
<td>174</td>
<td>325</td>
</tr>
<tr>
<td>F</td>
<td>233</td>
<td>300</td>
</tr>
</tbody>
</table>
The two studies gave similar plasma concentrations at around 65 mg kg BW/day and a consistent plasma concentration at 100 mg/kg BW/day. They have therefore been analysed together, as shown below in Figure 1, where these data are compared to plasma salicylate levels measured in rats.

**SAL plasma levels achieved in the rat:**

Very few studies have reported SAL plasma level following oral administration of SAL. Two studied have been identified where plasma level have been measured in rat at a dose level where foetal malformation were observed. These studies are described in Annex 3.

In the study of Tanaka et al (1973a), the mean plasma level following 7 days gavage administration of SAL at 150 mg/kg bw/day was 221 µg/mL at 3 hours following the last administration. This dose level was associated in the foetuses with a number of internal, external and skeletal anomalies.

The study of Tanaka et al (1973b) was conducted in the diet and is therefore more comparable to human exposure pattern than gavage studies. In this study, foetal anomalies were observed at 2000 ppm (estimated to be approximately equivalent to 167 mg/kg bw/day). At this dose level, mean achieved plasma level following 7 days exposure was 116 µg/mL in the serum. At 4000 ppm, a larger number of abnormalities were reported (32 among a total of 54 examined foetuses) together with clear maternal toxicity and foetal mortality. At this dose level, SAL plasma level was not measured.

**Comparison of SAL plasma levels from humans and rat studies:**

The plasma concentrations obtained in the rat studies by Tanaka et al have been plotted alongside the human data from the studies of Bochner et al and Gibson et al. (Figure 1). In order to show the comparison in terms of SAL dose, the human ASA doses have been converted to equivalent SAL dose (using a factor of 0.77 for molecular weight).

---

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>182</td>
<td>295</td>
</tr>
<tr>
<td>M</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>285</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>180</td>
<td>343.75</td>
</tr>
</tbody>
</table>

---

Fig. 1: SAL Plasma concentration (in µg/mL) in humans and rats as a function of administered dose (ASA dose converted to SAL for humans)
It can be seen from Fig. 1 that the SAL plasma concentrations reached in humans were much higher than those obtained in rats for equivalent administered dose as SAL. The mean SAL plasma level of 221 µg/mL achieved in the 150 mg SAL/kg bw/day group in the Tanaka (1973a) gavage study corresponds to a dose of about 56 mg SAL/kg bw/day (equivalent to 73 mg ASA/kg bw/day) for human adults. The SAL plasma level of 116 µg/mL achieved in the 2000 ppm (167 mg SAL/kg bw/day) group in the Tanaka (1973b) dietary study corresponds to a dose of about 32 mg SAL/kg bw/day (equivalent to 42 mg ASA/kg bw/day) for human adults.

These administered dose levels of 150 and 167 mg SAL/kg bw/day, delivering serum SAL concentrations of 221 and 167 µg/mL respectively in the studies of Tanaka (1973a & 1973b) represent the threshold for developmental toxicity of SAL in the rat (see Annex 3). It is therefore necessary to determine whether equivalent plasma concentrations were reached in the epidemiological studies in pregnant women.

**Was this dose achieved in the epidemiological studies with ASA?**

For a 60 kg woman, the serum concentration of 221 µg/mL, the threshold for developmental toxicity in rats, was shown in Fig. 1 to be achieved from a dose of about 73 mg ASA/kg bw/day (56 mg SAL/kg bw/day). This equates to consumption of approximately 2.7 g ASA/day (i.e. slightly more than five 500 mg tablets per day).

In the prospective study reported as Collins & Turner (1975), Turner & Collins (1975), described above and in Annex 2, ingestion of powders containing 384 or 510 mg ASA between 2 and 12 doses/day every day throughout pregnancy or at least once per week resulted in toxicity to the mother and consequently in the foetuses, however no increased malformation rate was observed despite the clear adverse effects on maternal health. In this study, with a dose range for ASA of 768-6120 mg per day, well over 2.7 gram/day was certainly used by many of these women.

In addition, ASA can produce foetal abnormalities in the rat even after a single dose (Kimmel et al, 1971; Gupta et al 2003), as would be expected, since there are specific periods of sensitivity for the development of different organ systems. Among the women in the different epidemiological studies reported in the epidemiology review of salicylate (SAC, 2011), many received standard doses of ASA (up to 4 g/day in divided doses, 1-2 tablets 2-3 times per day for 1 to 4 days). In the most recent and extensive study, Czeizel et al (2000)
reported that no increased congenital abnormalities were observed in groups of women (1065 cases and 1486 controls) having taken salicylic acid with a recommended dose of 2, 3 tablets of 500 mg ASA once or twice a day during an average of 4-5 days, i.e. a daily dose of 1 to 3 gram/day during gestational months 2-3. It is then highly likely that a significant proportion of women both in the Kozer et al (2003) meta-analysis and the Czeizel et al (2000) study have largely exceeded the dose of 2.7 grams/ day, estimated above as giving plasma concentrations of SAL showing developmental toxicity in the rat, during a sensitive period of their pregnancy.

Effects in Repeated Dose Toxicity Studies in Rats on Salicylates

Tanaka et al (1973a, 1973b) reported that developmental toxicity occurred at dose levels, which did not induce significant maternal toxicity. According to the limited parameters of maternal toxicity measured in these studies and in guideline developmental toxicity studies it is not possible to determine what more subtle effects related to the pharmacological action of salicylates may have been present in the maternal animals. Such effects would normally be established in repeated dose on general toxicity.

No guideline repeated dose toxicity study is available on SAL, however systemic toxicity has been evaluated from subchronic and chronic studies on Methyl Salicylate (MeS) (Webb, 1963) and subacute studies on ASA (Takahashi, 1985), EMEA (1999). These studies are reported in detail in Annex 5 It can be concluded from these studies that both ASA and SAL have similar haemorrhagic potency in rats, with threshold below the lowest dose tested, 116 mg/kg bw/day as SAL. The results of Takahashi (1985) demonstrate that haemorrhagic effects would be expected to have been present at the dose levels tested in the developmental toxicity studies of Tanaka et al (1973a, 1973b). These dose levels should therefore be considered as effect levels for maternal toxicity.

Conclusion:

Well-designed epidemiological studies on pregnant women having consumed ASA at different dosage from low dose for miscarriage prevention to excessive dose with clear toxicity to the mother have not demonstrated any clear increased risk of adverse pregnancy outcome in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay.

A large number of these women having taken ASA as a classical pain/fever release treatment or due to addictive behaviour have certainly reached equivalent or higher plasma level than those achieved in rats and linked, in this species, to congenital abnormalities.

It can therefore be considered that the human epidemiological data on ASA are also relevant for classification of SAL as it clearly demonstrates the absence of developmental toxicity in pregnant women at dose levels toxic to the mothers and delivering SAL serum concentrations at least equal to those where rat studies demonstrate clear teratogenic effects. The human epidemiological studies are therefore considered fully representative of human exposure.

The Consortium believe that these data support the proposition that SAL does not present a developmental toxicity hazard for humans and that classification as such is therefore not appropriate. This, together with rabbit or mouse data confirmed that the rat is not an appropriate species to evaluate developmental effects in human.
Daston (1990) indicated rat maternal and fetal blood levels following perfusion:

at a rate of 16.25 ml/rat/day, sufficient to deliver a dosage of 50 mg/kg/day.

*TABLE 1. Tissue distribution of salicylate in gestation day 12.5 and 20.5 rats in vivo*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Gestation day 12.5</th>
<th>Gestation day 20.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal plasma</td>
<td>35 +/- 2.7</td>
<td>41 +/- 8.5</td>
</tr>
<tr>
<td>Maternal serum</td>
<td>37 +/- 2.7</td>
<td>45 +/- 9.4</td>
</tr>
<tr>
<td>Embryo/fetus</td>
<td>6.3 +/- 0.9</td>
<td>16 +/- 4.0</td>
</tr>
<tr>
<td>Fetal blood</td>
<td>-</td>
<td>22 +/- 5.7</td>
</tr>
</tbody>
</table>

Carney (2004) data showed that Sa concentration in human fetus is 1.6 time that of mother, while in rat fetus concentration is 0.2 that of mother, indicating the particular effects in rats compared to humans. Circles added to the basic Fig.1.

*Fig. 1: Plasma concentration (in µg/mL) as a function of dose (in mg/kg bw/day)*

The human doses compared to rats fit the allometric scaling factor of 4.
Annex 1

ASA Prophylactic/Therapeutic Dose Levels

Several regimes are available for the medicinal use of ASA in therapeutic or prophylactic applications. Precise dosage recommendations vary, but typical ranges for adults, with their equivalent in terms of SAL concentration for a 60 kg person, are shown in Table 1.

**Table 1: ASA doses**

<table>
<thead>
<tr>
<th>Indication</th>
<th>Unit dose strength (mg)</th>
<th>Dose regime</th>
<th>Duration</th>
<th>Daily Dose as ASA (mg/kg/day) for 60 kg person</th>
<th>Equivalent Daily Dose as SAL (mg/kg/day; conversion factor 0.77)</th>
<th>SAC Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of rheumatic fever</td>
<td>350 – 500</td>
<td>Up to 6500 mg per day in divided doses</td>
<td>Short term (1-2 weeks then 60-70 mg/kg/day for 1-6 weeks)</td>
<td>Up to 108</td>
<td>Up to 83</td>
<td></td>
</tr>
<tr>
<td>Treatment of severe inflammatory conditions such as osteo- or rheumatoid arthritis, and SLE-associated arthritis.</td>
<td>350 – 500</td>
<td>3000-5400 mg per day in divided doses</td>
<td>Medium to Long-term</td>
<td>50-90</td>
<td>38.5 – 69</td>
<td>&quot;High dose&quot;</td>
</tr>
<tr>
<td>Treatment of mild pain or fever.</td>
<td>350 – 500</td>
<td>Up to 4000 mg/day 1-2 tablets 2-3 times per day</td>
<td>Short-term (typically 1 to 4 or 5 days)</td>
<td>11.7 – 66.7</td>
<td>9 – 51</td>
<td>&quot;Standard therapeutic dose&quot;</td>
</tr>
<tr>
<td>Prophylaxis for myocardial infarction, angina stroke etc</td>
<td>75 – 350</td>
<td>1 tablet per day</td>
<td>Medium to Long-term</td>
<td>1.25 – 5.8</td>
<td>1 – 4.5</td>
<td>&quot;Low dose&quot;</td>
</tr>
<tr>
<td>Prevention of multiple miscarriage, pregnancy-induced hypertension and other complications of pregnancy.</td>
<td>50 – 150</td>
<td>1-2 tablets once per day</td>
<td>Medium-term: 1st trimester or from 2nd and/or 3rd trimester</td>
<td>1 – 5</td>
<td>0.77 – 3.85</td>
<td></td>
</tr>
</tbody>
</table>
Annex 2

Human ASA Self-administration at High (abusive) Dose in Pregnancy in Australia

Collins & Turner (1975) and Turner & Collins (1975) reported on a prospective study of salicylate exposure in pregnancy in women who self-administered ASA in combination with other analgesics at high doses throughout the whole of their pregnancy. The study group of 144 exposed women represented 6.6% of the 2180 Australian-born women attending a Sydney antenatal clinic over a 28 month period. ASA users were identified from two consecutive urine tests positive for salicylate, with details on analgesic usage confirmed by interview immediately after delivery. The ASA-users were divided into two groups. Group 1, “Constant” takers, were women who reported consumption of from 2 to 12 proprietary analgesic powders containing ASA on every day of pregnancy. Group 2, “Intermittent” takers took the same doses at least once per week throughout pregnancy, most much more frequently than this but less than daily. Details of the proprietary powders (mainly from Stewart, 1978) and calculated exposure levels are shown in Table 2. Data on the women in each group and their durations of ASA exposure are summarised in Table 3.

Table 2: Composition of Proprietary ASA Powders available in Australia in 1970s and ASA/SAL dosage

<table>
<thead>
<tr>
<th>Formulation</th>
<th>ASA Unit dose strength (mg)</th>
<th>Self medication dose</th>
<th>Daily dose (mg/day)</th>
<th>Daily Dose as ASA (mg/kg/day) for 60 kg person</th>
<th>Equivalent Daily Dose as SAL (mg/kg/day; conversion factor 0.77)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bex Powders:</strong> ASA 384 mg Phenacetin 384 mg (Paracetamol from 1975) Caffeine citrate 146 mg</td>
<td>384</td>
<td>2-12 powders</td>
<td>768 – 4608</td>
<td>13 – 77</td>
<td>10 – 59</td>
</tr>
<tr>
<td><strong>Vincent’s Powders:</strong> ASA 510 mg Salicylamide 170 mg (Phenacetin prior to 1967) Caffeine citrate 170 mg</td>
<td>510</td>
<td>2-12 powders</td>
<td>1020 – 6120</td>
<td>17 - 102</td>
<td>13 – 79</td>
</tr>
</tbody>
</table>

Table 3: age, parity and ASA consumption

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>All clinic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>63</td>
<td>81</td>
<td>2180</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>29</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>Parity:</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>21</td>
<td>40</td>
</tr>
<tr>
<td>2-4</td>
<td>57</td>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>5 or more</td>
<td>33</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td>Duration of ASA consumption (Years)</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Less than 5</td>
<td>31</td>
<td>56</td>
<td>3.0</td>
</tr>
<tr>
<td>5-9</td>
<td>24</td>
<td>23</td>
<td>1.5</td>
</tr>
<tr>
<td>10-14</td>
<td>32</td>
<td>10</td>
<td>1.3</td>
</tr>
<tr>
<td>15 or more</td>
<td>13</td>
<td>11</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>6.6</td>
</tr>
<tr>
<td>ASA Useage</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Bex Powders</td>
<td>36</td>
<td>Not specified</td>
<td></td>
</tr>
<tr>
<td>Vincent’s powders</td>
<td>58</td>
<td>Not specified</td>
<td></td>
</tr>
<tr>
<td>Either powder</td>
<td>6</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>ASA tablets</td>
<td>Not reported</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>
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 Table 4. It was not possible to make a precise comparison between maternal and cord blood salicylate levels, but where maternal level was high, so was cord blood level. Since mean duration of labour in women of Groups 1 & 2 was approximately 5.5 hours (see Table 5), it is clear that many hours had elapsed since the last ASA dose and that therefore these serum levels do not represent peak values.

Table 4: Serum Salicylate levels

<table>
<thead>
<tr>
<th></th>
<th>Mothers</th>
<th>Babies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>81</td>
<td>76</td>
</tr>
<tr>
<td>Serum salicylate µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 10</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>11 – 30</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>31 – 50</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>51 – 70</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>71 – 90</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

The survey reported that the high levels of salicylate use throughout pregnancy were associated with a number of adverse effects on the mothers and their babies. The main maternal and foetal features of the study are summarised in Table 5.

Table 5: Incidence of main maternal and foetal features (as percentage unless expressed otherwise)

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Controls (matched with group 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking in pregnancy</td>
<td>86</td>
<td>65</td>
<td>54</td>
</tr>
<tr>
<td>Anaemia in pregnancy †</td>
<td>41</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>17</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Hospital admission in pregnancy</td>
<td>32</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td>Ante-partum haemorrhage</td>
<td>14</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Post-partum haemorrhage</td>
<td>12</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Transfusion at delivery</td>
<td>12</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Mean duration of pregnancy</td>
<td>39.7</td>
<td>39.8</td>
<td>38.7</td>
</tr>
<tr>
<td>Duration 36 weeks or less</td>
<td>5</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Duration 42 weeks or more</td>
<td>16</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Mean duration of labour</td>
<td>5.6</td>
<td>5.5</td>
<td>4.8</td>
</tr>
<tr>
<td>Complicated delivery</td>
<td>30</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>12</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Breech</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Forceps or ventouse</td>
<td>11</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Stillbirths</td>
<td>4/57</td>
<td>1/81</td>
<td>0</td>
</tr>
<tr>
<td>Neonatal deaths</td>
<td>0/53</td>
<td>3/80</td>
<td>0</td>
</tr>
<tr>
<td>Mean corrected birth weight</td>
<td>3283 g</td>
<td>3372 g</td>
<td>3502 g</td>
</tr>
<tr>
<td>Major congenital anomalies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular septal defect (VSD)</td>
<td>2/64 (3.1%)</td>
<td>4/82 (4.9%)</td>
<td>1/64 (VSD) (Frequency for all clinic patients: 2.4%)</td>
</tr>
<tr>
<td>Holt-Oram syndrome (genetic defect)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoplastic left heart syndrome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaphragmatic hernia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perforation of large bowel + 3 VSDs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Anaemia defined as haemoglobin 10.5 g per 100 ml or less at any antenatal visit
* Significantly lower than control birth-weight p < 0.005

A number of maternal effects were reported in this study. The frequency of anaemia in Group 1 women was twice that of controls. Gestation and labour were slightly prolonged in both groups of ASA takers, with increased incidence of complicated deliveries. The incidence of ante-partum and post-partum haemorrhage and transfusion at delivery for both ASA groups was greatly increased over controls, with incidence for Group 1 double that of Group
2. All these effects are explicable from the known pharmacological effects of ASA and indicate an adverse effect of high salicylate intake on the health of these women.

Contrary to the expectations of the researchers, there was no significantly increased incidence of congenital anomalies in the babies of ASA-exposed mothers despite exposure to these very high levels of salicylate on every day of gestation in the case of the babies of Group 1 women. On the other hand, mean birth weights of live-born Group 1 babies were significantly lower than controls and correlated with the duration of maternal ASA consumption (years). The incidence of still-births was also significantly increased for the babies of Group 1 women, as shown in Table 6. The still-births in salicylate users were not all clearly related to pregnancy complications, but all occurred in older women who had been taking salicylates for many years. These 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.

**Table 6: Still-births**

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

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

It can be concluded from this study that habitual consumption of high doses of analgesics including salicylates throughout pregnancy resulted in detrimental effects on the health of the mothers, including increased incidence of anaemia and of ante-partum and post-partum haemorrhage. The authors considered that the increased frequency of anaemia could have been the result of chronic gastric erosion, leading to microscopic blood loss combined with the increased iron requirements of pregnancy.

The levels of analgesic use described in the papers of Collins & Turner are almost unique to Australia during the middle of the 20th century. Stewart (1978) reported on the association of consumption of the analgesics described by Collins & Turner with end-stage renal disease. Distribution of analgesic consumption in the adult population of the different States is shown in Table 7.

**Table 7: Distribution of Regular Analgesic Consumption and End-stage Renal Failure**

<table>
<thead>
<tr>
<th>State</th>
<th>Adult Analgesic Users (%) (1 or more powder/tablet per day)</th>
<th>End-stage renal failure due to renal papillary necrosis (Yearly rate per million population)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Queensland</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>New South Wales</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Victoria</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Tasmania</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>South Australia</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>Western Australia</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Consumption of analgesics, particularly in the eastern States of Australia, was described by Stewart and other authors as “Analgesic Abuse”. Several reasons for consumption were given in two surveys. The majority of users (65% and 42%) stated that they took the powders
for headache. Once consumption was established, it is probable that they continued to take powders due to the caffeine-withdrawal headache when they stopped consumption. Others (6% and 31%) admitted to taking the drugs for their psychotropic effect. Only a minority took the drugs regularly for arthritis or chronic musculoskeletal pain.

The causative factor for the severe renal disease which was the major issue of concern has been a matter of debate, but is generally considered to have been principally Phenacetin, which was replaced in the proprietary powders either by Paracetamol (Vincent’s Powders in 1967) or Salcylamide (Bex’s Powders in 1975). The other major adverse effect of analgesic abuse was gastric erosion and peptic ulcer. An increase in incidence of gastric ulcer in young women in Australia was first reported in the 1940s and attributed to ASA consumption in the 1960s (Chapman & Duggan, 1969).

The adverse peri-natal effects reported by Collins & Turner, consisting of increased incidence of prolonged gestation, complicated deliveries and peri-natal mortality are readily explained as secondary consequences of the pharmacological effects of ASA on maternal health status. Even at these high dose levels, deleterious to the health of the mothers, where doses equivalent to approximately 10-80 mg/kg bw/day SAL were consumed, no significant increase in incidence of malformations was found.
Annex 3

Salicylate Developmental Toxicity Studies in Rats

Developmental toxicity studies on salicylates in rats have shown reduced foetal viability and delayed development at doses below those causing clear maternal toxicity and malformations at maternally toxic dose levels. These effects suggest that classification for developmental toxicity could be required. In contrast, studies in mice and dogs have reported such effects only at doses causing severe maternal toxicity, while in rabbits, studies conducted even at severely maternally toxic dose resulted only in a reduction in mean foetal weight. The only study conducted in monkey is of too low quality to be interpreted. It is not clear from these studies which species is the most relevant for prediction of the effects of human exposure to SAL, but rata data are taken as a worst case.

Two developmental toxicity studies in rats are available on SAL. In Tanaka et al (1973a), SAL was administered to pregnant Wistar rats by oral gavage at 75, 150 or 300 mg/kg bw/day on GDb-14. At 150 mg/kg/day there were no signs of marked maternal toxicity but increased neonatal mortality, with external and skeletal anomalies were reported. The NOAEL for development was 75 mg/kg bw/day. A group of 5 pregnant rats were administered SAL at 2000 ppm (equivalent to 100 mg/kg bw from GD 8-14 and sacrificed 3 hours after the final treatment for measurement of SAL concentration in maternal organs and foetuses. The results are shown in Table 8:

Table 8: Organ distribution of SAL after Gavage Administration in Pregnant Rats

<table>
<thead>
<tr>
<th>Organs / Days of treatment</th>
<th>8-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>221.28±12.76</td>
</tr>
<tr>
<td>Brain</td>
<td>24.86±2.46</td>
</tr>
<tr>
<td>Liver</td>
<td>83.92±5.88</td>
</tr>
<tr>
<td>Kidney</td>
<td>128.47±8.01</td>
</tr>
<tr>
<td>Adrenal</td>
<td>68.57±3.29</td>
</tr>
<tr>
<td>Ovary</td>
<td>71.57±2.05</td>
</tr>
<tr>
<td>Uterus</td>
<td>74.09±6.88</td>
</tr>
<tr>
<td>Placenta</td>
<td>85.62±3.87</td>
</tr>
<tr>
<td>Foetus</td>
<td>62.48±3.82</td>
</tr>
<tr>
<td>Amnionic fluid</td>
<td>62.29±12.52</td>
</tr>
</tbody>
</table>

In Tanaka et al (1973b) SAL was administered to pregnant Wistar rats (20 rats per dose level) on GDb-14 at 600, 1000, 2000 or 4000 ppm in the diet. Based on a dose conversion factor of 12 for pregnant rats, the dietary dose was approximately equivalent to 50, 83, 167 or 333 mg/kg bw/day. 15/20 animals were sacrificed on GD 20, At 4000 ppm, high foetal mortality, growth retardation and a high frequency of complex anomalies (craniolischis, pseudomacroglossia, myeloschisis, cracked skin, pes varus, oligodactyly, syndactyly, flexed tail) and skeletal anomalies were reported in association with maternal toxicity (severely reduced food consumption and bodyweight loss in the early part of the study). At 2000 ppm, significant foetal growth retardation and a low frequency of anomalies (myeloschisis, subcutaneous hematocoele, cracked skin, pes varus) were reported in the absence of significant maternal toxicity. 5/20 dams were allowed to give birth. The offspring were weaned on PND21 and were terminated on PND56. In the 4000 ppm group, only 6 pups were born alive from one dam, and these all died within one day after birth. There were no significant effects on litter size, weight or weaning rate in the lower dose groups, The maternal NOAEL was 2000 ppm (167 mg/kg bw/day) and developmental NOAEL was 1000 ppm (83 mg/kg bw/day). A group of 5 pregnant rats were administered SAL at 150 mg/kg bw from GD 8-14 and sacrificed 3 hours after the final treatment for measurement of SAL concentration in maternal organs and foetuses. The results are shown in Table 9:
Table 9: Organ distribution of SAL after Dietary Administration in Pregnant Rats

<table>
<thead>
<tr>
<th>Organs / Days of treatment</th>
<th>8-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>115.96±9.02</td>
</tr>
<tr>
<td>Brain</td>
<td>4.14±0.46</td>
</tr>
<tr>
<td>Liver</td>
<td>21.68±1.37</td>
</tr>
<tr>
<td>Kidney</td>
<td>60.89±4.87</td>
</tr>
<tr>
<td>Adrenal</td>
<td>28.06±3.12</td>
</tr>
<tr>
<td>Ovary</td>
<td>35.23±1.05</td>
</tr>
<tr>
<td>Uterus</td>
<td>28.10±1.54</td>
</tr>
<tr>
<td>Placenta</td>
<td>27.37±1.80</td>
</tr>
<tr>
<td>Foetus</td>
<td>13.86±1.20</td>
</tr>
<tr>
<td>Amnionic fluid</td>
<td>12.35±0.72</td>
</tr>
</tbody>
</table>

A number of developmental toxicity studies in rats are available for ASA, of which the most reliable is Gupta et al (2003). ASA was administered by oral gavage to pregnant rats at 50, 125 or 250 mg/kg bw/day (38, 96, 192 mg/kg as SAL) on GD6-17. Maternal bodyweight gain was significantly reduced in the mid and high dose groups, with food consumption reduced only at the high dose. Developmental effects at 250 mg/kg (192 mg/kg as SAL) were increased number of early resorptions and post-implantation loss, reduced foetal bodyweight, reduced number of viable foetuses and significant increase in number of malformations. At 125 mg/kg (96 mg/kg as SAL), the number of viable foetuses was decreased, with no malformations. The NOAEL for development was 50 mg/kg (38 mg/kg as SAL).

The studies of Tanaka et al (1973a, 1973b) on SAL showed a threshold for developmental toxicity in rats of around 150 mg/kg bw/day in the absence of clear maternal toxicity. The study of Gupta et al (2003) on ASA confirmed developmental toxicity at 192 mg/kg bw/day as SAL, with effect on viability but no malformations at 96 mg/kg bw/day as SAL, with significant reduction in maternal body weight gain at both dose levels. According to the limited parameters of maternal toxicity measured in guideline developmental toxicity studies it is not possible to determine what more subtle effects related to the pharmacological action of salicylates may have been present in the maternal animals. Such effects would normally be established in repeated dose on general toxicity, reported in Annex 5.
Annex 4

Assessment of “High dose” ASA as prescribed in pregnancy

ASA is one of a number of non-steroidal anti-inflammatory drugs (NSAIDs) which may be prescribed at high dose levels for the long-term treatment of a number of severe inflammatory conditions which can affect women of child-bearing age. Only limited information is available on the effects of such prescribed drug use in pregnancy. No case reports have been located suggesting any association between NSAID use for these conditions and occurrence of a malformed child.

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 incidence of 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 during the first trimester.

A small prospective study (Østensen & Østensen. 1996) of long-term intake of NSAIDs for anti-rheumatic therapy in patients with autoimmune rheumatic diseases reported no association of NSAID use with any teratogenic effects. Patients were recruited from a prospective study on pregnancy and rheumatic disease. A cohort of 88 pregnant patients with inflammatory rheumatic disease was divided into 2 groups, 45 who were treated and 43 not treated with NSAID during pregnancy. Possible long-term effects of NSAID on physical and mental development of the offspring were evaluated by telephone interview. Groups did not differ with regard to demographic data. Forty-nine pregnancies had been exposed to therapeutic doses of NSAID for a mean duration of 15.3 weeks. A comparison of pregnancies exposed with those not exposed to NSAID revealed no differences in pregnancy outcome, duration of labour, complications at delivery, or neonatal health. No significant differences were found between the groups with respect to health and development of offspring at follow-up. The authors concluded that this study of a limited number of pregnancies in rheumatic patients showed no teratogenicity or adverse effects of NSAID on the neonate, nor did it reveal harmful long-term effects caused by intrauterine exposure to these drugs. Since this was only a small study, without specific information related to ASA, it provides only limited support to the premise that ASA would not be considered a human developmental hazard.

ASA Overdose in Pregnancy

Case studies have reported foetal death in spite of treatment which preserved the life of the mother. Diffuse petechial haemorrhages of the lungs, heart, thymus and kidneys were found at autopsy of the stillborn infant of a woman who reportedly ingested 50 ASA tablets per day for a month (Palatnick, 1998). However, in the few cases where the gross maternal overdosage shortly before delivery resulted in the delivery of a live baby who showed features of salicylate poisoning after birth, haemorrhages have not been reported (Buck., 1983). 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). It is not possible to draw any conclusion from these studies due to the small number of cases available for assessment and the lack of information on period of gestation during which overdose took place.
Annex 5

Effects in Repeated Dose Toxicity Studies on Salicylates

No guideline repeated dose toxicity study is available on SAL. Systemic toxicity has been evaluated from subchronic and chronic studies on Methyl Salicylate (MeS), which is rapidly hydrolysed in vivo to SAL. In seventeen week and 2-year studies (Webb & Hansen, 1963) and in a ten-week study (Harrisson et al, 1963) MeS showed only an increase of cancellous bone in rats fed 2.0% in the diet, with slight effects at 1.0 or 0.5%. The no-effect level was 0.1% in the diet, equivalent to approximately 50 mg/kg bw/day for MeS, or 45.4 mg/kg bw/day as SAL. However the parameters measured in these old studies were limited. No examination of the stomach was reported to determine whether gastric erosion may have been present.

In 1- and 4-week studies on ASA (Takahashi, 1985), rats and mice were fed diets containing 0.3, 0.6 and 1.2% ASA (approximately 150, 300 and 600 mg/kg bw/day equivalent to 116, 231, 462 mg/kg bw/day as SAL). The purpose of the studies was to examine differences in haemorrhagic toxicity of ASA in rats and mice. The endpoints examined were therefore targeted for this purpose and did not cover all those specified in the OECD 407 guideline. In rats, bodyweight gain and food consumption were reduced in a dose-dependent manner. In mice, bodyweight gain was reduced only at the high dose level. In rats given ASA for 1 week, some haemorrhages and alopecia were observed at 0.6 and 1.2%. In rats given ASA at 1.2% for 4 weeks, haemorrhagic death, haemorrhagic anaemia, external haemorrhage, epistaxis and ear haemorrhage, alopecia and internal bleeding in testes, epididymes, bladder wall, lungs and parathyroid gland were observed. At 28-day sacrifice of survivors, contents of stomach and caecum were dark reddish-brown, lower weight gain, increased bleeding and changes in coagulation parameters were reported. In rats receiving 0.6% ASA, diarrhoea was observed in some animals. Cages were occasionally stained with drops of blood and epistaxis was observed. Massive haemorrhage was observed in two rats in the latter part of the experiment. At 0.3%, epistaxis was observed in one animal during the study and in 4/5 rats at sacrifice. No conspicuous signs of toxicity were seen in mice at any dose level. Prothrombin index and kaolin-activated partial thromboplastin time index were significantly decreased in both 1- and 4-week studies at all dose levels in a dose-related manner in rats, while in mice only kaolin-activated partial thromboplastin time index was significantly decreased at the high dose. Liver concentrations of both SAL and gentisic acid were moderately increased in a dose-related manner at both 1 and 4 weeks in rats, while gentisic acid was not increased in mice. Since haemorrhagic effects were reported at all dose levels in the rat study, no no-effect level was obtained. The LOAEL for haemorrhagic effects was 0.3% approximately 150 mg/kg bw/day equivalent to 116 mg/kg bw/day as SAL).

In a second experiment, haemorrhagic effects of ASA, SAL and gentisic acid were compared in rats and mice at a single dose level in a 1-week study. Dietary concentrations gave daily dose levels as shown in Table 8:

Table 8: Mean Daily Doses of ASA, SAL, Gentisic Acid

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/rat</td>
<td>mmol/kg</td>
</tr>
<tr>
<td>ASA</td>
<td>0.266</td>
<td>1.47</td>
</tr>
<tr>
<td>SAL</td>
<td>0.269</td>
<td>1.48</td>
</tr>
<tr>
<td>Gentisic acid</td>
<td>0.313</td>
<td>1.58</td>
</tr>
</tbody>
</table>

Results of this second experiment are shown in Table 9. Prothrombin index and kaolin-activated partial thromboplastin time index were significantly decreased with ASA in rats but not mice. These indices in rats were not decreased with statistical significance for SAL due to the large variance associated with abnormal readings in 2/5 animals. Nevertheless the values were similar to those for ASA, while indices for gentisic acid were not decreased. ASA
and SAL depressed bodyweight gain to a similar degree in rats, with little effect in mice, while genticisic acid had no effect. Relative liver weight in both rats and mice was increased with ASA and SAL but decreased with genticisic acid.

**Table 9: Effects of ASA, SAL, Genticisic Acid**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PT index (%)</td>
<td>K-PTT index (%)</td>
</tr>
<tr>
<td>Control</td>
<td>100±5</td>
<td>100±6</td>
</tr>
<tr>
<td>ASA</td>
<td>54±6 ***</td>
<td>68±9 *</td>
</tr>
<tr>
<td>SAL</td>
<td>64±17</td>
<td>71±14</td>
</tr>
<tr>
<td>Genticisic acid</td>
<td>122±4 *</td>
<td>118±4 *</td>
</tr>
</tbody>
</table>

* p<0.05   ** p<0.01   *** p<0.001

It can be concluded from these studies that ASA and SAL have similar haemorrhagic potency in rats, with threshold below the lowest dose tested, 116 mg/kg bw/day as SAL. In the dietary developmental toxicity study reported on SAL by Tanaka et al (1973a) the lowest dose showing developmental toxicity was 100 mg/kg bw/day, a dose level reported as not inducing significant maternal toxicity. The results of Takahashi (1985) strongly suggest that haemorrhagic effects would have been present at this dose level, and certainly present at 150 mg/kg bw/day in the gavage study of Tanaka et al (1973b).

Summaries of two repeat dose toxicity studies are available from a summary report on ASA in veterinary medicine (EMEA, 1999). In rats, equivalent doses of 0, 50, 150 and 500 mg/kg bw of ASA were given daily as ASA-DL-lysine and 500 mg as sodium acetylsalicylate (study duration not reported). The highest dose induced severe clinical abnormalities and mortality. No clinical signs were observed at 150 mg/kg. Congestion, petechiae, haemorrhages and punctiform lesions were observed in the stomach at 150 and 500 mg/kg. Necropsy examination revealed a dose-related hepatomegaly, with no histological expression. Kidney weights were increased in all males at all dose levels. A dose-related decrease in serum globulins was recorded, still significant at the lowest dose tested in females. In dogs, ASA-DL-lysine was administered at doses of 0, 50, 150, 250 and 500 mg/kg ASA-equivalent/kg bw/day for 3 months, and sodium acetylsalicylate was administered at doses of 0, 250 and 500 mg/kg bw/day. The highest dose induced rapid mortality and all animals died within 2 to 7 days post treatment initiation. Doses of 150 and 250 mg/kg induced vomiting and mortality. Vomiting was still observed at 50 mg/kg but at lower frequency. In this group (n=6) one dog presented gastric striae and two dogs presented focal atrophy of the mucosa with dedifferentiation of the epithelial lining and glandular epithelium. A slight decrease in the heart rate in all treated animals was recorded. No NOEL could be established from these studies.
References


Lewis RN, Schulman JD (1973) Influence of acetylsalicylic acid, an inhibitor of prostaglandin synthesis, on the duration of human gestation and labor Lancet 2:1159-1161


Comparative Distribution and Embryotoxicity of Acetylsalicylic Acid in Pregnant Rats and Rhesus Monkeys¹,²

J. G. WILSON, E. J. RITTER, W. J. SCOTT, AND R. FRADKIN

Children's Hospital Research Foundation and Department of Pediatrics,
University of Cincinnati, College of Medicine,
Cincinnati, Ohio 45229

Received August 27, 1976; accepted January 17, 1977

Comparative Distribution and Embryotoxicity of Acetylsalicylic Acid in Pregnant Rats and Rhesus Monkeys, WILSON, J. G., RITTER, E. J., SCOTT, W. J., AND FRADKIN, R. (1977). Toxicol. Appl. Pharmacol. 41, 67–78. Acetylsalicylic acid was given orally to pregnant rats on gestation Days 9–12 and to pregnant monkeys on Days 23–32 and to nonpregnant females of both species at doses of 100 and 150 mg/kg twice daily. Concentrations of salicylic acid were determined in maternal plasma, embryo, decidua, placenta, and chorionic and amniotic fluids at 1, 2, 4, 8, and 17 hr after the last dosage. Total salicylic acid concentrations in plasma after comparable doses were generally higher in rats than in monkeys and in nonpregnant than in pregnant rats, but concentrations varied in pregnant vs nonpregnant monkeys. Unbound salicylate in rat plasma ranged between 30 and 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 between 17 and 30% of the total plasma concentration and was to some degree paralleled by the concentration in the monkey embryo. The greater embryotoxicity of acetylsalicylic acid in the rat than in the monkey was correlated with higher concentrations and longer duration of concentrations in the respective embryos on a day-to-day basis.

The teratogenic potential of salicylates in rodents is clearly established by the demonstrated high sensitivity of rats (Warkany and Takacs, 1959), mice (Larsson and Eriksson, 1966), and hamsters (Lapointe and Harvey, 1964) to these compounds when given at daily doses of 250–1000 mg/kg at various times during pregnancy. Subhuman primates have been shown to be somewhat less susceptible, requiring larger doses than rats to attain appreciable embryotoxicity (Wilson, 1971, 1972). The present results, however, further substantiate the effects of aspirin in this regard in rhesus monkeys.

Data on human embryotoxicity associated with the taking of salicylates by pregnant women are meager. Occasional reports in the medical literature suggest that there may be increased pregnancy wastage, usually in the form of abortion, following repeated or

¹ Supported by National Foundation-March of Dimes Grant No. 1-385.
² Some of these data were presented in preliminary form at the 14th Annual Meeting of the Society of Toxicology in a symposium on “Comparative Toxicology” at Williamsburg, Virginia, March, 1975, and in a symposium on “New Approaches to the Evaluation of Abnormal Embryonic Development” at the Freie Universität, Berlin, September, 1975.

Copyright © 1977 by Academic Press, Inc. All rights of reproduction in any form reserved. Printed in Great Britain

ISSN 0041–008X
excessive use of salicylates during pregnancy (Jackson, 1948; Carter and Wilson, 1963) but direct causal relationships have not been established. Eight cases of presumed association between the taking of salicylate preparations during early pregnancy and the birth of variously malformed children have been reported (McNiel, 1973), but unresolved questions about dose and duration of dosage and the concurrent taking of other drugs render these cases no more than suggestive. Epidemiologic surveys have been inconclusive. In a retrospective study of 833 pregnancies producing malformed infants, Richards (1969) found that significantly more mothers ($p < 0.001$) took salicylates during the first trimester than did matched control mothers of normal infants. Nelson and Forfar (1971) in a similar survey of 458 mothers of defective children found that significantly more of them took aspirin than did control mothers during the first trimester. A partly prospective study by Saxén (1975) of 599 children with oral clefts revealed that 14.9% of their mothers had taken salicylates during the first trimester, but only 5.6% of the mothers of normal children had used these compounds ($p < 0.001$). Crombie et al. (1970), however, found that fewer aspirin-containing preparations were issued to mothers of malformed children than to women delivering normal babies.

The daily dose which produced a low level of teratogenicity when pregnant rhesus monkeys were treated during early gestation (Wilson, 1971, 1972) was 500 mg/kg, whereas 250 mg/kg caused extensive embryotoxicity in rats (Kimmel et al., 1971). Nevertheless, the fact that these disparate species were susceptible to developmental deviation under the influence of acetylsalicylic acid suggested that they would be interesting subjects for comparative study of the distribution of this drug during pregnancy. In a previous comparative study in pregnant rats and rhesus monkeys, hydroxyurea, which is highly embryotoxic at comparable doses in both was found to have somewhat different pharmacokinetics in the two species (Wilson et al., 1975). The present experiment is a similar pharmacokinetic study using acetylsalicylic acid, a drug with known differences in teratogenic potential in rats and monkeys.

**METHODS**

The methods for breeding rats and monkeys and for collecting embryonic and fetal materials have been described in detail elsewhere and are only briefly reviewed here for purposes of reference. Young adult female rats of a Wistar-derived strain (Royalhart) were considered to be at 0 time of gestation when sperm were found in vaginal lavage at 9 AM after being caged overnight with males of the same stock. Subsequent injections, collections of blood, and harvesting of embryos and fetuses were performed at stated days and hours thereafter. Acetylsalicylic acid was suspended in 0.3% aqueous solution of carboxymethyl-cellulose and given by gavage twice daily in a weight-adjusted volume ranging between 1 and 2 ml in total daily doses of 100–200 mg/kg on Days 9, 10, 11, and 12 of gestation. Plasma was prepared from blood removed by cardiac puncture under ether anesthesia at 1, 2, 4, 8, and 17 hr after treatment with acetylsalicylic acid. Plasma binding was measured by the ultrafiltration method for salicylates described by Spector et al. (1972) using freshly prepared plasma. Salicylic acid concentrations in plasma, placenta, and embryonic tissues and fluids were determined by the method of Chirigos and Udenfriend (1959) as modified by Kimmel et al. (1971), which is specific for salicylic acid. Tissues were homogenized in 0.01 N HCl and extracted into 1,2-dichloro-
ethane. This in turn was extracted into 0.5 M borate buffer, pH 10, and fluorescence was determined in an Aminco-Bowman spectrophotofluorometer at 400 nm, with activation set at 310 nm. Embryos from some females were removed 1, 2, 4, 8, or 17 hr after the last maternal gavage, weighed, and processed singly for salicylic acid determinations. Other pregnancies were allowed to continue to Day 20 when fetuses were removed and examined by routine methods to detect external, visceral, and skeletal abnormality.

Rhesus monkeys maintained in this laboratory for several months to a few years were bred and diagnosed for pregnancy by methods described by Wilson et al. (1970). The finding of motile sperm in vaginal lavage of females caged with experienced males on Days 11 to 13 of the menstrual cycle was considered to be time 0 of pregnancy. (Dating pregnancy in this manner may involve an error of ±24 hr.) Females known to be pregnant were given acetylsalicylic acid by gavage twice daily for 10 days on Days 23 through 32 of gestation (dosage was suspended in 10 ml of 0.3% aqueous carboxymethyl-cellulose). Blood for preparation of plasma was removed by venipuncture at 1, 2, 4, and 8 hr after gavage on Days 4 and 5 of treatment from some animals and at 1, 2, 4, 8, and 17 hr after the last gavage on Day 10 of treatment. Hysterotomy to remove the conceptus was also performed at these intervals after the last gavage. Whenever possible the chorion was removed intact so that samples of chorionic and amniotic fluids could be obtained for analysis. The embryo was examined externally under low magnification for heart beat and overt abnormality, weighed, and prepared for salicylic acid determination, as mentioned above.

Student’s t test for differences between means was used in analysis of data. All acetylsalicylic acid was powdered USP grade from Mallinckrodt.

RESULTS

Embryotoxicity in Rats

Table 1 summarizes dose-finding trials for general embryotoxicity as seen in the 20-day fetus. A dose of 100 mg/kg twice daily for 4 days produced no appreciable embryotoxicity and was chosen as the low dose for the subsequent comparative study. Significant but not overwhelming effects on intrauterine death, growth and malformation rates were caused by 150 mg/kg and this was chosen as the high dose. No sharply defined pattern of malformations could be associated with aspirin in the rat, although defects of the heart, brain, and axial skeleton were most frequent. Occasional maternal death and loss of weight were observed at 200 mg/kg.

Embryotoxicity in Monkeys

Table 2 summarizes the embryotoxicity of acetylsalicylic acid as seen in 32-day embryos and 100-day fetuses after treatment twice daily at 100 or 150 mg/kg for 10 days beginning on Day 23 of gestation. Both dosages may have caused a slight increase above the expected rate of intrauterine death (about 12% occurs spontaneously prior to 100 days in untreated animals of this colony) and both caused some transitory growth retardation on Day 32. Any early weight deficiency, however, appears to have been recovered in eight fetuses allowed to go to 100 days of gestation before removal.
In an earlier study larger daily doses of 200–250 mg/kg given two times per day for 3 consecutive days between Days 18 and 26 produced more pronounced embryotoxicity in 100-day fetuses (Wilson, 1971) than was observed here. Of eight female monkeys given this regimen, two aborted within 3 weeks after treatment, three produced mal-

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Total number of implants</th>
<th>Dead or resorbed (%)</th>
<th>Mean weight of survivors (g)</th>
<th>Survivors malformeda (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>95 (8)b</td>
<td>6</td>
<td>3.7</td>
<td>2</td>
</tr>
<tr>
<td>150</td>
<td>105 (8)</td>
<td>34</td>
<td>2.6</td>
<td>55</td>
</tr>
<tr>
<td>175</td>
<td>89 (7)</td>
<td>66</td>
<td>2.8</td>
<td>73</td>
</tr>
<tr>
<td>200</td>
<td>51 (4)</td>
<td>73</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>481 (41)</td>
<td>5</td>
<td>3.6</td>
<td>1</td>
</tr>
</tbody>
</table>

a Various malformations including cardiac, facial, brain, spinal, tail, and other skeletal defects were observed.
b Values in parentheses are the numbers of litters.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Day pregnancy terminated</th>
<th>Number of animals</th>
<th>Aborted or resorbed</th>
<th>Condition of offspring when removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>32</td>
<td>18</td>
<td>3</td>
<td>Nine growth retarded, six visibly normal</td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>4</td>
<td>0</td>
<td>Four normal</td>
</tr>
<tr>
<td>150</td>
<td>32</td>
<td>15</td>
<td>3</td>
<td>Two growth retarded, one grossly abnormal, one cranioschisis, eight visibly normal</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4</td>
<td>0</td>
<td>One cystic kidney, three normal</td>
</tr>
</tbody>
</table>
ASPIRIN DISTRIBUTION IN RATS AND MONKEYS

Maternal Plasma Concentrations of Salicylic Acid in Rats

The total plasma concentrations of salicylic acid, the principal metabolite of acetylsalicylic acid, appeared to reach a peak between 2 and 4 hr after oral administration, but remained relatively high for 8 hr after treatment at either dosage level in both pregnant and nonpregnant females (Table 3). Concentrations were consistently higher in nonpregnant than in pregnant females, although these were statistically different \( p < 0.05 \) only at 2 and 17 hr after 100 mg/kg and at 2 hr after 150 mg/kg. The percentage of total salicylic acid that remained unbound (free) in plasma was comparable in pregnant and in nonpregnant animals at either dosage for the first 2 hr, but thereafter the percentage in animals receiving the larger dose was generally somewhat higher than after the smaller dose. The latter observation suggests that the dose of 150 mg/kg may have exceeded available binding sites and consequently permitted a higher percentage of salicylic acid molecules to remain unbound after 2 hr.

Maternal Plasma Concentrations of Salicylic Acid in Monkeys

At comparable dosages and posttreatment intervals, plasma concentrations of salicylic acid were generally lower in monkeys than in rats (compare Table 4 with Table 3), and this reached statistical significance \( p < 0.05 \) at 4 hr and later as the concentrations in monkey began to decrease appreciably, whereas in rats a similar degree of lowering occurred only after 8 hr. Although plasma concentrations in pregnant and nonpregnant monkeys did not differ consistently, they were statistically higher \( p < 0.05 \) after 2, 4, and 8 hr at the 100-mg/kg dose in nonpregnant animals. The most striking

### TABLE 3

**Concentrations of Salicylic Acid in Plasma of Pregnant and Nonpregnant Rats at Intervals after Last of Eight Treatments Given Twice Daily on Days 9 through 12**

<table>
<thead>
<tr>
<th>Dose and status</th>
<th>Concentration (µg/ml) at specified hours after gavage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>100 mg/kg, pregnant</td>
<td></td>
</tr>
<tr>
<td>Total in plasma</td>
<td>169 ± 7</td>
</tr>
<tr>
<td>Unbound (%)</td>
<td>36</td>
</tr>
<tr>
<td>100 mg/kg, nonpregnant</td>
<td></td>
</tr>
<tr>
<td>Total in plasma</td>
<td>174 ± 27</td>
</tr>
<tr>
<td>Unbound (%)</td>
<td>36</td>
</tr>
<tr>
<td>150 mg/kg, pregnant</td>
<td></td>
</tr>
<tr>
<td>Total in plasma</td>
<td>185 ± 12</td>
</tr>
<tr>
<td>Unbound (%)</td>
<td>46</td>
</tr>
<tr>
<td>150 mg/kg, nonpregnant</td>
<td></td>
</tr>
<tr>
<td>Total in plasma</td>
<td>202 ± 20</td>
</tr>
<tr>
<td>Unbound (%)</td>
<td>37</td>
</tr>
</tbody>
</table>

* Each value is the mean ± SE of three to seven rats.

b \( p < 0.05 \) compared to corresponding concentrations in pregnant rats.
difference between rat and monkey plasma concentrations, however, was the low percentage of salicylic acid remaining unbound in the plasma. In all groups of monkeys in which adequate numbers of samples were taken, regardless of dosage or pregnancy status, this percentage rarely exceeded 30% and often was below 20%.

To determine whether the moderately prolonged duration (10 days) of treatment in monkeys might have influenced plasma concentrations by either accumulation or by a change in the rate of metabolism of acetylsalicylate, e.g., by enzyme induction, plasma samples were taken from some animals after 4 or 5 days of treatment for comparison with concentrations in plasma taken after 10 days of treatment on Day 32 of gestation.

### TABLE 4

**CONCENTRATIONS OF SALICYLIC ACID IN PLASMA OF PREGNANT AND NONPREGNANT MONKEYS AT INTERVALS AFTER THE LAST OF 20 ORAL TREATMENTS GIVEN TWICE DAILY ON DAYS 23 THROUGH 32**

<table>
<thead>
<tr>
<th>Dose and status</th>
<th>Concentration (µg/ml) at specified hours after gavage&lt;sup&gt;a&lt;/sup&gt;</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg/kg, pregnant</td>
<td>Total in plasma</td>
<td>139 ± 7</td>
<td>159 ± 6</td>
<td>116 ± 7</td>
<td>39 ± 5</td>
<td>3 ± 1</td>
</tr>
<tr>
<td></td>
<td>Unbound (%)</td>
<td>36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>100 mg/kg, nonpregnant</td>
<td>Total in plasma</td>
<td>121 ± 10</td>
<td>201 ± 34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>156 ± 24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86 ± 23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7 ± 2</td>
</tr>
<tr>
<td></td>
<td>Unbound (%)</td>
<td>25</td>
<td>17</td>
<td>16</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>150 mg/kg, pregnant</td>
<td>Total in plasma</td>
<td>169 ± 11</td>
<td>192 ± 10</td>
<td>197 ± 12</td>
<td>102 ± 13</td>
<td>79 ± 51</td>
</tr>
<tr>
<td></td>
<td>Unbound (%)</td>
<td>31</td>
<td>23</td>
<td>21</td>
<td>13</td>
<td>—</td>
</tr>
<tr>
<td>150 mg/kg, nonpregnant</td>
<td>Total in plasma</td>
<td>176 ± 14</td>
<td>182 ± 8</td>
<td>156 ± 9</td>
<td>90 ± 8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Unbound (%)</td>
<td>27</td>
<td>26</td>
<td>24</td>
<td>17</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each value is the mean ± SE of three monkeys, except as otherwise indicated.

<sup>b</sup> Based on only one measurement of free salicylic acid.

<sup>c</sup> p < 0.05 compared to corresponding concentration in pregnant monkeys.

No trend toward increased or decreased plasma concentrations was apparent and it was concluded that neither accumulation of salicylates nor change in metabolic rate occurred during the last several days of treatment in monkeys. Valid comparisons can therefore be made between plasma levels in the rat after 4 days of treatment and in the monkey after 10 days of treatment.

**Embryo Concentrations of Salicylic Acid in the Rat**

The concentration of salicylic acid in rat embryos roughly approximated the value of unbound metabolite in the maternal plasma at both dosages at most posttreatment intervals studied (Table 5). Although no observations were made at intervals of less than 1 hr, it is evident that no appreciable concentration gradient existed across the placenta for a significant period in either direction. In other words, passage across the
placenta appears to have been relatively rapid, as would be expected in case of simple diffusion.

The dosage of 100 mg/kg produced concentrations of salicylic acid in embryos that were maintained between 50 and 60 μg/g for 3 to 4 hr after treatment. Since this dosage was essentially nonteratogenic it can be inferred that the embryo can tolerate this concentration of salicylic acid for several hours per day (recalling that treatment was twice daily) between gestation Days 9 through 12 without ill effect. Conversely, the teratogenic dose of 150 mg/kg maintained embryo concentrations well above 60 μg/g for 8 hr or more after each treatment, or for a total period probably in excess of 16 hr each day. Thus a threshold for embryotoxicity can be crudely defined as that maternal dosage which maintains embryo dosage at greater than 60 μg/g for 16 or more hr per day during the known susceptible period.

Embryo Concentrations of Salicylic Acid in the Monkey

The concentration of salicylic acid in monkey embryos also approximated the value of unbound metabolite in maternal plasma at 2 hr after treatment and subsequently, but at 1 hr unbound plasma concentrations remained appreciably higher than embryo levels (Table 6). This may be indicative of slower diffusion across the monkey placenta than the rat placenta, a possibility in keeping with the greater thickness of the monkey placental membrane. (In the Fick equation the rate of diffusion is inversely proportional to thickness of the diffusion membrane.)

Mean embryo concentrations of salicylic acid in the monkey never exceeded 36 μg/g even at the larger dose of 150 mg/kg of acetylsalicylate, and it exceeded 20 μg/g only at 2 and 4 hr after treatment. This comparatively low-level, short-duration exposure in the monkey embryo to no more than 20–36 μg/g for perhaps 3 or 4 hr twice a day for 10 days may be at least partly responsible for the low level of embryotoxicity observed in the monkey at the doses used in the present experiment.

### TABLE 5

Comparison of Concentrations of Unbound Salicylic Acid in Maternal Rat Plasma and of Total Salicylic Acid in 12-Day Rat Embryos

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Concentration (μg/ml or g) at specified hours after gavage&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Plasma, unbound</td>
<td></td>
</tr>
<tr>
<td>Embryo</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Plasma, unbound</td>
<td></td>
</tr>
<tr>
<td>Embryo</td>
<td>63 ± 11</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each plasma value is the mean ± SE of three pregnant rats. Each embryo value is the mean ± SE of five to ten embryos from each of three litters.
TABLE 6

COMPARISON OF CONCENTRATIONS OF UNBOUND SALICYLIC ACID IN MATERNAL PLASMA AND OF TOTAL SALICYLIC ACID IN 32-DAY MONKEY EMBRYOS

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Concentration (µg/ml or g) at specified hours after dosinga</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma, unbound</td>
<td>50b</td>
<td>48b</td>
<td>46b</td>
<td>7b</td>
<td></td>
</tr>
<tr>
<td>Embryo</td>
<td>9 ± 4</td>
<td>22 ± 5</td>
<td>18 ± 5</td>
<td>3 ± 1</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma, unbound</td>
<td>53 ± 16</td>
<td>44 ± 4</td>
<td>42 ± 8</td>
<td>13 ± 3</td>
<td></td>
</tr>
<tr>
<td>Embryo</td>
<td>18 ± 5</td>
<td>36 ± 4</td>
<td>33 ± 21</td>
<td>15 ± 3</td>
<td></td>
</tr>
</tbody>
</table>

a Each plasma value is the mean ± SE of three pregnant monkeys and each embryo value is the mean ± SE of the embryos from the three pregnant monkeys at the respective dose and posttreatment interval.

* Only one measurement of free salicylic acid.

Fig. 1. Comparison of salicylic acid concentrations in monkey plasma and embryos and in rat plasma and embryos after a maternal dose of 150 mg/kg of acetylsalicylic acid. Each point for monkeys is the mean of three maternal or three embryo animals. In the case of rats, each point is based on three to seven maternal animals and on five to ten embryos from each litter. The vertical brackets represent the SE.
Comparative Distribution in the Monkey and Rat

Figure 1 illustrates the striking differences in distribution of salicylic acid in these two species at a dose of 150 mg/kg. Total concentrations in the maternal plasma remained generally higher in the rat than in the monkey, particularly at 4 and 8 hr following treatment. A larger proportion of the total metabolite in rat plasma was unbound and thus available for placental transfer, further metabolism, or urinary excretion. The higher concentration of free salicylic acid in rat plasma was closely paralleled by concentrations in the embryo. In the monkey, the plasma concentration of total metabolite fell rapidly between 4 and 8 hr and since it did not accumulate in the embryo, it is assumed that salicylic acid was removed from monkey plasma either by further metabolism or by more rapid excretion in the monkey than in the rat. Other explanations are possible, e.g., storage in nonplasma compartments, but in any case it should be recalled that monkey plasma concentrations were no more different after 4 or 5 days than after the full 10-day treatment span.

As already noted, at an equivalent maternal dosage the monkey embryos is exposed to a much smaller dosage on a day-to-day basis than is the rat embryo.

Distribution to Various Compartments of the Monkey Conceptus

As shown in Tables 4 and 6 concentrations of salicylic acid in the monkey embryo remained low compared with total concentrations in maternal plasma at all post-treatment intervals and at both dosage levels. To determine concentrations in other compartments of the conceptus, i.e., decidua, placenta, yolk sac, amniotic fluid, and chorionic fluid, attempts were made to harvest adequate samples of these tissues and fluids when embryos were removed at hysterotomy. These attempts were not always successful, owing to contamination with maternal blood or loss of chorionic and amnio-

<table>
<thead>
<tr>
<th>Dose and tissue</th>
<th>Concentration (μg/g) at specified hours after dosinga</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Decidua</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>Placenta</td>
<td>23 ± 8</td>
</tr>
<tr>
<td>Embryo</td>
<td>9 ± 4</td>
</tr>
<tr>
<td>150 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Decidua</td>
<td>73 ± 21</td>
</tr>
<tr>
<td>Placenta</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Embryo</td>
<td>18 ± 5</td>
</tr>
</tbody>
</table>

a Each value is the mean ± SE of three specimens from the pregnant monkeys in Table 6.
tic fluids during removal, although adequate samples of decidual tissue and placenta were usually available and the concentrations in these and in the embryos are presented in Table 7.

Concentrations in the decidua and placenta paralleled roughly, but at a higher level, those in the embryo. Actually they more closely followed total concentrations in the maternal plasma, the decidua being about 35% and the placenta about 25% of the plasma concentration (Table 4). These concentrations were probably proportional to the content of maternal blood in these tissues.

Data on salicylic acid content of the embryonic yolk sac and of chorionic and amniotic fluids are too meager and variable to permit more than broad generalizations. Surprisingly, concentrations in the yolk sac, a truly embryonic tissue, were invariably two to five times greater than in the remainder of the associated embryo at both doses and at all posttreatment intervals in the 15 specimens examined. This suggests accumulation or storage by the yolk sac epithelium. Concentrations in the accessory fluid compartments of the chorion and amnion were highly variable but tended to approximate that in the embryo during the first 4 hr, whereas at 8 hr all of four samples measured had appreciably higher concentrations than did the associated embryo. The latter observation suggests that these fluid compartments may act as a sump in which extraneous chemicals may slowly accumulate, possibly later tending to prolong embryonic exposure by backward diffusion into the embryo after blood concentrations in embryo and maternal plasma have decreased.

DISCUSSION

When general embryotoxicity of rats and monkeys to acetylsalicylic acid was compared at equivalent dosages it was evident that these species have somewhat different dose-response characteristics. In rats a dose of 100 mg/kg essentially had no effect on intrauterine development, whereas a dose of 150 mg/kg had appreciable effects in terms of intrauterine death, growth retardation, and rate of malformation. It was possible to establish a crude threshold of embryotoxicity in the rat for salicylic acid, namely, >60 µg/g of embryo for 16 or more hr per day between Days 9 and 12 of gestation. The dose-response situation in monkeys was less clear and no such threshold was identified.

It is of interest to examine the extent to which these differences in embryotoxicity can be correlated with differences in the distribution of aspirin in pregnant rats and monkeys. The total plasma concentrations of salicylic acid, the principal metabolite in plasma, were generally higher in rats than in monkeys at comparable doses and at most intervals after gavage. Probably of greater importance was the fact that a higher percentage of the total concentration remained unbound in the rat (30-50%) than in the monkey (<30%) within 8 hr after gavage, and that the concentration of free metabolite in the plasma was closely paralleled by concentrations in the embryos of either species. In general terms this meant that the rat embryo was exposed to about three times higher concentrations of salicylic acid for approximately twice as long as was the monkey embryo; in other words to a considerably greater level and duration of dosage each day. Thus, the statement in an earlier publication to the effect that the rat is teratogenically more sensitive to aspirin than the monkey (Wilson, 1971) must now be
questioned. The greater exposure of the rat embryo than of the monkey embryo at equivalent maternal dosages could account for some or all of the embryotoxicity.

An earlier study (Wilson et al., 1975) compared the distribution of hydroxyurea in pregnant rats and rhesus monkeys. This compound, thought itself to be the active teratogen, is mainly metabolized to urea. It was found to disappear from maternal plasma of both species somewhat faster than was salicylic acid in the present study. For example, a maternal iv dose of 100 mg/kg produced a mean plasma concentration in monkey of 92 µg/ml at 1 hr after treatment, and this was reduced to 29 µg/ml at 4 hr, with a half-life calculated to be about 120 min. A 100-mg/kg oral dose of aspirin produced a mean concentration of 139 µg/ml at 1 hr, which continued to rise to 159 µg/ml at 2 hr and was still at 116 µg/ml at 4 hr. Clearance of salicylic acid from monkey plasma is thus much slower than the comparable clearance of hydroxyurea. The difference in clearance rate between the two drugs was even more dramatic in the pregnant rat. A 100-mg/kg dose of hydroxyurea iv resulted in a plasma concentration of 43 µg/ml at 0.5 hr, of 15 µg/ml at 1 hr, and of 1.2 µg/ml at 2 hr, with a half-time estimated to be 15 min. A 100-mg/kg dose of aspirin in rats produced a plasma concentration of salicylic acid of 169 µg/ml at 1 hr and it was still at 133 µg/ml at 8 hr. The more rapid clearance of hydroxyurea from the plasma of both species may be attributed, in part at least, to the lower degree of binding of this drug (unpublished observations), than of aspirin to plasma proteins, which presumably would allow for more rapid filtration at the renal glomerulus.

The teratogenicity of a drug or other chemical agent, however, is not directly dependent on the level or duration of concentration in maternal plasma. More critical determinants of the teratogenic potential of a foreign chemical are: (1) its mode of action within the embryo and (2) the embryo dosage as a factor of both level and duration of concentration in the embryo. The placenta may also influence embryo dosage by controlling rates of entry and egress of foreign chemicals to and from the embryo. Although most drugs are said to cross the placenta by simple diffusion, the ease of passage of the parent compound as well as its metabolites is a function of molecular size, lipid solubility, polarity, etc. The mode of action of aspirin or of its metabolite salicylic acid in the embryo is unknown, but in producing its teratogenic effect (at 150 mg/kg) it is known to be present in the rat embryo at concentrations in excess of 60 µg/g for 16 or more hr per day. Yet, hydroxyurea produces a roughly equivalent teratogenic effect (at a 137-mg/kg maternal dosage) at embryo concentrations only slightly above 16 µg/g for less than 2 hr per day. It is thus apparent that teratogenic effects cannot be related simply to maternal plasma concentrations or to molar concentrations in the embryo for stated periods of time. The most critical factor, therefore, would seem to be the mechanism of action of the agent within the embryo; that is, how it impinges on the cells of the developing organism and how this influences the course of subsequent development.

REFERENCES


OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

SALICYLIC ACID

adopted by the SCCNFP during the 20th plenary meeting of 4 June 2002
1. Terms of Reference

1.1 Context of the question

Cosmetic products marketed in the EU may only contain those preservatives which are listed in Annex VI of the Cosmetics Directive 76/768/EEC, “List of preservatives which cosmetic products may contain”.

The preamble of the Annex states that preservatives marked with the symbol (+) may also be added to cosmetic products in concentrations other than those laid down in the Annex for other specific purposes apparent from the presentation of the products.

Salicylic acid and its salts bear the symbol (+) and can therefore be used in cosmetics at higher concentrations, as long as they are not employed as preservatives.

In its opinion of 17 February 1999 concerning the restrictions on materials listed in Annex VI of Directive 76/768/EEC on cosmetic products, the SCCNFP stated that those substances indicated by (+) in Annex VI, when incorporated into cosmetic formulations for non-preservative functions, should be subjected to the same restrictions in usage levels and warnings as when used for preservative effects.

If a preservative marked with the symbol (+) is added for non-preservative purpose to a cosmetic product in a concentration higher than that laid down in the Annex VI, data to substantiate its safety should be submitted to the SCCNFP.

1.2 Request to the SCCNFP

The SCCNFP was requested to review the data submitted to support the safety of salicylic acid and its salts, when used at concentrations other than those laid down in Annex VI to Directive 76/768/EEC as preservatives, for other specific non-preservative purposes apparent from the presentation of the products:

* Submission I: concerns the evaluation of the safety of salicylic acid for other specific non-preservative purposes: leave-on formulations (face and general creams) and rinse-off products (make-up removers, shower gels, shampoos and hair conditioners) at a level of 2 %, leave-on hair care products at 1 % salicylic acid level and the use of salicylic acid as a preservative in other cosmetic products at the 0.5 % concentration.

The SCCNFP was also requested to answer the following question:

* Submission II: can salicylic acid and its salts safely be used for non-preservative purposes in cosmetic rinse-off hair products at a maximum concentration of 3 %?
1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission’s general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods (doc. n° SCCNFP/0546/02).

The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

In the interest of consumer’s health protection, the SCCNFP highlights the important requirement of ensuring that files for evaluation are submitted complete.

The files should include, as well as the results procured by the applicants themselves, all relevant published literature and other findings to the applicant’s best ability, and also “grey material” available elsewhere. Subsequently, should additional data or information be acquired by Industry and/or other agencies, this should be transmitted to the Commission, for review as necessary.
2. Toxicological Evaluation and Characterisation

2.1. General

Salicylic acid is a white crystalline powder originally introduced for oral therapeutic purposes by Rev. Edmund Stone in 1763. Wintergreen leaves, willow and sweet birch bark, bacteria, fungi and fruits represent its natural occurrence. Salicylic acid and its derivatives, have been available by a synthetic process since the mid 1800s.

Salicylic acid and its salts are currently in Annex VI part 1 number 3 and are restricted to a maximum concentration of 0.5 % calculated as acid function and submitted to the following restrictions:

- limits and requirements: “do not use in preparations for children under 3 years old, excepting shampoos”

- warnings to be printed on the label: “do not use for children care under 3 years old” (only concerning products which could be in contact with the skin for a long time)

2.1.1. Chemical name

Salicylic acid

2.1.2. Synonyms

o-hydroxybenzoic acid
2-hydroxybenzoic acid

2.1.3. Trade names and abbreviations

No names are available

2.1.4. CAS no.

69-72-7

2.1.5. Structural formula

![COOH

OH](image)
2.1.6. Empirical formula

Emp. Formula : C₇H₆O₃
Mol weight : 138.12

2.1.7. Purity, composition and substance codes

No data are available

2.1.8. Physical properties

Melting point 158-160°C
Boiling point 211°C at 20 mm Hg
Vapor pressure 5 mm Hg at 136°C
Density 1.443 at 20°C
Partition coefficient Log P<sub>ow</sub> : 0.35
Flash point 157°C

2.1.9. Solubility

In water 2.17 mg/ml at 20°C
Soluble in : ethanol, diethyl ether

2.2. Function and uses

**Cosmetic uses**

Salicylic acid used as preservative (up to 0.5 %) in cosmetic products. At a level up to 1 % in leave-on hair products and up to 2 % salicylic acid is used in leave-on cosmetics products (face and general creams) for its exfoliating and cleansing properties and in rinse-off products (shower gels, shampoos, hair conditioners, make-up removers).

**Dermatological uses**

Salicylic acid is widely used in the treatment of many common dermatological conditions because of its keratoplastic properties.

**Other fields of use**

Salicylic acid is used: as a preservative in food, as a chemical raw material for the synthesis of dye and salicylates derivatives (aspirin), as an antiseptic and antifungal by topical application in veterinary medicine.
TOXICOLOGICAL CHARACTERISATION

Important note :

Following oral administration, acetylsalicylic acid (aspirin) is rapidly hydrolysed to salicylic acid. Considering the great number of available and published toxicological data concerning acetylsalicylic acid, when compared to salicylic acid, the studies performed with this ingredient have been considered to have also some importance for salicylic acid.

However, concerning pharmacokinetic data, studies have shown a different mechanism of action between acetylated versus non-acetylated salicylates. Acetylsalicylic acid inhibits prostaglandin synthesis by irreversible acetylation of the cyclo-oxygenase, whereas the interaction of salicylic acid with the cyclo-oxygenase is transient and reversible, suggesting a minimal inhibiting activity.

2.3. Toxicity

2.3.1. Acute oral toxicity

Animal data

Acute toxicity has been investigated following various routes :

The oral LD50 of salicylic acid were 400-3700 mg/kg for the Rat. Ref. : 6, 66

The oral LD50 of formulations containing salicylic acid up to 2% were 10-20 g/kg for the rat, that is equivalent to 200 to 400 mg/kg bw for the pure substance. Ref. : 82, 83, 84

Human data

In human the oral lethal dose for sodium salicylate is estimated between 20 and 30 g in adults. Ref. : 32

Toxic effects were reported when 10 g or more of salicylates are given orally in single dose or divided doses within a period of 12 to 24 hours. Children under the age of 3 years are more sensitive than adults to salicylates. Ref. : 25

2.3.2. Acute dermal toxicity

The topical application of acetylsalicylic acid powder at a dosage of 2 g/kg to Rabbit did not induced any sign of erythema or oedema on both the intact and abraded skin of the animals. The dermal LD50 was estimated greater than 2 g/kg in Rabbit. Ref. : 85
2.3.3. Sub-chronic toxicity

2.3.3.1 Sub-chronic dermal toxicity

Animal data

A 14-day percutaneous study was performed in four groups of 3 male and 3 female New Zealand White rabbits administered topically at 2 g/kg/day of salicylic acid-containing solutions. The concentrations tested were 0%, 2%, 10% and 25% (corresponding to 0, 40, 200 et 500 mg/kg/day) of salicylic acid in a vehicle solution (8% propylene glycol butyl ether in ethanol). After a 7-hour period of daily exposure, the application site was washed with water and dried. No death was observed during the study. Dose-related slight to marked erythema and oedema were noted for all dosage groups. Desquamation was most often noted in the 25% salicylic acid group; fissuring of varying degree was observed in all dosage groups. Eschar was noted in the 10% and 25% dosage groups; exfoliation was noted on day 13 in a 25% dosage group. Atonia was predominantly observed in the animals treated with 10% and 25% salicylic acid. These signs were generally noted on or between days 7 to 14. The changes in the body weights of animals were considered as not remarkable during the study. Concerning clinical findings, no visible abnormalities were noted at necropsy in any animal beyond the dermal irritation observed at the test sites. Under the experimental conditions adopted, the test articles were considered as dermal irritants by the investigators.

Ref. : 110

Two 91-day studies were performed in New Zealand White rabbits in order to assess the subchronic cutaneous and systemic toxicity of two cleansing formulations containing 0.5% salicylic acid. 2 ml/kg of the test article, corresponding to 10 mg/kg, was applied to intact skin of the rabbits, with 7 hours daily exposure, 5 times a week. The neat or 50% w/v in distilled water diluted product was applied. Controls were treated with distilled water. The following observations were performed during both studies: clinical data (food consumption, faeces, behavior), daily dermal irritation observations, body weights records, mean haematology values (neutrophil, monocytes, basophil, leucocytes and lymphocytes counts), gross pathology findings (organ lesions, skin lesions), organ weights and histopathology findings.

No death was observed during the study. No statistical differences were found in mean body weight and in organ weight. Transient dermal irritation including erythema, oedema, atonia, desquamation and fissuring, varying up to moderate intensity and transient slight to moderate desquamation were observed and considered related to the treatment. No systemic toxicity was observed as confirmed by the clinical evaluation, the clinical chemistry, haematological and histopathological examinations. The tested products were considered slightly and transiently irritating to the skin when applied neat or at a concentration of 50% w/v to the intact skin of Rabbit.

Ref. : 111, 112

Two 91-day subchronic cutaneous toxicity studies were performed in New Zealand White rabbits treated with cleansing formulations containing 0.5% to 6% of salicylic acid in propylene glycol butyl ether/ethanol (vehicle), corresponding to topical doses of 10, 20, 40 or 120 mg/kg of
salicylic acid. Two controls group were included, one with untreated animals, one with vehicle treated animals. The tested product was applied once daily during a seven hour period, five days per week at a dosage volume of 2 ml/kg to the intact skin of the animals. A first 28-day period was followed by an interim sacrifice of five animals per group; the remaining animals continued on study to the 91-day termination. The observations recorded during the study were: clinical signs, dermal irritation, body weights, ophthalmoscopic examinations, haematological parameters (haematocrit, haemoglobin, erythrocyte/leucocyte and platelet counts, coagulation times), biochemical parameters (ASAT, ALAT, alkaline phosphatase, glucose, urea nitrogen, bilirubin, cholesterol, albumin, globulin, total protein, creatinine, electrolytes, phosphorus, calcium), urological parameters (volume, specific gravity), serum salicylate analysis, macroscopic and microscopic examinations, organ weights.

All animals survived after 28 days and 91 days of treatment. There were no test article-related effects on appearance, behaviour, body weights or ophthalmoscopic examinations. Slight to marked erythema, desquamation, fissuring, oedema and slight to moderate atonia were noted at the site of application. The greatest severity for all findings, particularly scab formation, and desquamation, was observed most predominantly in the high dose group and during the first 28 days of the treatment. After 91 days of treatment, the severity and frequency of hyperkeratosis, acanthosis and dermal inflammation were greatest in the high-dose group. The differences noted in body weight gain and in the body weight change values were not considered treatment-related. No test article-related toxicologic findings were detected in any haematological, biochemical or urological parameters. Serum salicylate was noted in all groups at 1 hour after dosing; the maximum levels occurred between 2.5 and 7 hours after dosing. A low incidence of trace to mild myocardial degeneration was observed in all treatment groups and the vehicle control group at the terminal sacrifice. However no dose-response relationship was retained with respect to either lesion incidence or severity.

Under the experimental conditions adopted, the tested formulations were considered irritant.

Ref. : 113, 114

Human data

Mild chronic salicylate intoxication is defined as salicylism and was described after topical application.

Ref. : 15, 29, 50, 151, 162, 183

This event is rare and depends among various factors such as the age of the patient, the intensity of the skin damage, the concentration of salicylic acid in the formulation, the surface of application. Ointments containing salicylic acid 3 to 6 % have caused nausea, dyspnæae, hearing loss, confusion and hallucinations in 3 patients with extensive psoriasis. They had two soap and water baths daily combined with UV therapy and six ointment applications. Under these conditions, the symptoms developed in 4 days and were associated with significant salicylic acid plasma levels (46 to 64 mg/100 ml). Symptoms disappeared rapidly after discontinuation of the ointment applications.

Ref. : 175
Two fatal cases of percutaneous salicylate poisoning, caused by the treatment of a fungal infection with an alcoholic solution containing 20 % salicylic acid, were described.  

Ref. : AR3

Salicylism can be developed within a short period of treatment. A case was reported after 2 days of treatment, with 10 % salicylic acid, in a man with a widespread psoriasis that covered 80 % of his body surface.

Ref. : AR2

The application of salicylic acid to extensive areas, particularly in children, may involve a risk of toxicity from absorption. Children are particularly susceptible.

Ref.: 79, AR4, AR5

Salicylate plasma levels can be indicative of salicylic acid intoxication. Symptoms occur at plasma level of 35 mg/100 ml or higher.

Ref. : 14

The correlation between body salicylate and clinical severity of the intoxication is poor, that can be associated with the variability of the protein binding and the blood pH. Severe manifestations are linked with diseased skin, multiple applications on large body areas of formulations containing high concentrations of salicylic acid.

2.3.4. Chronic toxicity

2.3.4.1 Chronic oral toxicity

Animal data

In the submission, there is only information on acetylsalicylic acid-related toxicity following oral administration. With regard to the rapid hydrolysis of acetylsalicylic acid to salicylic acid after oral absorption, the available animal data concerning acetylsalicylic acid have been considered.

Ref. : 69, 76

A 200-day comparative study was performed in rats (2 groups of 10 animals) in order to assess the long term toxicity of acetylsalicylic acid versus acetaminophen, and their possible interaction when combined. Acetylsalicylic acid alone is discussed in this report. This ingredient was administered orally by gavage at the concentration of 200 mg/kg/day, a volume of 10 ml/kg was administered. Clinical tests, as body weight gain, were performed at monthly intervals; during that time, alkaline phosphatase, lactic dehydrogenase analysis, volume, pH and osmolarity of urine were recorded; aspartate and alanine aminotransferase were measured from blood samples. Macroscopic and microscopic examinations were performed after dosing for 200 days.

Two deaths were observed during the study with acetylsalicylic acid and were not considered related to the treatment. A mildly toxic effect on the kidney (without any sign of pathology) was recorded; the osmolarity of the treated group was significantly higher than control in the fourth
and fifth months. No significant changes compared to the control were related to a potential toxicity of acetylsalicylic acid at the dose level used.

Ref. : 169

**Human data**

Ingestion of acetylsalicylic acid tablets is the major cause of salicylate poisoning in adults. In children, infants and neonates other causes were described in the literature (teething gels to gums, breast milk, placental transfer)

Ref. : 1, 16, 63, 81

Oral doses of acetylsalicylic acid of 100 mg/kg or higher induce salicylism. Plasma levels are indicative of salicylic intoxication, symptoms are occurring at plasma levels of 35 mg/100 ml or greater.

Ref. : 14

### 2.4. Irritation & corrosivity

#### 2.4.1. Irritation (skin)

**Animal data**

The compound salicylic acid was moderately irritant to minimally irritant when applied up to 2% product formulations or alcohol solutions to intact or abraded rabbit skin under occlusion or semi-occlusion for up to 24 hours. The pH of the tested products was between 2.8 and 4.0.

Ref. : 88, 91, 93, 94

**Skin irritation studies in rabbit**

<table>
<thead>
<tr>
<th>salicylic acid %</th>
<th>Test conditions</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not indicated (laundry additive) pH 4.0</td>
<td>0.5 g semi-occlusive patch for 4 hours</td>
<td>Moderately irritating</td>
<td>91</td>
</tr>
<tr>
<td>2% in alcohol solution pH 2.65</td>
<td>0.5 g occlusive patch for 24 hours</td>
<td>Minimally irritating</td>
<td>88</td>
</tr>
<tr>
<td>2% in alcohol solution pH not indicated</td>
<td>0.5 ml occlusive patch for 24 hours</td>
<td>Non irritating</td>
<td>93</td>
</tr>
</tbody>
</table>
Evaluation and opinion on: Salicylic Acid

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Test conditions</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25% in hydroalcoholic cleanser</td>
<td>0.5 ml occlusive patch for 24 hours</td>
<td>Non irritating</td>
<td>94</td>
</tr>
<tr>
<td>0.5% in hydroalcoholic cleanser pH 2.81</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Repeated open applications of 0.25% to 5% hydroalcoholic solutions (no correspondence available between weight of tested substance and surface unit) of salicylic acid with pH between 2.3 and 3.0 were performed to the skin of guinea pigs for up to 5 consecutive days. Mild to no irritation was noted.

Ref.: 86, 87, 89

**Skin irritation studies in guinea pig**

<table>
<thead>
<tr>
<th>Salicylic acid %</th>
<th>pH</th>
<th>Test conditions</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5% in alcohol solution; pH 2.34</td>
<td></td>
<td>0.15 ml open application for 3 hours, twice a day for 4 consecutive days</td>
<td>Mildly irritating</td>
<td>86</td>
</tr>
<tr>
<td>5% in alcohol solution pH 2.32</td>
<td></td>
<td></td>
<td>No peak for skin irritation noted</td>
<td></td>
</tr>
<tr>
<td>0.5% in hydroalcoholic cleanser; pH 2.90</td>
<td></td>
<td>0.25 ml open application for 23 hours, once daily for 5 consecutive days</td>
<td>Minimal skin irritation</td>
<td>87</td>
</tr>
<tr>
<td>0.25% in hydroalcoholic cleanser; pH 2.7–2.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5% in hydroalcoholic cleanser; pH 2.70</td>
<td></td>
<td>0.25 ml open application for 23 hours, once daily for 5 consecutive days</td>
<td>Non irritating</td>
<td>89</td>
</tr>
</tbody>
</table>

**Human data**

After repeated application of formulations containing up to 2% salicylic acid, it is possible to categorize salicylic acid as a mild transient irritant.

<table>
<thead>
<tr>
<th>Duration</th>
<th>salicylic acid %</th>
<th>Test conditions</th>
<th>Results (classified as)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
<td>2%</td>
<td>Occlusive and semi occlusive patches diluted</td>
<td>Test substance (15%) non irritating</td>
<td>137</td>
</tr>
<tr>
<td>cream</td>
<td>pH not indicated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In submission II additional human skin irritation data are provided for the use of salicylic acid in rinse-off products at an increased concentration from 2 % to 3 %.

In a 12 days cumulative irritation study with a shampoo containing 3 % salicylic acid applied continuously under a patch test (as a 4 % dilution) the formulation showed a potential for irritation under the drastic test conditions..

Ref. : 187

When shampoos (prototype or commercial formulations) containing 3 % salicylic acid were compared in exaggerated use repeat application patch test (4 studies were conducted) to shampoo formulations containing up to 2 % of salicylic acid versus placebo, there were no statistical differences in combined irritation or transepidermal water loss (measured with an Evaporimeter™). So at 3 % in a rinse-off shampoo formulation salicylic acid does not appear to be more irritant than the other components of the formulations.

Ref. : 188, 189, 190, 191

<table>
<thead>
<tr>
<th>Time</th>
<th>Type of Product</th>
<th>Concentration</th>
<th>pH</th>
<th>Occlusive Patch Duration</th>
<th>Application Frequency</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 days</td>
<td>Surfactant-based product</td>
<td>Concentration not indicated</td>
<td>pH not indicated</td>
<td>Occlusive patch 24 hours 7 times/week</td>
<td>Probably mildly irritating in normal use conditions</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>
2.4.2. Irritation (mucous membranes)

Animal data

Numerous formulations (non-alcoholic “NA” and hydroalcoholic) containing salicylic acid 0.5 % to 2 % have been evaluated in a modified Draize test: the Low Volume Eye Test. The formulations tested were considered by the investigators as mild irritant when instilled into the Rabbit eye.

Ref. : 33, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104

<table>
<thead>
<tr>
<th>Test material</th>
<th>Average Score</th>
<th>Median days to clear</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH cleansing milk with 0.05% SA pH 5.4</td>
<td>2.7</td>
<td>1</td>
<td>Redness and discharge</td>
<td>95</td>
</tr>
<tr>
<td>NH toner with 0.2% SA pH 5.7</td>
<td>2.7</td>
<td>2</td>
<td>Redness and discharge</td>
<td></td>
</tr>
<tr>
<td>NH moisturizer with 2% SA</td>
<td>5.3</td>
<td>4</td>
<td>Conjunctivitis</td>
<td>96</td>
</tr>
<tr>
<td>Hydrogel with 2% SA</td>
<td>3.3</td>
<td>3</td>
<td>Conjunctivitis</td>
<td>97</td>
</tr>
<tr>
<td>NH cleanser with 2% SA pH 3.09</td>
<td>4.3</td>
<td>4</td>
<td>Iritis (1/3), conjunctivitis (2/3)</td>
<td>98</td>
</tr>
<tr>
<td>NH cleanser with 2% SA pH 3.09</td>
<td>2.0</td>
<td>3</td>
<td>Conjunctivitis (2/3)</td>
<td></td>
</tr>
<tr>
<td>NH cleanser with 2% SA pH 3.09</td>
<td>6.3</td>
<td>4</td>
<td>Iritis (1/3), conjunctivitis (3/3)</td>
<td></td>
</tr>
<tr>
<td>NH moisturizer with 2% SA</td>
<td>1.3</td>
<td>2</td>
<td>Conjunctivitis (2/3)</td>
<td>99</td>
</tr>
<tr>
<td>NH cream with 1.5% SA</td>
<td>2.7</td>
<td>3</td>
<td>Conjunctival swelling and redness (2/3)</td>
<td>100</td>
</tr>
<tr>
<td>NH cream with 1.5% SA</td>
<td>0.7</td>
<td>1</td>
<td>Conjunctivitis (1/3)</td>
<td></td>
</tr>
<tr>
<td>NH cream with 1% SA</td>
<td>0.7</td>
<td>1</td>
<td>Conjunctivitis (1/3)</td>
<td></td>
</tr>
</tbody>
</table>
Low Volume Eye Tests in rabbit (continued)

<table>
<thead>
<tr>
<th>Test material</th>
<th>Average Score</th>
<th>Median days to clear</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH moisturizer with 2% SA, pH 2-3</td>
<td>2</td>
<td>2</td>
<td>Conjunctivitis (2/3)</td>
<td>101</td>
</tr>
<tr>
<td>NH moisturizer with 2% SA, pH 4-5</td>
<td>2</td>
<td>2</td>
<td>Conjunctivitis (2/3)</td>
<td></td>
</tr>
<tr>
<td>NH moisturizer with 2% SA</td>
<td>0.7</td>
<td>1</td>
<td>Conjunctival redness (1/3)</td>
<td>102</td>
</tr>
<tr>
<td>NH moisturizer with 2% SA</td>
<td>1.3</td>
<td>1</td>
<td>Conjunctival redness and swelling (1/3)</td>
<td></td>
</tr>
<tr>
<td>NH moisturizer with 1.5% SA</td>
<td>2</td>
<td>3</td>
<td>Conjunctival redness (3/3)</td>
<td></td>
</tr>
<tr>
<td>NH moisturizer with 0.5% SA</td>
<td>0</td>
<td>0</td>
<td>No effects observed</td>
<td></td>
</tr>
<tr>
<td>NH cream with 2% SA,</td>
<td>3.3</td>
<td>2</td>
<td>Conjunctivitis (3/3)</td>
<td>103</td>
</tr>
<tr>
<td>Hydro after-shave with 2% SA</td>
<td>1.3</td>
<td>4</td>
<td>Conjunctival discharge and redness (2/3)</td>
<td>104</td>
</tr>
<tr>
<td>Hydro after-shave with 2% SA</td>
<td>1.3</td>
<td>2</td>
<td>Conjunctival redness (2/3)</td>
<td></td>
</tr>
</tbody>
</table>

SA: salicylic acid ; NH: Non Hydroalcoholic

**Human data**

Different formulations containing salicylic acid were evaluated for their eye irritation potential:

- 2% salicylic acid in cream after a single periocular application induced a transient subjective irritation (stinging, burning, itching) that was confirmed by ophthalmic investigation. The irritation resolved within 96 hours. The applied cream to the periocular area was able to migrate into the eyes.

  Ref. : 118

- A twice daily application for five days of a 2% salicylic acid cream to normal and sensitive eye volunteers induced mild to moderate bulbar conjunctival inflammation and a mild to moderate corneal superficial punctuate keratopathy. Irritation increases as the length of exposure. All eyes returned to normal within two days after the end of exposure.

  Ref. : 150
- From a cream containing 1.5 % salicylic acid, with a pH of 2.5 to 2.8, after a 3 day periocular study, a mild conjunctival inflammation and mild or no superficial punctate keratopathy were reported.
  Ref. : 117, 118

- The same formulation was evaluated in a 4 week in-use study, with application around the eyes twice a day for 28 days. Results indicated a mild ocular irritation.
  Ref. : 148

- In a 14 day in-use study comparing a 1.5 % salicylic acid cream and a cream without salicylic acid, the superficial conjunctival and corneal superficial keratopathy were mild and similar for all the formulations (including the one without salicylic acid).
  Ref. : 149

- In a large 6 week home use-test study with a cosmetic formulation containing up to 2 % salicylic acid (pH 3.0 to 3.09) 4 volunteers in a group of 64 users of salicylic acid were subject to a mild, transient irritation, in a group of 48 volunteers using the control product without salicylic acid 3 were subject to similar effects.
  Ref. : 119

From these studies, it can be concluded that the intensity of the eye irritation with salicylic acid containing formulation is strongly related to the composition and formulation of the matrix and the capacity to migrate into the eyes.

2.4.3. In-use skin test

Human data

Under the conditions of use in the market place, the studied products have demonstrated to have a low potential for skin irritation on the face of the panellists.

<table>
<thead>
<tr>
<th>Duration Matrix</th>
<th>salicylic acid % pH</th>
<th>Test conditions</th>
<th>Results (classified as)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks hydroalcoholic solution</td>
<td>0.5 % pH 2.82</td>
<td>Daily application forehead and nose</td>
<td>No evidence of skin irritation</td>
<td>142</td>
</tr>
<tr>
<td>6 weeks no alcoholic lotion</td>
<td>2 % pH not indicated</td>
<td>Home use test</td>
<td>Mild, transient and/or sporadic reactions even for the products without salicylic acid</td>
<td>140</td>
</tr>
<tr>
<td>6 weeks cream no alcohol</td>
<td>2 % pH not indicated</td>
<td>Home use test 50 % of the included panellists had self assessed sensitive skin</td>
<td>Little or no irritation potential</td>
<td>147</td>
</tr>
</tbody>
</table>
Evaluation and opinion on : Salicylic Acid

| 12 weeks cream with no alcohol hydroalcoholic gel and lotion | not indicated pH not indicated | Neat product 2 times/day 5 days/week | The cream showed improvement in irritation and dryness versus lotion | 141 |
| 14 weeks non alcoholic lotions and moisturizers with/without salicylic acid | 2% pH 2.28 | Home use test | 12/194 adverse effects : itching, stinging, redness, mild erythema, burning feeling, irritated upper eye-lid, skin reaction on finger | 139 |

### 2.5. Sensitisation

#### Animal data

Potential allergic contact sensitization has been investigated according to the modified Buehler test protocol using the Guinea-pig:

- 20 animals had hydro-alcoholic solutions of salicylic acid, acetyl salicylate, methyl salicylate or hexadienyl acetyl salicylate (25% w/v) applied for 6 hours, once a week, for three weeks. After a 2-week rest period the animals were challenged with the same concentrations. Under the experimental conditions adopted none of the animals exhibited signs of sensitisation.

Taking into account the available data, the mentioned references seem to correspond to only one performed study.

Ref. : 105, 106, 107, 108, 156

#### Human data

The results of human repeated insult patch tests conducted with formulation up to 2% salicylic acid confirm that topical application does not cause skin sensitisation. In 3 studies, some subjects (*) were showing a positive response to an ingredient of the product formulation. None of the subjects were sensitive to salicylic acid. ** pH value not given.

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Salicylic acid Concentration / pH</th>
<th>Patch test</th>
<th>Positive responses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic lotion</td>
<td>0.5% / **</td>
<td>Occlusive</td>
<td>3/84*</td>
<td>121</td>
</tr>
<tr>
<td>Non alcoholic cream</td>
<td>1% / 2.87</td>
<td>?</td>
<td>0/86</td>
<td>122</td>
</tr>
<tr>
<td>Hydroalcoholic gel</td>
<td>0.5% / **</td>
<td>Occlusive</td>
<td>0/89</td>
<td>123</td>
</tr>
<tr>
<td>Hydroalcoholic foam</td>
<td>0.5% / 4.5-5.5</td>
<td>Occlusive</td>
<td>0/101</td>
<td>120</td>
</tr>
<tr>
<td>Hydroalcoholic cleanser</td>
<td>0.5% / 2.82</td>
<td>Occlusive</td>
<td>1/86*</td>
<td>124</td>
</tr>
<tr>
<td>Non alcoholic liquid make up</td>
<td>0.55% / **</td>
<td>Occlusive</td>
<td>0/98</td>
<td>125</td>
</tr>
</tbody>
</table>
Other published data suggest that topically applied salicylic acid is not a contact allergen.

Ref. : 13, 74, 152

In submission II additional human skin irritation data are provided for the use of salicylic acid in rinse-off products at an increased concentration from 2 % to 3 %. A 12 days cumulative irritation study with a shampoo containing 3 % salicylic acid applied continuously under a patch test (as a 4 % dilution) showed no evidence of sensitization in a challenge patch test applied after a resting period of 16 days.

Ref. : 187

2.6. Reproduction toxicity

In the submission, there is only information on acetylsalicylate-related reproductive toxicity following oral administration. Taking into account the rapid hydrolysis of acetylsalicylic acid to salicylic acid after oral absorption, the available animal data concerning acetylsalicylic acid have been considered.

Ref. : 69, 76

Effects of prenatal administration of 200 mg/kg/day acetylsalicylic acid suspended in 1% tragacanth gum have been studied in rat. The substance has been administered twice daily by gastric intubation during the last 6 days of gestation. The control group received the vehicle. Behaviour and weight were recorded, gross examinations of the dams were performed after delivery.

A good tolerance of the treatment was noted during pregnancy. A prolongation of pregnancy and parturition time was observed. Two dams died during the labour period. A lethal effect on the
foetuses has been recorded: 7 dead pups out of 65 in the treated group. Only one dead pup out of 112 was seen in the control group. Foetal deaths were attributed to prolonged parturition caused by the effects of acetylsalicylic acid on prostaglandins synthesis.

Ref. : 173

The effects of sodium salicylate on parturition and neonatal viability were studied in mated female rats. The animals were administered orally by gavage twice daily on gestation days 15 through 21 at dosage levels of 20, 80 and 200 mg/kg/day, at a volume of 20 ml/kg. The positive control group received acetylsalicylic acid at a total daily dosage of 261 mg/kg, the control group received the vehicle only: 0.5% low viscosity carboxymethylcellulose.

Administration of 200 mg/kg/day sodium salicylate and of 261 mg/kg/day acetylsalicylic acid induced maternal toxicity: agonal clinical signs and/or reduction of body weights and food consumption. These signs were generally associated with prolonged parturition and difficulty in delivery. A significant increase in mean gestation length was noted with acetylsalicylic acid. Corresponding adverse effects on offspring survival for the affected dams were noted. However, there was no evidence of systemic or maternal toxicity, and no adverse effects on offspring survival or growth in the mid-dose group (80 mg/kg/day) and in the low-dose group (20 mg/kg/day).

Under the experimental conditions adopted, the NOAEL (No-Observable-Adverse-Effect-Level) of sodium salicylate has been found to be 80 mg/kg/day when administered orally to mated rats corresponding approximately to 69 mg/kg/day of salicylic acid.

Ref. : 115

2.6.1 Two-generation reproduction toxicity

No data are available

2.6.2 Teratogenicity

Numerous studies concerning fetotoxic and teratogenic potential of acetylsalicylic acid and salicylic acid have been performed in animals. When these compounds were administered by oral or by parenteral route at various time during pregnancy at daily doses of 75 to 500 mg/kg in rats, mice and monkeys, fetal malformations (skeletal anomalies, cleft lip), resorptions and perinatal death were recorded.

Ref. : 27, 54, 166, 167, 172, 176, 181, 182

Teratogenicity studies:

<table>
<thead>
<tr>
<th>Species</th>
<th>Test article</th>
<th>Route of exposure</th>
<th>Dosage</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>Methyl salicylate</td>
<td>Subcutaneous Injections Days 9, 10 and 11 of gestation</td>
<td>0.1 to 0.5 ml</td>
<td>47/116: reabsorption of the young 45/298 offspring: cleft lip, eye defects, hydrocephaly, exencephaly 75/298 offspring: skeletal deformities</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(no detail about concentration dosage)</td>
<td></td>
</tr>
</tbody>
</table>

Ref. : 176
### Evaluation and opinion on: Salicylic Acid

<table>
<thead>
<tr>
<th>Species</th>
<th>Test article</th>
<th>Route of exposure</th>
<th>Dosage</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>Acetyl salicylate</td>
<td>Oral Days 8 and 9, or 9 and 10 of gestation</td>
<td>500 mg/kg/day</td>
<td>Cleft lip: increase incidence Various malformations Effect level: 500 mg/kg/day</td>
<td>172</td>
</tr>
<tr>
<td>Rats</td>
<td>Sodium salicylate</td>
<td>Oral Days 9 and 11 of gestation</td>
<td>250 or 500 mg/kg/day after metal salt treatment (Fe, Mn, Cu)</td>
<td>Dose dependent malformations Increased maternal serum concentration of salicylic acid Effect level: 250 mg/kg/day Teratogenic effect of sodium salicylate potentiated by metal salts (Mn)</td>
<td>55</td>
</tr>
<tr>
<td>Rats</td>
<td>Acetyl salicylate or salicylate</td>
<td>Oral Days 8 to 14 of gestation</td>
<td>75, 150, 300 mg/kg/day</td>
<td>Rapid death after 300 mg/kg/day dosing Significant abnormalities for both test article at 150 mg/kg/day 75 mg/kg/day: low incidence of skeletal (3/26 extra ribs, 2/29 in controls) and external malformations (1.8%) LOEL: 75 mg/kg/day (Lowest dose Observable Effect Level)</td>
<td>166</td>
</tr>
<tr>
<td>Rats</td>
<td>Salicylic acid</td>
<td>Oral Days 8 to 14 of gestation</td>
<td>0.06% to 0.4% in diet (50 to 200 mg/kg/day)</td>
<td>0.4% group: body weight loss, toxic symptoms, significant mortality and growth retardation in fetuses 0.2% group: growth retardation 0.1% and 0.06% groups: no significant effects</td>
<td>167</td>
</tr>
<tr>
<td>Rats</td>
<td>Acetylsalicylic acid</td>
<td>Oral Days 9 to 12 of gestation</td>
<td>100 and 150 mg/kg twice daily</td>
<td>100 mg/kg: no foetotoxicity 150 mg/kg: death and reabsorption in 34% of the embryos</td>
<td>182</td>
</tr>
</tbody>
</table>

### Teratogenicity studies (continued):

<table>
<thead>
<tr>
<th>Species</th>
<th>Test article</th>
<th>Route of exposure</th>
<th>Dosage</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey</td>
<td>Acetylsalicylic acid</td>
<td>Oral Days 23 to 32 of gestation</td>
<td>100 and 150 mg/kg twice daily</td>
<td>Transient growth retardation with both dosages 150 mg/kg/day: malformations in 3/15 fetuses 100 mg/kg/day: no malformations</td>
<td>182</td>
</tr>
<tr>
<td>Rats</td>
<td>Acetylsalicylic acid</td>
<td>Oral Days 7 to 17 of gestation</td>
<td>50, 100 and 200 mg/kg/day</td>
<td>Decrease in maternal body weight gain in all groups 200 mg/kg/day: increase in resorption and fetal malformations dose-related decrease in average fetal body weight for the 100 and 200 mg/kg groups 50 and 100 mg/kg groups: no abnormal foetuses, reduced fetal weights</td>
<td>73</td>
</tr>
</tbody>
</table>
2.7. Toxicokinetics (incl. Percutaneous Absorption)

2.7.1. Metabolism and pharmacokinetics

Human data

Salicylic acid and derivatives are weak acids, after oral administration they are found in the unionized form in the stomach. They are well absorbed in human from the gastrointestinal tract and rapidly distributed throughout the extracellular fluid and most tissues. High concentrations are found in the liver and the kidneys (organs of biotransformation and excretion) and 50 to 80% of salicylic acid in plasma is bound to albumin and other proteins. Salicylic acid is excreted by renal excretion as an unchanged chemical entity (10%) or after conjugation with glycine (salicyluric acid 75%), with glucuronic acid (salicyl acyl and phenolic glucuronides 5%) and/or after hydroxylation (gentisic acid < 1%).

Ref.: 30

Pharmacokinetics of acetylsalicylic is largely documented. Acetylsalicylic acid is hydrolyzed in the stomach and in the blood to salicylic acid and acetic acid. The biological half life of acetylsalicylic acid is only 20 minutes. After the oral administration of 0.6 g of acetylsalicylic acid, only 27% of the total plasma salicylate are still acetylated after 30 minutes.

Ref.: 30

2.7.2. Percutaneous Absorption

Animal data

Salicylic acid percutaneous absorption was studied in several animal species which are mainly non relevant according to the methodology for prediction of human skin permeability:

- Rabbits: salicylic acid was applied at the concentration of 6% incorporated in several formulations: hydrophilic ointment (O/W), hydrophilic petrolatum (W/O), petrolatum, polyethylene glycol. Salicylic acid was most effectively absorbed from the hydrophilic ointment. The dose applied was 7.5 g/animal, the plasma level pick was 12 mg/100 ml at 4.5 hours after the application of the hydrophilic ointment, it was 8 mg/100 ml at 6 hours for the hydrophilic petrolatum and 6.5 mg/100 ml at 4 hours with petrolatum. No absorption was detected from the propylene glycol excipient Sodium salicylate was studied in parallel from the same excipients, its absorption is considerably smaller than the acid salicylic one, but is also better from the hydrophilic ointments than from the others.

Ref.: 165

- Rabbits: 10 g of a 10% salicylic acid hydrophilic ointment with or without urea were applied on the ventral skin of the animals (91 cm²). Peak plasma salicylic concentrations were between 10 and 18 mg/100 ml and were attained within four to six hours. Urea had not the percutaneous absorption enhancer effect that was initially expected.

Ref.: 7
Guinea pigs: percutaneous absorption of salicylic acid was studied from four oily vehicles applied on 2.25 cm²: liquid paraffin, oleic acid, hexadecyl alcohol and isopropyl myristate. When salicylic acid had a strong affinity to the vehicle (high solubility), the absorption was poor. The higher flux was reached from liquid paraffin (14.6 % of the dose between 1 and 6 hours - 500 µg/ml for a volume of 25 ml). The absorption was about 10 times greater when the skin was damaged by tape stripping.

Ref. : 177

Guinea pigs: percutaneous absorption was investigated in vivo from solutions at different pH. The absorption rate of the non ionised form (pH 2 and pH 3) is respectively 10 times and 5 times higher than the rate of the ionised form (pH 4 solution).

Ref. : 3

Rats: various concentrations (1, 5 and 10 %) of salicylic acid in hydrophilic ointment were applied repeatedly at daily or weekly intervals during 7.5 hours. The surface of application was 3 cm², the amount applied under occlusion was 2 g. A gradual decrease in the salicylic acid penetration was observed following weekly applications of either 5 or 10 % concentrations. The penetration flux of 1 % was not modified under the same conditions. For the daily dosage with 5 and 10 %, the penetration flux increased after 2 days of treatment and declined thereafter. For the 1 % salicylic acid concentration, the penetration increased slightly after 3-4 days of treatment. These data were related to skin histological modifications.

Ref. : 155

Human data:

In vitro across human skin the absorption of salicylic acid at a concentration of 2 % was studied from 6 different formulations (hydro-alcoholic and non-alcoholic vehicles). Diffusion was greater for the hydro-alcoholic (35 %) than from the non alcoholic excipients (propylene glycol, cream)

Ref. : 109

A comparative pharmacokinetics study, oral dosing versus topical administration, was conducted in order to determine the systemic salicylic acid burden after topical use of a leave-on formulation containing 2 % salicylic acid (hydro-alcoholic vehicle, non-alcoholic cream). The application sites were the face and the neck, the amounts applied were 1.25 g to 1.50 g (i.e. 25 mg of salicylic acid). 40 subjects received the topical formulations daily during 16 days. A group of 12 volunteers received one daily dose of a “baby aspirin formula” containing 81 mg of acetylsalicylic acid. Plasma salicylate levels were compared (peak plasma level, time to peak, area under the curves). The peaks of salicylate levels for the topical application were 1/20th for the cream to 1/10th for the hydro-alcoholic formulation of the one obtained after oral dosing. The AUC were respectively 1/8th to 1/5th that of the oral treatment. The topical pharmacokinetics was not affected by the skin type

Ref. : 116
Conclusion on percutaneous absorption

Salicylic acid is readily absorbed when applied on the skin. The absorption is strongly dependent on the vehicle composition, pH, structure of the skin, conditions of the application on the skin (single dose, repeated doses, occlusion). The absorption from topically applied 2 % salicylic acid containing products is in the range of 20 % of the applied dose.

After topical human administration of 1.25 g-1.50 g of a 2 % salicylic acid containing formulation (corresponding to 25 mg of salicylic acid) daily for 16 days, the peak salicylate levels were between 1/10th and 1/20th those obtained after the oral administration of 81 mg of acetyl salicylic acid (aspirin baby dose).

2.8. Mutagenicity/Genotoxicity

Studies have been performed in order to assess the mutagenic/genotoxic potential of salicylic acid and acetylsalicylic acid. These results are summarised in the following tables 1, 2 and 3.

2.8.1. In vitro mutagenicity in Bacteria and Yeast (Table 1)

<table>
<thead>
<tr>
<th>Methods</th>
<th>Test article</th>
<th>Metabolic activation</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ames tests</td>
<td>salicylic acid</td>
<td>with</td>
<td>negative</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>acetylsalicylic acid</td>
<td>without</td>
<td></td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>500 µg/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ames tests</td>
<td>salicylic acid</td>
<td>No data available</td>
<td>negative</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>3 to 8 \times 10^{-5} M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis assay</td>
<td>salicylic acid</td>
<td>without</td>
<td>positive</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>acetylsalicylic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.8.2. In vitro mammalian clastogenicity and DNA damage (Table 2)

<table>
<thead>
<tr>
<th>Methods</th>
<th>Test article</th>
<th>Metabolic activation</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured CHO cells (3 hour exposure)</td>
<td>salicylic acid 1.5 to 25 mg/ml</td>
<td>with and without</td>
<td>negative</td>
<td>164</td>
</tr>
<tr>
<td>Chinese hamster lung cells (48 hour exposure)</td>
<td>salicylic acid 1.0 and 1.25 mg/ml</td>
<td>Without</td>
<td>positive</td>
<td>40</td>
</tr>
</tbody>
</table>
The *in vitro* submitted studies for salicylic acid and for acetylsalicylic acid include results of experiments whose methodology is not reported, they are mainly represented by a list of results related to many chemicals. The results reported do not comply with the guidelines defined by the SCCNFP.

### 2.8.3. *In vivo* clastogenicity/mutagenicity (Table 3)

<table>
<thead>
<tr>
<th>Method</th>
<th>Test article</th>
<th>Animal species</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila sex-linked recessive lethal assay</td>
<td>Acetylsalicylic acid 10 mM</td>
<td><em>Drosophila melanogaster</em></td>
<td>negative</td>
<td>56</td>
</tr>
</tbody>
</table>

### 2.9. Carcinogenicity

**Animal data**

- Salicylic acid was tested as part of a skin tumour promotion study using uninitiated mouse skin. Salicylic acid 20% in a dioxane solution was applied topically (one drop of about 25 µl) to 31 female “Sutter” mice, 2-3 months of age, treated twice weekly for 12 weeks. There were no deaths or papillomas throughout the study. However, as no post-mortem examination was performed at the end of the treatment period, the results were considered of limited value for evaluation of possible carcinogenic properties of the substance.

  Ref. : 9

- Carcinogenicity studies have been performed to assess the carcinogenic potential of acetylsalicylic acid in mice at 1 and 5% and in rats at 0.25% and 2% in drinking water. The results were negative on both studies. Considering these results, salicylic acid, a metabolite of acetylsalicylic acid, was considered to be devoid of such a potential.

  Ref. : 77

Salicylic acid is the main metabolite of acetylsalicylic acid (aspirin) and there is sufficient evidence in animal models that acetylsalicylic acid prevents cancer.

  Ref. : AR 6

**Human data**

No data are available for salicylic acid.

- Salicylic acid is the main metabolites of aspirin (acetylsalicylic acid). Epidemiological studies have shown that acetylsalicylic acid reduces the risk of colorectal cancer.

  Ref. : AR 6

- Thun *et al.* reported that chronic use of acetylsalicylic acid decreases susceptibility to bowel cancer.

  Ref. : 57
In another report, salicylic acid has been shown to interact with phenolsulphotransferase and it has been proposed that this could be one of the pathways by which acetylsalicylic acid reduces cancer risk.

Ref.: 58

Recently it has also been reported that users of acetylsalicylic acid had a moderately reduced risk of gastric cancer.

Ref.: AR1

**Hazard evaluation**

Only one animal study on the carcinogenicity of salicylic acid has been found. The study is of limited value for evaluation of possible carcinogenic properties of the substance. However, it has been found both in epidemiological studies and in animal experiments that acetylsalicylic acid reduces skin cancer risk. Since salicylic acid is the main metabolite of acetylsalicylic acid, the cancer preventive effect of acetylsalicylic acid may be caused by its metabolite salicylic acid.

### 2.10. Special investigations

*In vitro* eye irritation studies were performed. The ocular irritation potential for prototype anti-dandruff shampoo formulations containing 2% or 3% salicylic acid was compared to marketed anti-dandruff shampoos (1.8 to 3% salicylic acid) and regular shampoos formulations. Two types of *in vitro* assays were performed: Bovine Corneal Opacity and Permeability assay (BCOP) and Cell Viability Assays (NRR and MT).

- **Bovine Corneal Opacity and Permeability assay (BCOP)**: Products were applied to excised bovine corneas under either in-use or maximalizing conditions for an assessment of ocular irritation relative to that of currently marketed products. Opacity changes of the test cornea or changes in corneal permeability after exposure to the product were measured to assess the ocular irritation potential.
  
  The results are summarized in the table: In Vitro Eye Irritation Results.

  Ref.: 192

- **Cell viability assays**: Two assays were used to measure viability of normal human keratinocytes after exposure to products relative to that of currently marketed products: the Neutral Red Release Assay (NRR) and the Mat-Tek Epi-ocular™ (MT).

In both assays, BCOP and NRR, no significant differences were noted between shampoos formulations containing or not salicylic acid. In the Epi-ocular assay, the prototype formulations showed a higher predicted eye irritation potential, comparatively to the commercial shampoo formulations with or without salicylic acid. The results are summarized in the table: In Vitro Eye Irritation Results.

Ref.: 193, 194
In Vitro Eye Irritation Results:

<table>
<thead>
<tr>
<th>Product ref.</th>
<th>Description</th>
<th>salicylic acid (%)</th>
<th>BCOP (In vitro score/classification)</th>
<th>NRR\textsubscript{50} (mg/ml)</th>
<th>MT (ET\textsubscript{50}) (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>« TEST » Shampoos</td>
<td>2.0% salicylic acid</td>
<td>2.0</td>
<td>0.57/non-irritant</td>
<td>0.76</td>
<td>11.3</td>
</tr>
<tr>
<td>1853</td>
<td>3.0% salicylic acid</td>
<td>3.0</td>
<td>1.7/non-irritant</td>
<td>0.80</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Commercial Shampoos</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1516 Baby Shampoo</td>
<td>0</td>
<td>0.6/non-irritant</td>
<td>3.87</td>
<td>47.8</td>
<td></td>
</tr>
<tr>
<td>2012 Adult Shampoo « A » (Normal)</td>
<td>0</td>
<td>0.74/non-irritant</td>
<td>0.82</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>2014 Adult Shampoo « A » (Dry/damaged)</td>
<td>0</td>
<td>1.8/non-irritant</td>
<td>0.80</td>
<td>33.6</td>
<td></td>
</tr>
<tr>
<td>2247 Adult Shampoo « B » (Normal)</td>
<td>0</td>
<td>1.8/non-irritant</td>
<td>1.11</td>
<td>27.2</td>
<td></td>
</tr>
<tr>
<td>2248 Adult Shampoo « B » (Dry/damaged)</td>
<td>0</td>
<td>1.6/non-irritant</td>
<td>0.82</td>
<td>23.3</td>
<td></td>
</tr>
<tr>
<td>1570 Adult Shampoo « C » (Normal/oily)</td>
<td>0</td>
<td>1.5/non-irritant</td>
<td>0.79</td>
<td>24.8</td>
<td></td>
</tr>
<tr>
<td>1039 1.8% salicylic acid</td>
<td>1.8</td>
<td>2.91/non-irritant</td>
<td>1.65</td>
<td>27.9</td>
<td></td>
</tr>
<tr>
<td>1034 3.0% salicylic acid</td>
<td>3.0</td>
<td>0.9/non-irritant</td>
<td>1.04</td>
<td>21.6</td>
<td></td>
</tr>
</tbody>
</table>

In the experimental conditions adopted, the obtained results showed that the concentration of salicylic acid, up to a level of 3%, and used in rinse-off shampoo formulations does not appear to significantly influence the ocular irritation potential.

2.11. Conclusions

In cosmetics salicylic acid is currently used at concentrations up to 2% in leave-on formulations (face and general cream) and in rinse-off products (make-up removers, shower gels, shampoos and hair conditioners), at concentrations up to 1% in leave-on hair care products and at 0.5% as preservative in other cosmetic products. Studies have been submitted to support the use of salicylic acid at a level of 3% in rinse-off hair-care formulations.

Irritant potential:

- Product formulations or alcohol solutions containing up to 5% salicylic acid (skin irritation studies) and up to 2% salicylic acid (low volume eye tests) with pH between 2.3 and 5.7 were mildly to no irritating to the skin or to the eye of the animals treated. These results were confirmed by \textit{in vitro} eye irritation studies using formulations containing salicylic acid at a concentration up to 3.0%.
After repeated application in human under occlusive or semi-occlusive patches of formulations containing up to 3 % salicylic acid with a pH range 2.5 – 3.8, it can be possible to categorized salicylic acid as a mild transient irritant.

Allergenic potential :

- According to the modified Buehler test protocol using the Guinea pig, salicylic acid was not considered as a sensitising agent. However there are neither data related to the experimental potential risk under maximising conditions nor to the confirmation of absence of risk to Human.  
- The results of human repeated insult patch tests conducted with formulation up to 2 % salicylic acid confirm that topical application does not cause skin sensitisation. Salicylic acid is not known as a sensitiser. 
- No information is available concerning the phototoxicity or photoallergenic potential of salicylic acid in animal.

Potential systemic toxicity :

- Carcinogenicity studies were performed with acetylsalicylic acid ; this substance was not carcinogenic. Most of the results from genotoxic studies do not comply with the actual SCCNFP notes of guidance. 
- No systemic toxicity was noted from subchronic dermal toxicity studies conducted in the rabbit at the highest dosage of 120 mg/kg/day salicylic acid formulations ; dermal irritation was the main recorded observation. 
- The chronic oral toxicity study performed in rat with acetylsalicylic acid at a concentration of 200 mg/kg/day during 200 days, showed no significant toxic effects compared to the control group at this dose level. 
- In humans, toxic effects were reported when 10 g or more of salicylates are given orally in single dose or divided doses within a period of 12 to 24 hours. Children are more sensitive than adults to salicylates. Reye’s syndrome in children is associated with the ingestion of acetylsalicylic acid 
- Numerous reproductive studies have been performed with acetylsalicylic acid or salicylic acid in various animal species. A NOAEL of sodium salicylate administered orally to mated rats has been established to 80 mg/kg/day corresponding to 69 mg/kg/day of salicylic acid. The results also showed that following oral administration salicylic acid is not teratogenic nor embryotoxic up to 75 mg/kg/day in rodents and up to 100 mg/kg/day in Monkey. Above these dose levels, foetal malformations (skeletal malformations, cleft lip, growth retardation), resorptions and perinatal death were recorded with the compounds salicylic acid or acetylsalicylic acid. 
- Salicylic acid is readily absorbed when applied to human skin. The absorption is strongly dependent on the vehicle composition, pH, structure of the skin, conditions of the application on the skin (single dose, repeated doses, occlusion). The human percutaneous absorption from topically applied 2 % salicylic acid containing products is in the range of 20 % of the applied dose of salicylic acid. The topical application of salicylic acid to extensive areas, particularly in children, may involve a risk of toxicity by absorption due to their specific surface of exposure / body weight ratio.
2.12. Safety evaluation

Calculation of the margin of safety was performed according to the different uses of salicylic acid in cosmetic products.

**CALCULATION OF THE MARGIN OF SAFETY**

**Salicylic acid**

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Exposure</th>
<th>Ingredient Applied</th>
<th>Body Weight</th>
<th>Absorption</th>
<th>Systemic Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Leave-on skin care product / face cream)</td>
<td>1.6 g</td>
<td>32 mg</td>
<td>60 kg</td>
<td>20%</td>
<td>0.11 mg/kg bw</td>
</tr>
<tr>
<td>(Leave-on skin care product / hand cream)</td>
<td>2.4 g</td>
<td>48 mg</td>
<td>60 kg</td>
<td>20%</td>
<td>0.16 mg/kg bw</td>
</tr>
<tr>
<td>(Leave-on hair products)</td>
<td>1 g</td>
<td>10 mg</td>
<td>60 kg</td>
<td>20%</td>
<td>0.03 mg/kg bw</td>
</tr>
<tr>
<td>(All rinse-off products)</td>
<td>0.72 g</td>
<td>14.4 mg</td>
<td>60 kg</td>
<td>20%</td>
<td>0.07 mg/kg bw</td>
</tr>
</tbody>
</table>
Dermal absorption per treatment \( I \times A \) = 2.9 mg
Systemic exposure dose (SED) \( I \times A / 60 \text{ kg} \) = 0.05 mg/kg bw

(A shampoo)

Based on an exposure of 0.08 g, containing at maximum 3 %

| Maximum amount of ingredient applied | I (mg) | = 0.24 mg |
| Typical body weight of human | = 60 kg |
| Maximum absorption through the skin | A (%) | = 20 % |
| Dermal absorption per treatment | \( I \times A \) | = 0.05 mg |
| Systemic exposure dose (SED) | \( I \times A / 60 \text{ kg} \) | = 0.01 mg/kg bw |

(All other remaining cosmetic products / Salicylic acid as a preservative)

Based on an exposure of 12 g, containing at maximum 0.5 %

| Maximum amount of ingredient applied | I (mg) | = 60 mg |
| Typical body weight of human | = 60 kg |
| Maximum absorption through the skin | A (%) | = 20 % |
| Dermal absorption per treatment | \( I \times A \) | = 12 mg |
| Systemic exposure dose (SED) | \( I \times A / 60 \text{ kg} \) | = 0.20 mg/kg bw |

Overall SED \( 0.11 + 0.16 + 0.03 + 0.05 + 0.01 + 0.20 = 0.56 \text{ mg/kg bw} \)

No observed adverse effect level (75 mg/kg) NOAEL = 75 mg/kg bw
(rat oral teratogenicity study)

Margin of Safety \( \text{NOAEL} / \text{SED} \) = 133

2.13 Opinion

On the bases of the information provided for consideration, the SCCNFP considers that salicylic acid is safe for “other uses” than as a preservative, at a concentration up to 2.0 % for the leave on and rinse-off cosmetic products and at a concentration up to 3.0 % for the cosmetic rinse-off hair products.
2.13. References

The references in italic were not used for the risk assessment


41. Johnson & Johnson, 12-day cumulative irritation,

42. Johnson & Johnson, 12-day cumulative irritation,

43. Johnson & Johnson, 14-day cumulative irritation,

44. Johnson & Johnson, HRIPT,
Evaluation and opinion on: Salicylic Acid

45. Johnson & Johnson, HRIPT,
46. Johnson & Johnson, HRIPT,
47. Johnson & Johnson, HRIPT,
48. Johnson & Johnson, HRIPT,
49. Johnson & Johnson, HRIPT,


82. Procter & Gamble. Limit test for acute oral toxicity in rats, BCS0070, 1993a

83. Procter & Gamble. Limit test for acute oral toxicity in rats, BCS0025, 1993b

84. Procter & Gamble. Up/down procedure for an LD50, HBE BTS 0026, 1989a

85. Procter & Gamble. Acute percutaneous toxicity in rabbits, ECM BTS 206, 1976b

86. Procter & Gamble. Skin irritation study in guinea pigs, P81081, 1982a

87. Procter & Gamble. Skin irritation study in guinea pigs, P81069, 1982b

88. Procter & Gamble. Primary skin irritation study in rabbits, P79006, 1979a

89. Procter & Gamble. Skin irritation study in guinea pigs, P83012, 1983

90. Procter & Gamble. Primary skin irritation study in rabbits, BYCR0484, 1986

91. Procter & Gamble. Primary skin irritation/corrosion study in rabbits, ECM BTS 2085/02, 1995a

92. same as ref. 91

93. Procter & Gamble. Primary skin irritation study in rabbits, P80027, 1980

94. Procter & Gamble. Primary skin irritation study in rabbits, P80087, 1982c

95. Procter & Gamble. Low volume eye irritation study in rabbits, BTS 0606/01, 1996

96. Procter & Gamble. Low volume eye irritation study in rabbits, BCS0070, 1993c

97. Procter & Gamble. Low volume eye irritation study in rabbits, BCS0025, 1993d

98. Procter & Gamble. Low volume eye irritation study in rabbits, SC 95A003, 1995b

99. Procter & Gamble. Low volume eye irritation study in rabbits, BCS0139, 1993e

100. Procter & Gamble. Low volume eye irritation study in rabbits, SC95A013, 1995c

101. Procter & Gamble. Low volume eye irritation study in rabbits, BS94A056-20, 1994a
102. Procter & Gamble. Low volume eye irritation study in rabbits, SC95A005, 1995d
103. Procter & Gamble. Low volume eye irritation study in rabbits, CS95A012, 1995e
104. Procter & Gamble. Low volume eye irritation study in rabbits, BD94A110-5G, 1995f
105. Procter & Gamble. Guinea pig closed patch test study, TGPSE 84, 1975
106. Procter & Gamble. Delayed contact hypersensitivity in guinea pigs, ECM BTS 206, 1976d
107. Procter & Gamble. Delayed contact hypersensitivity in guinea pigs, ECM BTS 206, 1976e
108. Procter & Gamble. Delayed contact hypersensitivity in guinea pigs, ECM BTS 206, 1976f
109. Procter & Gamble. In vitro skin penetration human skin, BCS0062(S3), 1994b
110. Procter & Gamble. 14-day percutaneous subchronic toxicity, BCS0062, 1993f
111. Procter & Gamble. 91-day subchronic percutaneous toxicity, IBSE0002, 1990a
112. Procter & Gamble. 91-day subchronic percutaneous toxicity, IBSE0001, 1990b
113. Procter Gamble. 28-day percutaneous study, BCS0062S, 1994c
114. Procter & Gamble. 91-day subchronic percutaneous toxicity, BCS0062S, 1994d
115. Procter & Gamble. Perinatal toxicity study in rats, BCS0062(S2), 1994e
116. Procter & Gamble. Percutaneous pharmacokinetic study, BCS0099, 1994f
117. Procter & Gamble. Ocular irritancy evaluation study, CRL25895, 1995g
118. Procter & Gamble. Periocular application study, CR95010, 1995h
119. Procter & Gamble. Ophthalmologic safety evaluation study, SC95C016, 1995i
120. Procter & Gamble. HRIPT, IBSE0002, 1989b
121. Procter & Gamble. HRIPT, BCS0025, 1993I
122. Procter & Gamble. HRIPT, HBE BTS 0327/01, 1993j
123. Procter & Gamble. HRIPT, BCS0070, 1993k
124. Procter & Gamble. HRIPT, BCS0105, 1995j
125. Procter & Gamble. HRIPT, SC95C014, 1995k
126. Procter & Gamble. HRIPT, SC95C015, 1995l
127. Procter & Gamble. HRIPT, SC95C002, 1995m
128. Procter & Gamble. HRIPT, SC95C008, 1995n
129. Procter & Gamble. HRIPT, CFTSE97/002, 1997
130. Procter & Gamble. 21-day cumulative irritation, BCS0133, 1993q
131. Procter & Gamble. 21-day cumulative irritation, BCS0025, 1993r
132. Procter & Gamble. 5-day cumulative cleanser. SC94C006, 1995r
133. Procter & Gamble. Back irritation, CR94037, 1995s
134. Procter & Gamble. Facial appearance/irritation. CR94062, 1994/5h
135. Procter & Gamble. Dermatologic safety evaluation, SC95C016, 1995v
136. Procter & Gamble. Facial appearance/irritation, CR93012, 1993s
137. Procter & Gamble. Facial appearance/irritation, BYCR 1046/03, 1993t
138. Procter & Gamble. 21-day cumulative irritation, BCS0093, 1993w
139. Procter & Gamble. 21-day cumulative irritation, BCS0093, 1993v
140. Procter & Gamble. HRIPT, BCS0080, 1993x
141. Procter & Gamble. HRIPT, BCS0138, 1994k
142. Procter & Gamble. 6-week facial irritation, BCS0070(S3), 1993z
143. Procter & Gamble. Dermatologic and ophtalmic safety study, SC95C037, 1996
144. Procter & Gamble. Periocular application study, CR95039, 1995z
145. Procter & Gamble. Periocular application study, 1995114, 1996b
173. Tuchman-Dupleissis H., HisS D., Mottot G. and Rosner I. Effects of prenatal administration of acetylsalicylic acid in rats. Toxicology, 1975, 3: 207-211.


**Additional references**

AR6. Vaino H. et al., Cancer Epidem Biomark Prevent, 6, 749-753, 1997