Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Styrene

EC number: 202-851-5
CAS number: 100-42-5

ECHA-RAC-CLH-O-0000002714-75-01/F

Adopted

28 November 2012

28 November 2012
CLH-O-0000002714-75-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: styrene

EC number: 202-851-5

CAS number: 100-42-5

The proposal was submitted by **Denmark** and received by the RAC on **10/10/2011.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

Denmark has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **10/10/2011.** Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **24/11/2011**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Bert-Ove Lund**
Co-rapporteur, appointed by the RAC: **Benjamin Piña**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **28 November 2012** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion that **styrene** should be classified and labelled as follows:

Classification and labelling in accordance with CLP

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	601-026-00-0	styrene	202-851-5	100-42-5	Flam. Liq. 3 Acute Tox. 4* Eye Irrit. 2 Skin Irrit. 2	H226 H332 H319 H315	GHS02 GHS07 GHS08 Wng	H226 H332 H319 H315		*	D
Dossier submitters proposal	601-026-00-0	styrene	202-851-5	100-42-5	**Add:** STOT RE 1 **Add:** Repr. 1B	**Add:** H372 (nervous system) **Add:** H360D	Replace Wng with Dgr	**Add:** H372 (nervous system) **Add:** H360D			
RAC opinion	601-026-00-0	styrene	202-851-5	100-42-5	**Add:** STOT RE 1 **Add:** Repr. 2	**Add:** H372 (hearing organs) **Add:** H361d	Replace Wng with Dgr	**Add:** H372 (hearing organs) **Add:** H361d			
Resulting Annex VI entry if agreed by COM	601-026-00-0	styrene	202-851-5	100-42-5	Flam. Liq. 3 Acute Tox. 4* Eye Irrit. 2 Skin Irrit. 2 STOT RE 1 Repr. 2	H226 H332 H319 H315 H372 (hearing organs) H361d	GHS02 GHS07 GHS08 Dgr	H226 H332 H319 H315 H372 (hearing organs) H361d		*	D

Classification and labelling in accordance with DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
Current Annex VI entry	601-026-00-0	styrene	202-851-5	100-42-5	R10 Xn; R20 Xi; R36/38	Xn R: 10-20-36/38 S: (2-)23	Xn; R20: C ≥ 12,5 % Xi; R36/38: C ≥ 12,5 %	D
Dossier submitters proposal	601-026-00-0	styrene	202-851-5	100-42-5	**Add:** Xn; R48/20 **Add:** Repr. Cat. 2; R61	T R: 48/20-61		
RAC opinion	601-026-00-0	styrene	202-851-5	100-42-5	**Add:** Xn; R48/20 **Add:** Repr. Cat. 3; R63	Xn R: 48/20-63		
Resulting Annex VI entry if agreed by COM	601-026-00-0	styrene	202-851-5	100-42-5	Repr. Cat. 3; R63 R10 Xn; R20-48/20 Xi; R36/38	Xn R: 10-20-36/38-48/20-63 S: (2-)23-36/37-46	Xn; R20: C ≥ 12,5 % Xi; R36/38: C ≥ 12,5 %	D

SCIENTIFIC GROUNDS FOR THE OPINION

Specific target organ toxicity (CLP) – repeated exposure (STOT RE) and repeated dose toxicity (DSD)

Summary of the Dossier submitter's proposal

The present proposal concerns one of the endpoints previously agreed at TC C&L i.e., repeated dose toxicity (CLP STOT RE 1; H372 "Causes damage to the nervous system through prolonged or repeated exposure by inhalation" and DSD Xn; R48/20 "Harmful: danger of serious damage to health by prolonged exposure through inhalation").
Styrene is a transitional substance and was discussed in the TC C&L group prior to the transfer of responsibility for classification and labeling to ECHA. At a TC C&L meeting in September 2007, it was agreed to classify with R48/20 (equivalent to STOT RE 1 under CLP) for repeated dose effects.
The key supporting studies are summarised below (copied from the Danish proposal).

Ototoxicity: Styrene-induced chronic impairment of auditory function has been demonstrated in a number of animal studies and several human studies. This has been substantiated by morphological evidence of hair cell loss in the rat cochlea as well as by functional investigations in humans. The available data suggest that humans are sensitive to this effect and that styrene is more potent than toluene.
Effects on colour vision: Several human studies show that low-level exposure to styrene (< 50 ppm) may impair colour vision. Some of the human studies may have underestimated the risk because some individuals were exposed to very low levels of styrene (< 8 ppm). Some studies argue that the effect is reversible, but scientifically this has not been documented. ACGIH[1] as well as several other Occupational TLV[2]-authorities have reduced the TLV of styrene to 20 ppm because loss of colour discrimination was considered to be a serious effect.
Neurotoxicity: Several different neurotoxicological investigations (including e.g., EEG, peripheral nerve conduction velocity, and ototoxicity) have been performed in both experimental animals and in humans. Styrene causes irreversible changes in the central nervous system of animals as documented in a substantial number of papers reviewed in the EU-RAR.
The proposed classification is STOT RE 1, with the hazard statement H372 "Causes damage to the nervous system through prolonged or repeated exposure via inhalation".

Comments received during public consultation

Industry stakeholders submitted several published studies that had not been included in the CLH dossier. These studies added useful information, but were not considered to affect the interpretation of findings described in the overall database. A general comment expressed by industry was that although the proposal draws on the studies already evaluated in the EU RAR, the dossier submitter has omitted relevant qualifying comments and negative criticism of studies important for their proposal. The RAC has therefore also considered the detailed industry comments and consulted the EU RAR.
All comments received on repeated dose toxicity were in support of classification with STOT RE 1 based on the evidence of ototoxicity. However, industry did not agree that effects on colour vision supported this classification, because (for example) two recent studies have not found any effects on colour vision in exposed workers (Seeber *et al*. 2009, Vyskocil *et al*. 2012). Industry is of the view that even if changes in colour discrimination were caused by styrene, these changes were reversible and so slight that they cannot be considered adverse health outcomes of styrene exposure.

[1] American Conference of Governmental Industrial Hygienists
[2] Threshold Limit Value

Assessment and comparison with the classification criteria

Key data and arguments that are relevant to the proposal
Clear evidence of ototoxicity has been seen in 10 repeated dose toxicity studies at concentrations of 600 ppm (2598 mg/m^3) and above where rats were exposed to styrene by inhalation. The evidence includes findings of hearing loss in the mid-frequency range (10-20 kHz) and histopathological evidence of destruction of the outer hair cells of the cochlea.
One study compared the effects in active and resting rats, and found that styrene-induced ototoxicity tends to occur at lower exposure concentrations in active than in resting rats (presumably because of higher systemic exposure at the higher ventilation rate). Styrene-induced ototoxicity has also been studied in the absence or presence of noise, and the findings indicate that simultaneous noise increases the ototoxicity of styrene.
The effect of styrene on hearing has also been observed in studies conducted in a number of occupational settings. The occupational co-exposure to other agents affecting hearing (noise and solvents) decreases the power of the epidemiological studies. However, the three largest studies on occupational exposure to styrene indicated effects on hearing at concentrations occurring in these occupational settings, i.e., below 50 ppm. Thus, there is some evidence to suggest that humans are more sensitive to the ototoxicity of styrene than rats, and that noise and other ototoxic solvents may potentiate the ototoxicity of styrene. In conclusion, the RAC finds ototoxicity to be well documented in rats, and that humans also are likely to be sensitive to this adverse effect of styrene.
The dossier also refers to effects on colour vision in humans as a basis for the classification proposal. There are a number of studies in occupational settings on the effects of styrene on colour vision. It is quite clear that styrene has an effect on colour vision, decreasing the ability to discriminate colour in humans. Studies submitted during the public consultation included a meta-analysis study (Paramei *et al*. 2004) which, based on the studies available at that time, concluded that there were effects on colour vision, as well as two more recent studies which did not show such effects (Seeber *et al*. 2009, Vyskocil *et al*. 2012; the latter published and submitted after the public consultation). Some studies indicate the effect to be reversible, whereas others indicate the irreversibility of this effect. Although the effect is clear, it is difficult to judge the extent to which it can be considered adverse.
The proposal also refers to neurotoxicity as a basis for the STOT RE 1 classification, but as no specific neurotoxicity studies are mentioned in the documents, the RAC did not comment on neurotoxicity in relation to this proposal. However, the RAC notes that the European Commission has acknowledged that styrene exposure above 50 ppm over a period of 5-10 years may induce chronic encephalopathy in occupationally exposed humans (Information notices on occupational diseases: a guide to diagnosis; European Commission 2009), indicating that classification based on neurotoxicity may be warranted. Due to lack of such data in the CLH dossier, the RAC could not address neurotoxicity in the current opinion.

Conclusion on classification

The CLP criteria state that a substance should be placed in STOT RE if it causes significant toxicity in humans or if, on the basis of evidence from studies in experimental animals, it can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Examples of significant toxicity relevant to this opinion mentioned in the CLP include "significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, **hearing** and sense of smell)".

The RAC is of the view that appropriate studies in experimental animals in combination with reliable epidemiological studies provide evidence for adverse effects on hearing (ototoxicity), and that this is a sufficient basis for classification with STOT RE. Regarding the category, the RAC finds that the evidence for ototoxicity occurring in humans at concentrations below 50 ppm warrant classification in category 1, even though the concentrations needed to cause ototoxicity in rats would suggest a lower category. In this case the human data are considered to be more relevant when deciding on the category, since reliable human toxicity data normally lead to classification in category 1 (there are no guidance values for human data).
The effects of styrene on colour vision in humans can be viewed as supportive of the STOT classification, but the degree to which this effect can be considered to be adverse is difficult to establish based on the proposal. Therefore, the RAC does not find that this effect, as described in the CLH proposal, as such is a sufficient basis for classification.
The proposed classification is STOT RE 1, which is supported by the RAC. However, the RAC considers that the hazard statement should be rephrased as follows, since the affected organs have been clearly identified, and could be affected via different routes of exposure (at least after oral and inhalation exposure): H372 "*Causes damage to the hearing organs through prolonged or repeated exposure*" (corresponding to Xn; R48/20 according to the DSD).

Reproductive toxicity

Summary of the Dossier submitter's proposal

The present proposal includes classification for developmental toxicity (Repr. 1B; H360D "May damage the unborn child when exposed via inhalation" according to CLP, and Repr. Cat. 2; R61 "May cause harm to the unborn child" according to DSD).
Previous discussions
Styrene is a transitional substance and was discussed in the TC C&L group at a number of meetings. For reproductive toxicity no agreement could be reached at that time, and the case was handed-over to ECHA[3].
Key supporting studies
In a well-conducted OECD and GLP-compliant two-generation study in rats, which included developmental neurotoxicity assessment in F2 offspring, a pattern of developmental delays both before and after weaning (decreased body weights, delays in attaining some pre-weaning developmental landmarks, slight shift in the normal pattern of motor activity and delayed preputial separation), was evident mainly in the F2 pups of the high exposure group (500 ppm). In addition, decreased swimming abilities on post-natal day (PND) 24 and reductions in forelimb grip strength on PND 60 were found in both sexes. These data indicate that neuromotor functions were affected and are assessed as being mainly a direct consequence of the styrene exposure. Significantly decreased pup body weight during the lactation period was found at 150 ppm in the absence of maternal toxicity. The results of this study show that exposure to 500 ppm styrene causes developmental toxicity manifested as a pattern of developmental delays, including delayed neurological development, and developmental neurotoxicity effects on post-weaning behaviour, especially related to neuromotor functions. In contrast to the earlier investigations at 300 ppm, the exposure to 500 ppm induced some maternal toxicity (reductions in body weights of 7-8% and degeneration of the nasal olfactory epithelium). However, it is considered unlikely that the developmental toxicity is a non-specific secondary effect of the maternal toxicity.

[3] Follow up III of the meeting of the Technical Committee on Classification and Labelling in Arona, 26-28 September 2007 (Ispra, 29 May 2008)

Comments received during public consultation

No new experimental studies specifically addressing the toxicity of styrene were submitted during the Public Consultation, but industry stakeholders provided extensive comments, mainly addressing the interpretation of the studies, accompanied by additional references to published literature to support their arguments. A general comment expressed by industry stakeholders was that although the proposal draws on the studies already evaluated in the EU RAR, the dossier submitter has omitted relevant qualifying comments and negative criticism of studies important for assessing reproductive toxicity. The RAC has therefore also considered the detailed industry stakeholders comments and consulted the EU RAR. Overall, the RAC agrees with industry that the data are not sufficient for classification with Repr. 1B. Some responses to the industry stakeholders comments are included in the Appendix to this opinion.

Other stakeholder comments

Very diverging views on this proposal were received, with one Member State and one Labour Union supporting the proposal, two Member State instead supporting classification as Repr. 2, one Member State expressing this as being a borderline case between Repr. 2 and no classification, and two Member States saying that there should be no classification for developmental toxicity. Those disagreeing with the proposal felt that the observed effects were not convincing or consistent between endpoints or generations, being rather mild, and were probably caused by maternal toxicity.

Assessment and comparison with the classification criteria

Key data and arguments that are relevant to the proposal
The proposal high-lights the effects in the two-generation study in rats as the main reasons for classification. The following findings in offspring are stressed in the dossier;

- a decreased pup growth in F2 offspring at 150 and 500 ppm (7-10% and 10-13%, respectively),
- a decreased relative pituitary gland weight (22% in F2 males),
- a decreased forelimb grip strength (24-28%), and
- an increased time to escape in straight channel swimming trials (38% in males).

The RAC notes the dose-dependent decrease in pup weights in the second generation offspring (F2), and although there were some effects on the F1 maternal body weight at the top dose (reduction by 7-8%), the reduced growth of the pups at the mid dose supports that this could be a direct effect on the offspring. There were no effects on the weights of the first generation offspring (F1), even though F0 maternal weights were clearly affected (7-8%).
The relative pituitary weight was clearly decreased in males at the top dose, and of such a magnitude to indicate this to be an adverse effect even in the absence of any pathological findings. It has been argued in the PC comments that the large variability in the weight of the pituitary between animals at PND 21 (but not in adults) makes it difficult to draw firm conclusions from the mean values observed on PND 21. On the other hand, if it is the developmental rate of the pituitary that is affected, the lack of pathological findings may be consistent with the decreased weight. Although this finding may constitute some evidence of developmental effects, the robustness of this finding is decreased by the lack of pituitary effects in F2 females (PND 21) or the F1 generation (PND 21 offspring or adults).
Forelimb grip strength was reduced (24-28%) in both sexes at 500 ppm at day 60 (but not on days 22 and 45). The magnitude of the effect on day 60 was larger than the effect

on body weight, potentially indicating a neuromuscular effect. However, it cannot be ruled out that the effect is caused by the decreased growth rate. Hind limb grip strength was decreased (18%) at day 45, but only in 500 ppm exposed males, and without any effects on days 22 and 60. Overall, the grip strength tests give some evidence of neuromuscular effects, but the findings are clearly weakened by only being observed on one of the three occasions when it was studied, and by the observed effects on forelimb and hind limb grip strength not occurring on the same occasions. Discrepancies between effects on fore and hindlimb strength have, however, been reported in other studies (Maurissen *et al*., 2003).
Time to escape in straight channel swimming trials is assumed to reflect swimming ability and motivation to escape. The time to escape was increased by 38% in males of the 500 ppm group at day 24. Effects of a similar magnitude were observed in the females, but were not statistically significant (information from the EU RAR). Findings of similar effects on day 62 when it was studied again would have strengthened this observation. However, the dossier notes that the positive controls PTU and methimazole also only affected this parameter on day 24 but not on day 62. It is not explained in the dossier why PTU and methimazole can be considered as "positive controls".
Observations of delayed development at 300 ppm in the two rat inhalation developmental studies are referred to as supporting information. The RAC notes that in both studies there are observations of effects on time of eye opening, righting reflex, and incisor eruption, and that these effects fit the pattern of effects observed in the two-generation study. The use of pair-fed control dams in one of these studies indicates that the effects are not caused by a decreased pup growth rate. The two developmental studies have some methodological deficiencies and can only be used as supportive studies.

Conclusion on classification

The CLP criteria state that a substance should be placed in Category 1B 'Presumed human reproductive toxicant', when the data; "*provide clear evidence of an adverse effect on….. development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate*".
For styrene, there are indications of effects on development but the rather inconsistent effects (e.g., decreased pup growth in F2 but not in F1, decreased grip strength only at some time points, and effects on swimming trials at day 24 but not on day 62) cannot qualify asthe 'clear evidence' required by the CLP. Furthermore, some relationship between decreased pup growth and the other effects cannot be completely ruled out. Thus, in the opinion of the RAC, classification with Repr. 1B, H360 (CLP) is not appropriate. As the criteria for DSD are very similar to the CLP criteria, classification with Repr. Cat 2; R61 according to the DSD is likewise not warranted.
The CLP criteria state that a substance should be placed in Category 2 'Suspected human reproductive toxicant' when the data provide; "*some evidence from humans or experimental animals, possibly supplemented with other information…..on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification*. *Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects*".
The generally well performed two-generation study provided some evidence of long-lasting, delayed pup development, as exemplified by dose-dependently decreased F2 pup body weights at 150 and 500 ppm (10-13% at 500 ppm), a decreased pituitary weight in male 500 ppm F2 pups (22%), and decreased grip strength (24-28% forelimb grip strength) and swimming abilities at 500 ppm. In the weight of evidence assessment

made by the RAC, it has been taken into consideration that styrene did not affect other parameters studied in the two-generation study. Two developmental studies in rats, in the absence of maternal toxicity, also indicated a delayed development of newborn pups (delayed eye opening, righting reflex, and incisor eruption), and decreased pup weights (8-11% at day 1 and 15% at day 21 at 300 ppm in one study and 8% at day 21 at 300 ppm in the other study) although there are some deficiencies in these studies. There might be a relationship between decreased pup growth and the other findings, but it is noted that the effect in the two-generation study on the pituitary weight, and decreased grip strength cannot be fully explained by the decreased growth rate. The possibility of the effects being caused by general pup toxicity rather than by specific developmental toxicity is discussed in the comments, but the RAC finds it difficult to distinguish between the two based on the available data.
Maternal toxicity was also discussed in the comments received during the public consultation as potentially explaining the observed effects. Maternal effects were only noted at the top dose (500 ppm) in the two-generation study. They consisted of nasal toxicity and a reduced body weight gain, such that the final body weights of the females were 7-8 % lower than control weights in both F0 and F1. It is not likely that the maternal nasal toxicity can explain the effects noted on the pups. Likewise, the reduced maternal weight gain does not seem to be of a sufficient magnitude to constitute marked maternal toxicity or to explain the pup effects.
Whether the pup effects were caused by the pre- or postnatal exposure has also been raised, and it is acknowledged that it is always difficult to determine when such effects have been initiated. However, in the two-generation study, F2 pup body weights were reduced already on day 0 ("*decreases in body weight…were observed…throughout the pre-weaning period (PND 0-21*"). In the two developmental toxicity studies (where treatment of the dams stopped prior to birth), pup body weights were reduced and developmental landmarks were delayed, occurring later during the pre-weaning phase, indicating that the effects were attributable to the gestational exposure. Placental transfer of styrene has also been shown in mice.
In adult rats, styrene causes ototoxicity (loss of hearing) and toxicity to the nasal epithelium. In humans, styrene causes hearing loss, affects colour vision and long-term exposure may also lead to brain damage (chronic encephalopathy). In rat pups, styrene consistently affects the growth of the pups, resulting in delayed development of the offspring. There are also indications of neurological/neuromuscular deficits in the offspring, and although there are inconsistencies in these data, these effects should be interpreted in the context of the neurotoxic effects of styrene on adult animals. There is evidence of developmental toxicity noted in three different studies.
Overall, the RAC is of the opinion that there is sufficient evidence of developmental effects to warrant classification as Repr. 2, H361d (CLP).
The criteria also state that if the effects are considered to be of low or minimal toxicological significance (e.g., small effects on foetal weights, or small differences in postnatal developmental assessments), classification may not necessarily be the outcome. The types of effects observed in the styrene studies might initially suggest that this is a borderline case for classification, but the RAC considers that the overall pattern of long-lasting developmental delays and neurological/neuromuscular deficits fulfill the requirements for classification with Repr. 2, H361d (CLP). As the criteria for reproductive toxicity classification under DSD are very similar to the CLP criteria, classification with Repr. Cat 3; R63, is warranted according to the DSD.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the dossier submitter; the evaluation performed by the RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and the RAC (excl. confidential information)

References

Maurissen JPJ, Marable BR, Andrus AK, Stebbins KE(2003). Factors affecting grip strength testing. *Neurotoxicology and teratology*, 25, 543-553.

Paramei GV, Meyer-Baron M, Seeber A (2004). Impairments of colour vision induced by organic solvents: A meta-analysis study. *Neurotoxicol* 25:803-16

Seeber A, Bruckner T, Triebig G (2009). Occupational styrene exposure, colour vision and contrast sensitivity: a cohort study with repeated measurements. *Int. Arch. Occup. Environ Health* 82:757-70

Vyskocil A, El Majidi N, Thuot R, Beaudry C, Charest-Tardif G, Tardif R, Gagnon F, Ska B, Turcot A, Drolet D, Aliyeva E, Viau C (2012). Effects of Concentration Peaks on Styrene Neurotoxicity in the Fibreglass Reinforced Plastics Industry Phase II. *IRSST report R-728*

Appendix

Response to comments on effects on pup body weights

The industry stakeholders noted the following points:

- in addition to the effects on pup body weights, maternal body weights were also lower relative to controls, although generally not statistically significant,
- due to the variability in pup body weights, the statistically significant effects may still be chance findings,
- the apparently decreased body weights of the exposed pups are not actually caused by decreases in the exposed animals but rather are explained by the unusually high body weights of the control pups,
- with reference to a publication by Piersma *et al*. (2011), effects seen only in F2 pups and not in F1 pups must be chance findings,
- decreased pup body weights
    - were due to non-specific general toxicity and are not evidence of specific developmental toxicity,
    - may be caused by impaired maternal care, which may be a consequence of maternal olfactory degeneration and transient narcotic effects,
    - are of minor toxicological relevance and not a reason for classification.

The RAC has studied the detailed comments from industry, and agrees that the effects on body weights in F2 pups are rather small, but also notes that it is consistent in F2 pups exposed to 500 ppm styrene over time, and larger than the potential effects on the dams. There are therefore no reasons to not trust the statistical evaluation of the data, or to speculate on chance findings. One cannot totally rule out that the decreased pup body weight is caused by impaired maternal care, but on the other hand, there are little firm data to substantiate that olfactory degeneration or, if occurring, slight transient narcotic effects could lead to decreased growth of the offspring. The RAC is of the opinion that the decreased pup growth provides some evidence of developmental effects of styrene.

Response to comments on effects on relative pituitary gland weight

The comments from industry stakeholders note:

- the great variability in pituitary gland weights in PND 21 pups, making comparisons of weights very uncertain,
- that effects were only noted in F2 pups and not in F1 pups or adult F1 animals,
- that with reference to a publication by Piersma *et al.* (2011), effects seen only in F2 pups and not in F1 pups must be considered as chance findings,
- that if there is an effect on pituitary gland weight, the effect cannot be regarded as severe,
- that the lack of histopathological alterations in the pituitary gland raises questions regarding the relevance of the effect on pituitary weight,
- that if there is a decreased pituitary weight, it is a consequence of non-specific delay of development and not evidence of specific developmental toxicity.

The RAC has studied the detailed comments, and agrees that the variability of the pituitary gland weight at PND 21, combined with the fact that the effect only occurs in F2 males, makes the original finding less robust. Because of the magnitude of the effect (22%) and its possible relationship to a delay of development (the lack of histopathological findings would support a delayed development rather than pituitary toxicity), the finding cannot be disregarded. The RAC is of the opinion that the decreased pituitary gland weight gives some evidence of developmental effects of styrene.

Response to comments on effects on grip strength

The comments from industry stakeholders note:

- the large variation in grip strength between animals, making comparisons between groups very uncertain, exemplified by increased grip strength in the 150 ppm group at day 45,
- that grip strength is not linearly correlated with body weight over time, making the body weight correlated conclusions uncertain,
- that the decreased body weight-correlated grip strength might be explained by larger decreases in body weight at earlier ages than when the grip testing was performed,
- the lack of consistency between results at different time points, as well as in hind-limb versus fore-limb grip strength data,
- that the data from this study are within the historical control data from that laboratory
- that the decreased grip strength does not correlate with any histopathological effects, this leads to a conclusion of doubtful significance of this minor effect, which may possibly be explained by a non-specific delay in development.

The RAC has noted the lack of consistency between time points, which is a weakness in the argument for this being a toxicologically significant effect. However, although the grip strength varies considerably, this is a well-established parameter and it is difficult to ignore the findings of a statistically significant effect on grip strength. The body weight correction might not be perfect, but seems sufficiently reliable to draw the conclusion that the effects on grip strength are larger than the effects on body weight. Histopathological findings could have strengthened the findings on grip strength, but the lack of pathology is not a sufficient reason to disregard the grip strength findings. A comparison with historical control data may be relevant when the effects lie at the borderline of biological and/or statistical significance, but the RAC notes that in view of the magnitude of the effect in this case (24-28% for forelimb grip strength), the concurrent controls should be given greater weight than historical control data. Overall, the RAC is of the opinion that the decreased grip strength gives some evidence of developmental effects of styrene, although not warranting classification Repr. 1B as proposed by the DS.

Response to comments on swimming trials

The comments from industry stakeholders note:
- that the increased swimming time in the short swim trial (straight channel; 10 sec) is of doubtful biological significance considering that there were no effects on swimming time in the longer swimming trial (time to escape; 50-150 sec),
- that the effect was confined only to the first of four trials,
- that historical control data would indicate an unusually short swimming time for the control group rather than long times for the groups exposed to styrene,
- that long swimming times can be related to lower body weights.

This led industry to the conclusion that if there is an effect, it has minor toxicological relevance, and could be caused by a non-specific delay of development associated with maternal toxicity combined with "chance" variation in data.
The RAC has noted that swimming time was affected only in the short swim and not in the long swim, but as no mechanism has been identified this is not considered a reason to disregard the effects. Furthermore, although the effect mainly arises in the first trial, the data for the first trial is rather convincing as a dose-related effect (approximately 13-16-17-21 and 15-21-18-24 seconds in males and females, respectively, of the control-50-150-500 ppm groups). It is likely that swim time correlates with body weight, but the effects at 500 ppm seems larger than expected based on the observed changes in body weights. Regarding historical control data, the RAC notes that if the effect would be caused by unusual control data, statistically significant effects would have been expected in all the exposed groups and not only in the 500 ppm group. Overall, while accepting

that the data are not entirely consistent, the RAC is of the opinion that the increased swim time gives some evidence of developmental effects of styrene.