

## Colour Discrimination

### Summary of the ESR hazard assessment for styrene

Toxicokinetics - A substantial amount of information is available on the toxicokinetics of styrene in humans, following exposure by the inhalation route; information on percutaneous absorption in humans is also available.

In humans, inhaled styrene vapour (at concentrations of 10-200 ppm) is well absorbed across the respiratory tract. Thus, a value of 100% for absorption via the inhalation route of exposure is taken forward to the risk characterisation.

Dermal absorption of the liquid has been estimated to be approximately 2% of the applied dose in an *in vitro* study using human skin samples. This value is taken forward to the risk characterisation. Dermal uptake of the vapour appears to make only a small contribution (5% or less) to the total body burden arising from combined inhalation and dermal exposure to the vapour. In studies of workers exposed to styrene in the air and also potentially to liquid styrene on the skin, uptake of liquid styrene via the skin appeared to make very little contribution to body burden. No information is available on oral absorption in humans, but from the physicochemical properties of styrene and experimental animal information, one would expect extensive absorption from the gastrointestinal tract. Thus, a value of 100% for oral absorption is taken forward to the risk characterisation. Following absorption, it can be predicted from experimental animal data that styrene is widely distributed in humans, and needle biopsy investigations have shown that styrene certainly locates in adipose tissue; there was a correlation between the amount of body fat and the total body burden of styrene.

The rate of absorption following inhalation is much higher (2-3 fold) in mice than in rats. The absorption rate in humans is approximately the same as in rats. The rate of styrene uptake in the upper respiratory tract is partly dependent on its metabolism, and was decreased when animals were pre-treated with a P450 inhibitor.

In humans, styrene is eliminated from the body relatively rapidly, primarily in the urine. However, there is some evidence for modest accumulation in human adipose tissue on repeated daily exposure. From studies in mice, there is evidence that styrene is also rapidly eliminated from blood following either single or repeated inhalation exposure. A study in pregnant mice has shown that styrene and/or its metabolites can cross the placenta into the foetus.

The metabolism of styrene has been studied thoroughly in mice, rats and humans. A number of metabolic pathways have been identified. The evidence suggests that these pathways are active in mice, rats and humans, although there are species differences in their relative importance.

Styrene is metabolised extensively in all species. The first step in the metabolism of styrene involves oxidation of the aromatic ring or side-chain. The main route in each species is the oxidation of the side chain to give the epoxide, styrene-7,8-oxide (SO). A number of studies have demonstrated the involvement of P450 in this step and provided information on the specific P450 isoforms involved in the production of SO. For the lung, the evidence shows that CYP2F2 is the specific isoform involved in the bioactivation of styrene in this tissue. The SO produced is enantiomeric and is produced in the R- and S-forms, probably as a result of metabolism by different P450 isoforms. Different ratios of R-SO to S-SO are found in different tissues and different species. Mouse Clara cells produce about 3 times more of the R-enantiomer than the S-enantiomer, while rats produce more of the S-enantiomer, and humans, like rats, produce more of the S-form. SO is either metabolised further by conjugation with glutathione to give mercapturic acids, or is hydrolysed by epoxide hydrolase (EH) to phenylglycol. This is subsequently metabolised to mandelic, phenylglyoxylic and hippuric acids. P450 and EH are both microsomal enzymes in the endoplasmic reticulum. Therefore, SO produced *in situ* by P450 may potentially be rapidly detoxified if there is sufficient EH present.

Other metabolic pathways can lead to phenylacetaldehyde (PA) and phenylacetic acid (PAA) (via side-chain  $\beta$ -oxidation and hydroxylation), to phenylethanol and acetophenone (via side-chain  $\alpha$ -oxidation and hydroxylation), oxidation of the aromatic ring to give 4-vinylphenol (4-VP), and products of ring opening. These metabolites are excreted in the urine. There are studies which have demonstrated that P450 enzymes are also involved in both the side-chain and ring oxidation of styrene and that 4-VP is further metabolised in lung microsomes by specific P450 isoforms to extremely reactive downstream products (e.g. an epoxide and a hydroquinone derivative). Subsequently these derivatives are conjugated with glutathione, but at present there is no information on the relative rates of 4-VP metabolites detoxification between different species.

It is clear that metabolism involving SO as an intermediate is a major route in rodents and humans. However, there are some notable species differences. In humans, almost all of styrene (95%) is metabolised to SO and further metabolised by EH; approximately 5% of styrene is metabolised via the phenylacetaldehyde pathway. No more than trace amounts (<1%) of SO-GSH conjugates or ring-oxidized metabolites of styrene (4-VP) occur in humans exposed to styrene. Further metabolism of SO by EH is important but less extensive in rodents than in humans (68-72% in rats and 49-59% in mice). In rodents, conjugation of SO with GSH is an important route accounting for up to a third of the SO removal. The most significant difference between mice and rats is in relation to the production of phenylacetaldehyde (12-22% in mice against 3-5% in rats) and products of ring-oxidation (4-VP; 4-8% in mice against <1% in rats).

The tissue specific metabolism of styrene suggests that *in situ* metabolism within each tissue may be a more important determinant of toxicity than the overall systemic metabolism and blood levels of styrene metabolites. The implication of this is that the specifics of the local metabolism in a target tissue

must be considered when extrapolating findings in animals to assess the likely hazards and risks in the equivalent human tissues.

Overall, human tissues – apart from the liver - produce very little SO, if any, and have a greater capacity to hydrolyse SO with EH than rodents. This difference is most pronounced in human nasal and lung tissues where production of SO is minimal or undetectable, and is also associated with a greater capacity to hydrolyse SO by EH. The mouse lung and nasal tissues produce the greatest amount of SO among the species tested, and, in general, have less EH activity, suggesting that significantly high local concentrations of SO will be present in these tissues. It is also evident that other toxic metabolites, particularly 4-VP and its reactive downstream products, are produced to a far higher extent in mouse lung than in rat (14-79% of the mouse concentrations) or human lung (1.5-5% of the mouse concentrations). It is unclear at present whether or not these species differences in the formation of 4-VP metabolites in the lung are a reflection of the different numbers of Clara cells (the metabolically active lung cells) present in the different species.

Acute toxicity - In humans, there is some acute inhalation toxicity information available indicating effects of styrene on the central nervous system (CNS) function. From the studies that have been reported there has been no convincing evidence of an effect on neurobehavioural test performance with exposures in the range 0.5 – 150 ppm; however, some impairment in test performance appeared with exposures of 200, 350 and 376 ppm for period of 30 – 90 minutes. Higher concentrations (800 ppm in one study) have produced signs and symptoms of pronounced CNS depression. No other acute toxicity information is available from human studies.

In rats and guinea pigs styrene is of moderate-low acute toxicity via the inhalation and oral routes. Two inhalation studies in rats have reported a 4-hour LC<sub>50</sub> of 2770 ppm (11.8 mg/l) and “some deaths” at 2149 ppm (9.1mg/l) for up to 40 hours. An oral LD<sub>50</sub> of approximately 5000 mg/kg has been reported in rats. In contrast, mice (at least some strains of mice) are much more sensitive to a single exposure to styrene, with cellular damage in the respiratory epithelium at 40 ppm (the lowest concentration tested) and fatal hepatocellular damage at 250 ppm and above in acute inhalation studies. The most likely explanation for this species difference is the greater potential for build-up of the reactive styrene oxide metabolite in mouse, compared to rats or humans. An acute oral study in hamsters also indicates styrene-induced hepatotoxicity at 600 mg/kg (but not at 450 mg/kg). No acute dermal toxicity studies have been performed in experimental animals, but one would predict low acute toxicity, with the possible exception of some strains of mouse. In view of the fact that humans (volunteers and workers) have been exposed without serious effects to acute exposure conditions that have proved toxic and even lethal to the more sensitive mouse strains, and considering the known toxicokinetic differences between the mouse and the human in the activation/deactivation of styrene, the mouse is considered to be a poor and unreliable model for the acute toxicity of styrene in humans. Therefore, for the purposes of risk characterization, information from the human volunteer

studies will be used. The most useful reference point in relation to short-term single exposure is the observation that no CNS depression was seen in humans exposed to 100 ppm for 7 hours.

Irritation - Although limited, the available data suggest that liquid styrene is not significantly irritating to the skin after a single exposure, but that repeated exposure does cause irritation. The available evidence suggests that liquid styrene can produce eye irritation. Exposure to airborne styrene vapour can also cause eye irritation. Exposures of up to approximately 200 ppm for one hour were without effect but concentrations of 375 ppm and above are clearly irritating. The NOAEC of 200 ppm for 1-hour exposure will be used in the risk characterisation for eye irritation caused by exposure to the vapour.

It is clear that exposures to airborne styrene can cause respiratory tract (nasal) irritation. No effects were seen at 100 ppm for 1 hour and only one subject out of nine reported nasal irritation at 216 ppm, suggesting little or no significant irritation at this concentration. Nasal irritation was more evident at 375 ppm and above, in several studies. For the purpose of risk characterisation, the NOAEC of 216 ppm for 1- hour exposure will be used.

Sensitisation - The reporting of the available animal skin sensitisation data is inadequate, precluding a clear conclusion being drawn from the studies themselves. However, given that widespread exposure to styrene has led to only one reported possible case of skin sensitisation, this extensive human experience indicates that styrene is not a significant skin sensitiser and negates the need for any further animal testing with respect to this endpoint. Similarly, there has been extensive inhalation exposure in humans, which has resulted in only two case reports of asthma, each of which has unconvincing aspects to it. This suggests that styrene has no significant asthmagenic potential.

Repeated dose toxicity - There is a large amount of information from studies in humans. In the few studies that have reported on patterns of mortality from non-malignant disease in occupational groups repeatedly exposed to styrene, the authors of the studies have signalled findings that have been proposed to be worthy of further exploration. However, a critical appraisal of the studies, taken together, suggests that they present no convincing evidence that styrene exposure has enhanced the incidence of mortality from any particular disease.

In the available worker health survey studies, consistent evidence of an increase in the self-reporting of symptoms of eye and nasal irritation and CNS disturbance (drowsiness, headache, lightheadedness) comes through. Unfortunately, the quality of the exposure data means that it is not possible to relate these effects to reliable levels of styrene exposure, particularly as it is possible that these effects are related more to short-term peaks in exposure, rather than to workshift averages. No reliable evidence for any other effects of styrene is furnished by these studies. From the studies available there is no convincing evidence of a clear, interpretable and toxicologically significant effect of styrene having occurred in exposed workers, in relation to haematological, immunological, hormonal or renal endpoints.

Because styrene is highly lipid-soluble and, like many other organic solvents, at certain concentrations, produces acute CNS effects, concern about the long-term toxicity of styrene has been focused on its potential for damaging the nervous system. To that end, for the last 40-45 years, several studies investigating the potential neurotoxicity of styrene in exposed workers have been undertaken in factories in many parts of the world. The majority of these studies have suggested that styrene has substantive effects on the nervous system in humans such that the generally uncritical recitation of the results from these investigations has created over the years the label that styrene is a potent neurotoxicant. However, despite this extensive investigation of styrene potential neurotoxicity, a critical review of the available data has shown that there is no clear relationship between repeated exposure to styrene and persisting damage to the nervous system.

Several studies have been conducted on EEG patterns in styrene-exposed workers. Overall, the collective findings do not provide robust evidence for the absence or presence of styrene-induced EEG changes in exposed workers. Furthermore, although the approach is valuable in that it provides measures of nervous system function that are independent of the level of collaboration of the subject, no clear criteria for interpretation of the health significance of any EEG changes that might have occurred are available. If styrene was the causal agent of EEG changes in workers covered by these studies, the most likely interpretation is that the effects were due to the general CNS depressant action of styrene.

The available nerve conduction studies have produced inconsistent results for different groups of workers exposed to similar levels of styrene. Overall, it is not clear if styrene exposure can produce a decrease in nerve conduction velocity; furthermore, if it can, the underlying basis for the effect and whether it represents a consequence of short-term or long-term exposure, could not be deduced from the available information. Also, the clinical significance of this effect is questionable, as all subjects appeared to be healthy workers.

A few studies are available which were designed to investigate potential effects of styrene on hearing function. When confounding factors such as age and noise exposure were taken into account, no relationship between styrene exposure and hearing loss was found. There is limited evidence that styrene exposure may have caused minor effects on vestibular reflexes in some workers. However, the quality of the exposure data is such that it is not possible to relate these effects to reliable levels of styrene exposure. Therefore, although these human data cannot be used for risk characterisation purposes, nevertheless they indicate that the observations of ototoxicity in animals may be relevant to humans.

In one study to investigate olfactory function, other than an adaptive increase in the threshold for odour detection of styrene itself, no olfactory deficit was evident in workers exposed to up to 77 ppm (8-hour TWA).

Studies on colour vision provide a sufficiently consistent body of evidence to support the view that styrene does cause an impairment of colour discrimination relative to age-matched controls. Generally, the impairment was of the tritan (blue-yellow) type, although some workers also had evidence of red-green colour vision deficiency. However, there is a lack of information

on whether the impairment is related to short-term or repeated long-term exposures to styrene. There is also a lack of information on the magnitude of impairment on colour discrimination in workers exposed to styrene concentrations > 100 ppm (8h TWA), although performance in colour discrimination tests at these high exposures is likely to be subject to confounding because of transient CNS depression or eye irritation. Studies suggest that an impairment of colour discrimination would not be expected with 8 h TWA exposures below 20 ppm. However, although an effect of styrene exposure on colour discrimination has been observed at higher exposure levels ( $\geq$  50 ppm, 8h TWA), given the very mild nature (the affected workers were not even aware of any deficit) and the likely reversibility of the effect which appears not to affect performance in jobs that require good colour vision, it is deemed that the slight impairment in colour discrimination observed should not be considered as an adverse health outcome of styrene exposure. It can be concluded that since the effects observed at 50 ppm (8h TWA) are not yet adverse, this exposure value can be considered a clear NOAEC.

Numerous workplace studies using neurobehavioural testing are available. It is noted that a range of significant confounding factors has not always been addressed. The results obtained have been variable, with some studies reporting effects and others no effects for workers within similar exposure ranges. Performance was adversely affected in several studies in only a small proportion (1-3 out of 6-20) of the tests administered. Also, different types of neurobehavioural test (representing various functional domains of the CNS) were apparently sensitive to styrene exposure in different studies. This lack of a clearly consistent effect on particular functional domains indicates that there is only weak evidence for a causal relationship. In some studies, there is a possibility that effects similar to those detected in volunteer studies (i.e. slightly slower response times) were observed and these might be related to brief peaks of styrene exposure of hundreds of ppm that had occurred during the previous shift. In view of this, it is not possible to discern a clear dose-response relationship for any of the effects observed in neurobehavioural tests in workers. Furthermore, at present there is not a clearly established, widely accepted interpretational framework into which these results can be fitted. Where apparent deficits in neurobehavioural test performance have been measured, the underlying toxicological processes involved, the consequences of the health and safety of the individual, and the effect of styrene in comparison with that of other experiences and phenomena regularly encountered in everyday life, have not been established.

Taking all of these points into consideration, the rapporteur proposes that the crucial issue in relation to the impact of styrene on the nervous system is the need to avoid acute CNS depressant effects and associated symptomatology.

In relation to findings in experimental animals, a variety of repeated inhalation exposure studies in different animal species are available. However, among these species, the rat and mouse have been the most extensively investigated. Four well characterised target sites of toxicity have been identified: the nasal epithelium (in rats and mice), the lung (in the mouse), the liver (in the mouse) and the ear (in the rat).

In relation to nasal epithelium damage, a NOAEC has not been identified, with chronic inflammatory changes (mild in degree and confined to the olfactory epithelium with no effects on Bowman's glands or olfactory nerve fibres), having been seen in the rat with long term daily exposure to 50 ppm styrene, the lowest concentration explored in a 2-year study. Nasal toxicity has also been reported in mice. In a similar 2-year study, respiratory metaplasia of the olfactory epithelium, hyperplasia and hypertrophy of the Bowman's gland and atrophy of the olfactory nerve fibres were observed starting from 20 ppm, the lowest concentration tested. It is clear that the nasal lesions induced by styrene exposure are a lot more severe in the mouse compared to the rat. Over the years a number of investigative studies have been undertaken to characterise and explain these species differences and to investigate the relevance of these findings to humans. The results of these investigations have shown that the differences in nasal toxicity between rat and mouse can be explained by the greater ability of the rat nasal epithelium to detoxify reactive metabolites of styrene formed *via* CYP2F2 metabolism. These reactive/toxic intermediates include styrene oxide and most probably the downstream metabolites of 4-VP. Detoxification of toxic species by epoxide hydrolase is 10-fold higher in the rat olfactory tissue as compared to the mouse while glutathione S-transferase activity is approximately 3-fold higher in the rat nasal tissue as compared to the mouse. It is important to consider that cytochrome P450-catalysed metabolic activation of styrene to styrene oxide in human nasal epithelial tissue appears to be negligible (see toxicokinetics section). Also, since uptake of styrene by nasal tissue is enhanced by the ability of the tissue to metabolize styrene, the lack of the primary step of styrene metabolism in human nasal tissue serves as a further reason for dismissing the relevance to humans of these rodent nasal lesions. Besides these metabolic differences, anatomically, there are significant differences in the nasal passages of rodents and humans, which result in significant different volumes and airflow patterns. Thus, although inhaled styrene may be deposited in the nasal passages of humans, it is likely that high levels will not be deposited in the olfactory area. Furthermore, human investigations have shown that exposure up to 77 ppm (8h TWA) styrene as occurring in the UP-resin industry is not associated with impairment of olfactory function, and in several human health surveys of workers exposed to styrene up to approximately 700 ppm, no nasal lesions have been described (see RDT, human studies section). Hence, it can be concluded that rodent nasal epithelium damage induced by styrene is not of relevance in relation to the potential repeated dose effects of styrene in humans at relevant levels of exposure.

In relation to the lung, no effects were seen in rats exposed up to 1000 ppm, but in mice a NOAEC was not identified, with damage to the lung epithelium (hyperplasia in the terminal bronchioles, focal bronchiolar-alveolar hyperplasia, fibrosis extending to the alveolar ducts and extension of non-ciliated cells from the terminal bronchiole to the alveolar duct) having been seen from 20 ppm, the lowest exposure concentration tested in a 2-year study. Work with the cytochrome P450 inhibitor 5-P-1-P has shown that metabolic activation of styrene to styrene oxide and other reactive metabolites (e.g the downstream metabolites of 4-vinylphenol) and the subsequent detoxification of styrene oxide are crucial elements of this toxic response. This

is supported by the observation that it is the metabolically active Clara cells that are the initial focus of damage. Early biochemical changes, sustained cell damage and regenerative cell proliferation were observed in lung Clara cells of mice exposed to 40 and 160 ppm styrene for up to 4 weeks. In this context it is important to consider that cytochrome P450-catalysed metabolic activation of styrene in human lung tissue appears to be negligible (see toxicokinetic section) and that the number of Clara cells in human lung is very low. Hence, it can be concluded that these lung tissue findings in mice reflect a toxic response that will not occur to any significant extent in humans at relevant levels of exposure.

In relation to the liver, exposures in the range 150-350 ppm have produced fatal hepatotoxicity in mice. Again, it appears that the metabolic activation of styrene to styrene oxide is a crucial stage in the hepatotoxicity process. In this context, it is evident that the toxicokinetic information available suggests that the mouse would be more susceptible than humans to styrene induced hepatotoxicity. This is borne out by the observation of mouse fatalities at exposure levels to which humans have been regularly exposed in the past, with no reports of hepatotoxicity or death.

In relation to effects on the ear, clear evidence of ototoxicity (both functional and histological) has been seen in rats repeatedly exposed to styrene by inhalation at concentrations of 600 ppm and above. In three different studies, no such effects were seen at 200 ppm, 300 ppm or 500 ppm, although in the latter two cases the studies were only of four weeks' duration. Ototoxicity has been seen at similar exposure levels with other aromatic organic solvents, such as toluene and xylene. Comparative studies using rats and guinea pigs exposed to 1000 ppm for 5 days indicate an obvious species-difference, as similar findings were not observed in guinea pigs. The underlying toxicological mechanism has not been clearly elucidated. This effect should be regarded as of potential relevance to human health. Overall, with no ototoxic effects having been seen at 200 ppm for 13 weeks, risk characterisation should be performed against this NOAEC.

In one single investigation, effects on the density of the large amacrine cells and on the content of neuramines and glutathione of the retina of rats exposed repeatedly to 300 ppm styrene for 12 weeks have been reported. However, it is considered that, in the absence of any associated histopathology, the biochemical changes observed are unlikely to represent a toxic response.

Overall, the available animal inhalation repeated dose toxicity studies have identified ototoxicity as the most relevant and sensitive effect of styrene repeated inhalation exposure with a NOAEC of 200 ppm for 13 weeks.

Most of the available repeated oral exposure studies have been performed in rats and mice. Information from a carcinogenicity bioassay in the rat has shown no evidence of treatment related toxicity in animals administered 1000 mg/kg/day for two years; a marked increase in mortality was evident at 2000 mg/kg/day. In another 2-year study, styrene did not produce any clear evidence of toxicity when administered in drinking water at a dose of 21 mg/kg/day, the highest dose level tested. However, it is noted that potential effects on the ear were not investigated in these studies. Ototoxicity, a clearly

established effect of styrene in the rat, was seen in one study at 800 mg/kg/day (and perhaps, although less convincingly, at 400 mg/kg/day) for 2 weeks.

In mice, the most reliable information comes from a cancer bioassay, in which increased mortality was seen at the highest dose of 300 mg/kg/day; a NOAEL of 150 mg/kg/day was identified from this study. The one significant observation from the remaining studies is that of toxicity towards the lung epithelium, adding further support to the concept that the lung toxicity of styrene in mice, following oral and inhalation exposure, results from local metabolism of styrene to styrene oxide and to other reactive metabolites (e.g. the downstream metabolites of 4-vinylphenol).

Overall, the most reliable reference point for risk characterisation in relation to repeated oral exposure is the NOAEL of 150 mg/kg/day identified from a 2-year cancer bioassay in the mouse. But in extrapolation to humans careful consideration has to be taken of the specifics of mouse metabolism and the high sensitivity of this species for liver toxicity as compared to e.g. the rat.

No repeated dermal studies are available, although low systemic toxicity would be predicted in most conventional experimental species with the possible exception of some strains of the mouse.

Mutagenicity - With respect to mutagenicity, a large number of studies have been published which have aimed to investigate the genotoxic potential of styrene in humans by examination of various endpoints in styrene exposed workers. Very low levels of DNA adducts were found in some styrene exposed workers but it has been stated that such low levels should be viewed with caution. There is also some evidence of DNA damage (SSBs) induced in styrene exposed workers. Both these endpoints are indicative of exposure but are not necessarily associated with heritable effects. The results of several studies on another indicator endpoint of unclear health significance, SCEs, did not provide evidence of a positive response, despite these being induced in animals exposed to styrene. There are also many studies investigating endpoints (gene mutations, chromosome aberrations and micronuclei) known to lead to heritable effects. The number of studies assessing gene mutation is very limited and no conclusions can be drawn from them. Although 5 studies appear to present evidence that styrene may be weakly clastogenic in humans, there are 11 robust negative studies also. Together with a lack of evidence of a dose-response relationship and the negative response for induction of micronuclei when studied concurrently in two of the positive chromosome aberration studies, no clear conclusion on *in vivo* clastogenicity of styrene in humans can be made.

Overall, given the lack of evidence of consistent relationships between exposure levels and study outcome, the lack of any consistent profile of endpoints and the absence of information on the relevance of the types of adducts seen and their mutagenic potential *in vivo*, there is no convincing evidence that styrene has shown mutagenic activity in humans.

The overall picture presented by the *in vitro* assay results available is that at least in some test systems (including Ames tests and *in vitro* chromosome

aberration studies in mammalian cells), styrene does possess some genotoxic potential *in vitro*. Metabolic activation (presumably to styrene oxide) is required for this activity. Styrene has been exhaustively studied in clastogenicity studies in animals up to dose levels producing severe toxicity in some cases. There is no convincing evidence of styrene clastogenicity when the quality of the studies and the plausibility of the test results are considered. Equivocal results were obtained after exposure to high doses causing lethality. However, overall, negative results were obtained from *in vivo* chromosome aberration and micronucleus studies in the rat, hamster and the mouse following single or repeated exposures via the inhalation, oral and intraperitoneal route in the tissues examined (bone marrow, peripheral lymphocytes, splenocytes and whole blood). Furthermore, a recently published micronucleus test in bone marrow cells of mice conforming to the current OECD guideline was clearly negative.

The general pattern of SCE results in the wide range of tissues examined (lymphocytes, splenocytes, bone marrow, alveolar macrophages, regenerating liver cells) from both the rat and the mouse following inhalation or i.p exposure to styrene has been positive. However, it is important to note that in most cases concomitant chromosome aberration and/or micronucleus assays involving the same animals and in some cases the same tissues were carried out and that negative results were obtained for these indicators of chromosome damage. Therefore, this clearly reduces the significance of the SCE findings in relation to mutagenicity.

The binding of styrene metabolites to DNA was very low and did not indicate any specificity for the target tissue (mouse lung). Induction of alkali-labile single-strand breaks has also been produced *in vivo* in rats and mice exposed to styrene. Again the significance of these findings is unclear, given the repeated failure of styrene to demonstrate mutagenic activity in standard clastogenicity assays.

In summary, the available data suggest that styrene is weakly positive in indicator tests detecting SCEs, DNA strand breaks and DNA adducts. In contrast, an *in vivo* UDS test performed in accordance with international guidelines did not reveal a genotoxic effect of styrene in mouse liver.

Overall, based on standard regulatory tests, there is no convincing evidence that styrene possesses significant mutagenic/clastogenic potential *in vivo* from the available data in experimental animals.

Carcinogenicity - In relation to carcinogenicity, several cohort and case-control studies covering workers exposed to styrene in the GRP and styrene production industries are available. In these studies there was no clear and consistent evidence for a causal link between specific cancer mortality and exposure to styrene. In the styrene-butadiene rubber industry, several studies have pointed to an increased risk of cancer of the lymphatic and haematopoietic systems. However, detailed analysis of these data, together with the general toxicological picture for styrene and butadiene, suggests that where increases are due to occupational exposure, it is butadiene, not styrene, that is the more likely causative agent. In conclusion, the mortality studies available provide no evidence for a causative association between styrene exposure and the induction of cancer in humans.

In animals, the carcinogenic potential of styrene has been explored in rats and mice, using the inhalation and oral routes of exposure. A carcinogenic effect of styrene towards the lung is evident in the mouse. This has been shown in a well-conducted lifetime inhalation study in CD1 mice at exposure concentrations of  $\geq 20$  ppm styrene and, somewhat less convincingly, in an oral study in mice of the B6C3F<sub>1</sub> strain. The inhalation study, which included extensive histopathological examination, showed that the tumours (prevalently adenomas) were preceded by cytotoxicity characterised by early Clara cell toxicity followed by progressive bronchiolar epithelial hyperplasia and bronchiolar-alveolar hyperplasia.

In the rat, styrene has not exhibited any clear evidence of carcinogenic potential. In individual studies there have been isolated findings of statistically significantly higher incidences of various particular tumour types in particular groups of styrene-treated animals, compared with the in-study controls. However, the findings have been within historical background ranges, not reproducible between studies, in some cases have not shown an upward trend with increasing dose, and have not been associated with evidence of underlying styrene-induced changes at the site in question.

On the question of the relevance of the mouse lung tumours for human health, consideration of the available toxicokinetic information and data from single and repeated inhalation exposure studies in experimental rodents suggests the following as the most plausible toxicological mechanism for the mouse lung tumours. Styrene is metabolised by cytochrome P450 enzymes in the metabolically active Clara cells of the bronchiolar epithelium of the mouse, producing cytotoxic metabolites of styrene including styrene 7,8 oxide (SO) and oxidative metabolites of 4-vinylphenol (4-VP). These metabolites cause early Clara cell toxicity/death and sustained regenerative bronchiolar cell proliferation which, in turn, leads to compensatory bronchiolar epithelial hyperplasia and ultimately tumour formation. Clara cell toxicity could also be a consequence of the long term depletion of glutathione, because of conjugation with SO. Genotoxicity of SO (an EU-category 2 and IARC group 2A carcinogen) or other reactive styrene metabolites is unlikely to be involved in tumour development as minimal binding of styrene metabolites to DNA has been detected in mouse lung with no species- or tissue-specificity.

All of the key events of this postulated mode of action are less operative in the non-responsive rat (which does not develop lung tumours at exposure concentrations up to 1000 ppm) and even less operative in humans.

The number of Clara cells (being responsible for both the formation of toxic metabolites and the target for their toxic action) is very low in humans, even less than in rats. While Clara cells comprise about 85% of bronchiolar epithelium in mice and 25% in rats, in humans such cells are rare.

The enzyme CYP2F2 required for the formation of the Clara cell toxicants such as SO (including the highly pneumotoxic R-enantiomer) and the downstream metabolites of 4-VP occurs at most only to a negligible extent in humans.

In human lung, detoxification of SO (if formed at all in human pulmonary tissue) takes place predominantly *via* epoxide hydrolase (located on the

endoplasmic reticulum in close proximity to the toxifying cytochrome P450s). The close proximity of the “detoxifying” enzymes to any “toxifying” enzymes ensures the efficient removal of any toxic metabolites. Rodents use both epoxide hydrolase and glutathione-S-transferase as detoxification pathways with the mouse relying on glutathione conjugation more so than the rat. As glutathione S-transferase is located in the cytosol, this makes this detoxification pathway less efficient than the epoxide hydrolase pathway. In comparison to the rodent species, in humans, SO detoxification proceeds nearly exclusively via epoxide hydrolase and glutathione S-transferase accounts for only 0.1% of SO detoxification.

Taking account both of the toxification to SO and its detoxification, PBPK-modelling has shown that the SO content of human lungs must be very small, if there is any.

Formation of 4-VP and its downstream metabolites in human lung occurs at a much lesser extent (1.5-5% of the mouse concentrations) than in rat lung (14-79% of the mouse concentrations) and at an even lesser extent than in mouse lung.

As indicated by PBPK-modelling, glutathione depletion caused by SO does not occur in humans. Also, as reactive downstream metabolites of 4-VP are formed in human lung only to a very small extent, the 4-VP metabolic pathway is not expected to cause any glutathione depletion in human pulmonary tissue.

There is no evidence that long term exposure to styrene has produced lung damage in humans.

Hence, overall, the weight of evidence appears to indicate that the consequences of long term exposure to styrene in mouse lung cannot be replicated in the human situation. Although there are still some uncertainties in this postulated mode of action and in its relevance to humans, namely the in vitro nature of the information available on 4-VP and its downstream metabolites in human lung, the lack of data on the relative rates of 4-VP metabolites detoxification in different species and the relatively short duration (up to 14 days) of the studies investigating 4-VP-induced regenerative bronchiolar cell proliferation in mice, no alternative modes of action that logically present themselves can be supported by as significant a body of evidence as the one presented in this assessment. Consequently, it is felt that the level of confidence in the postulated mode of action can be reasonably high and it is reasonable to conclude that the lung tumours seen in mice are unlikely to be of any relevance for human health. Whether this mechanism could be operative in humans at other sites cannot be excluded. However, it is noted that several cohort and case-control studies of workers exposed to styrene have shown no evidence for a causative association between styrene exposure and cancer in humans at any site and no consistent evidence for styrene-induced toxicity in any organ has emerged from studies of exposed workers.

Reproductive toxicity - A range of epidemiological studies, particularly focusing on developmental effects, has been conducted but most of these

have been too small to be conclusive. Nevertheless, the studies have been generally negative and the available human data certainly provide no reliable evidence for styrene exposure-related adverse effects in relation to spontaneous abortions, congenital abnormalities, birth weight, menstrual disorders, fecundity, male or female fertility or sperm quality within the exposure ranges investigated. Overall, there is no clear evidence of an effect of styrene on human reproduction, but data are too limited to exclude the possibility for effects.

A well-conducted two-generation inhalation study found no effects on fertility and reproductive performance in rats exposed to up to 500 ppm (2165 mg/m<sup>3</sup> ≈ 300 mg/kg/day) styrene.

From the other relevant studies available, there is no convincing evidence that styrene can impair reproductive performance, produce testicular toxicity, sperm abnormalities or adversely affect the reproductive organs. Thus, taken together, the data available indicate that styrene does not have the potential to impair fertility and reproductive performance in animals.

Data from inhalation and oral developmental toxicity studies in a number of species are available, but most are either poorly designed or reported. There are no studies using the dermal route of exposure.

In the rat, inhalation exposure produced no evidence of significant effects on conventional parameters assessed in the foetus at non-maternally toxic exposure concentrations of up to 600 ppm styrene. A slight and reversible developmental delay has been reported in a number of studies at 300 ppm styrene in the absence of overt maternal toxicity. This has been confirmed by a recent, well conducted two-generation study in which, a pattern of developmental delay, including decreased body weights, was evident mainly in the F<sub>2</sub> pups of the high exposure group (500 ppm). Very slight developmental effects (reductions of 6-10% in pup body weight during the pre-weaning period only) were also observed in the F<sub>2</sub> pups of the mid-exposure group (150 ppm). It is noted that, in contrast to previous investigations, in this OECD- and GLP-compliant study the delay in pup development was seen in the presence of some maternal toxicity (slight reductions in body weight at 150 and 500 ppm and degeneration of the nasal olfactory epithelium at 500 ppm). No specific developmental neurotoxicity was seen in this study up to the highest tested concentration of 500 ppm.

Only studies in the rat are available using the oral route of exposure; generally, no significant effects on any of the conventional parameters assessed in the foetus were seen at dose levels up to 300 mg/kg/day, at which maternal toxicity was observed.

Overall, it can be concluded that styrene produces a slight developmental delay in animals at exposure levels (≥ 150 ppm ≈ 90 mg/kg/day), which are slightly toxic to the dams. However, no developmental effects have been observed at 50 ppm.

Taking into account all of the available information, it suggested that 50 ppm is taken forward to the risk characterisation as the NOAEC for potential effects of styrene on development. However, it should be emphasised that the mild

nature of the effects observed and the presence of maternal toxicity should be taken into account when evaluating the adequacy of the resultant Margins of Safety.