Comparison of genotoxic potency of styrene 7,8-oxide with γ radiation and human cancer risk estimation of styrene using the rad-equivalence approach

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Styrene is suspected to cause lympho-hematopoietic malignancies through the formation of styrene 7,8-oxide. However, we are still unable to calculate the cancer risk for workers exposed to styrene using epidemiological data. The aims of this study were to determine the blood dose after styrene exposure and to compare the genotoxic potency of styrene 7,8-oxide and γ radiation in order to calculate the cancer risk by means of the rad-equivalence approach. Leucocytes of 20 individuals were exposed to 0, 0.1, 0.2 or 0.3 mM styrene 7,8-oxide (1 h) or 1, 2 or 3 gray (= 100, 200, 300 rad) γ radiation. Genotoxicity was evaluated with the cytokinesis-block micronucleus assay. Comparison of the two slopes of the regression lines between micronuclei and dose revealed a genotoxic potency for styrene 7,8-oxide of 3.2 rad/mM, corresponding with a median value derived from mutagenicity studies (1, 37, 208 rad/mM). At exposure levels of 1 ppm styrene, a blood styrene 7,8-oxide concentration between $0.03 \times 10^{-6}$ and $0.42 \times 10^{-6}$ mM is to be expected using data of toxicokinetic models and human exposure studies. With the cancer risk per unit dose of γ radiation as benchmark, we calculated a lifetime risk of acquiring a fatal lympho-hematopoietic cancer of 0.17 in 10^4 workers (between $0.037 \times 10^{-5}$ and $5.0 \times 10^{-7}$) exposed to 20 ppm styrene during 40 years.

Introduction

Styrene is suspected to cause lympho-hematopoietic malignancies (1,2). However, we are still unable to calculate the cancer risk for workers exposed to styrene using human epidemiological data (3). The rad-equivalence method has been suggested as a basis for cancer risk estimation when no appropriate human cancer data are available to calculate cancer risk associated with occupational exposure (4–8). Cancer risks of some genotoxic chemicals have been calculated by the rad-equivalence method and have been shown to give results in accordance with cancer test data (6).

The rad-equivalence method starts from an exact determination of the target dose associated with an occupational exposure to the carcinogen (6). The target dose of the carcinogen in the immediate surrounding of DNA needs then to be expressed as an equivalent radiation dose. This conversion step requires an accurate determination of the relative genotoxic potency ($Q$) i.e. the dose of γ radiation that produces the same genotoxic response as a certain dose of the chemical (8–10). The cancer risk can then be calculated since the cancer risk per unit γ radiation has been well described (11,12). The lifetime risk for fatal lympho-hematopoietic cancer ($Rc$) associated with ionizing radiation has been estimated to be $\sim 0.04$ excess deaths per 10^3 persons exposed to 0.01 gray ($= 1$ rad) (11). Multiplying $Rc$ with $Q$ of the carcinogen under study and with the dose of the carcinogen yields the estimated cancer risk ($P$).

One of the major assumptions of the rad-equivalence model is that risks are estimated at doses or dose rates so low that the promoting and modifying influences of the agent under consideration are negligible. Under these conditions, it seems permissible to assume that other promoting and modifying factors affect an initiated cell to the same extent, irrespective of whether initiation was induced by the chemical or γ radiation (8). In order to apply the approach to styrene, we need to determine the target and/or blood dose associated with an exposure to styrene and a value of $Q$ for styrene 7,8-oxide.

Styrene (IARC, 2B: possibly carcinogenic to humans) is metabolized to the electrophile styrene 7,8-oxide, which is considered to be the ultimate mutagen (International Agency for Research on Cancer, 2A: probably carcinogenic to humans) capable of binding to DNA and hemoglobin (Hb) (3). Laboratory and human studies have shown that styrene exposure can cause DNA adducts of styrene 7,8-oxide, e.g. N-7-adducts on guanine (13–15). Styrene 7,8-oxide DNA adducts can induce gene mutations including point mutations and chromosome mutations (14,16–19).

The dose is defined as the integral over time of the dose rate of the initiating agent (20,21). For chemicals, the dose rate (mM/h) is equal to the concentration (in mM) of the mutagenic compound or the metabolite in target tissues. The dose of styrene 7,8-oxide in blood is considered as a surrogate for the target dose, i.e. the dose of the ultimate mutagen in the immediate vicinity of DNA (4–20). The styrene 7,8-oxide concentration can either be measured in blood directly or derived from adducts to Hb (4,22–25). An alternative method is to estimate blood styrene 7,8-oxide concentration by means of physiologically based toxicokinetic models (26–28). The model of Csanády et al. (27) also predict styrene 7,8-oxide adducts levels to Hb and DNA resulting from styrene or styrene 7,8-oxide exposure and show a good agreement with experimental data (15,29).

So far, four in vitro studies allow a comparison of the genotoxic potency of styrene 7,8-oxide with that of γ radiation. Nishi et al. (30) compared 6-thioguanine-resistant mutations in Chinese hamster V79 cells induced by styrene 7,8-oxide and γ radiation. The induction of 6-thioguanine-resistant mutations in Chinese hamster V79 cells has also been investigated in two separate but comparable experiments of Janssen and Ramel (31), cells exposed to X-rays, and Beije and Janssen (32), cells exposed to styrene 7,8-oxide. Hussain et al. (unpublished data) compared mutation frequencies of styrene 7,8-oxide and γ 

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radiation by studying forward mutation to streptomycin non-dependence in a streptomycin-dependent Escherichia coli SD4 strain. The aims of the current investigation were to determine the blood dose associated with an occupational exposure to styrene and an accurate determination of $Q$ for styrene 7,8-oxide in order to calculate the lifetime risk for fatal lympho-hematopoietic cancer of workers exposed to styrene by means of the rad-equivalence approach.

Materials and methods

Comparison of genotoxic potency of styrene 7,8-oxide and $\gamma$ radiation

For the estimation of the $Q$ factor of styrene 7,8-oxide, we took into account available mutagenicity data. Nishi et al. (30) compared 6-thioguanine-resistant mutation in Chinese hamster V79 cells induced by 0.88–0.8 mM styrene 7,8-oxide (3-h exposure) and 0.5–10 gray ($\approx$ 50–1000 rad) $\gamma$ radiation. Jenssen and Ramel (31) and Beije and Jenssen (32) studied 6-thioguanine-resistant mutations in Chinese hamster V79 cells. Cells were exposed to respectively X-rays (0–400 rad) (31) and to 0 (4 h), 0.52 and 1.04 mM styrene 7,8-oxide during 2 h (D. Jenssen, personal communication). Hussain et al. (unpublished data) compared forward mutations to streptomycin in E. coli SD4 cells exposed to styrene 7,8-oxide (0.25–1000 mM/h) and 0 to 1.6 gray (720 h) radiation. We applied the rad-equivalence approach in order to calculate the lifetime risk for fatal lympho-hematopoietic cancer of workers exposed to styrene by means of the rad-equivalence approach.

Data are presented as frequencies of micronucleated binucleates per 1000 binucleates. Additional, we carried out a comparative in vitro genotoxicity study (single-cell gel electrophoresis and micronuclei assays) with styrene 7,8-oxide and $\gamma$ radiation (33). In this paper, we only refer to the data of micronucleus frequencies in binucleated cells, since the single-cell gel electrophoresis assay is rather considered to measure repairable DNA damage and not fixed mutations (34).

In brief, after informed consent, 20 non-smoking students [15 females, five males, mean ($\pm SD$) age 22.0 ($\pm 2.0$)] volunteered to participate. Each participant filled in a questionnaire on alcohol and consumer habits, medical and family history, hobbies, etc. The study protocol was approved by the Bioethics committee of the Katholieke Universiteit Leuven. Blood samples were taken by venipuncture in heparinized Vacutainers. Mononuclear leukocytes were isolated from samples by Ficoll–Paque density gradient (34). Cultures of isolated leukocytes (15% fetal calf serum, 2% phytohemagglutinin in Ham’s F10) were immediately prepared and incubated at 37°C. After 24 h, the isolated leukocytes were suspended in the medium containing final concentrations of 0.1, 0.2 or 0.3 mM styrene 7,8-oxide (Chemical Abstracts Service number: 96-52-2) and $\gamma$ radiation (0.022/rad) and styrene 7,8-oxide (0.48/mMh) in the studies of Jenssen and Ramel (31) and Beije and Jenssen (32), respectively. The study of Hussain et al. (personal communication, unpublished data) showed that

\[Q_\text{mncb: frequencies of micronucleated binucleates per 1000 binucleates.}\]

Repeted measures analysis of variance (ANOVA) was carried out to assess the genotoxicity at different doses. Per agent, a linear regression model was built with the MNCB as dependent parameter and dose as independent parameter. Linear regression analyses were carried out with Statistica package, version 6.1 (Statsoft, Tulsa OK, USA). The level of significance was set at $P < 0.05$ for all statistical analyses.

Results

Comparison of genotoxic potency of styrene 7,8-oxide and $\gamma$ radiation

In this study, we evaluated the micronuclear frequencies in lymphocytes induced by styrene 7,8-oxide and $\gamma$ radiation. Per agent, three doses have been tested, together with a common control. Results are represented in Table I. Repeated Measures ANOVA revealed a significant influence of dose on MNCB in the cells treated with styrene 7,8-oxide and $\gamma$ radiation (both $P < 0.001$). $Q$ is defined as the response increment per dose unit $\gamma$ radiation and can be deduced from the slopes of the regression line between MNCB and the respective dose per agent (8). Figures 1 and 2 represent scatter plots with the linear regression lines of MNCB for styrene 7,8-oxide and $\gamma$ radiation, respectively. The ratio of the slopes of styrene 7,8-oxide (24.21/mMh) and $\gamma$ radiation (0.66/rad), respectively, yielded a value for $Q$ of 37 rad/mMh.

This estimation for $Q$ of styrene 7,8-oxide is in line with the mutagenicity data of Nishi et al. (30). They found an increase in the number of 6-thioguanine-resistant mutants per $10^6$ viable cells over the control values of 0.22 per $\mu$g/ml and 24 per gram for styrene 7,8-oxide and $\gamma$ radiation, respectively. These values correspond to a genotoxicity potency factor $Q$ of 37 rad/mMh (0.0092 mg/gliter = 110.14 rad/mMol/liter = 37 rad/mMh). In contrast, a lower $Q$ factor of 22 rad/mMh was found by comparing the regression slopes of the dose-response curves for the induction of 6-thioguanine-resistant mutations by X-rays (0.022/rad) and styrene 7,8-oxide (0.48/mMh) in the studies of Jenssen and Ramel (31) and Beije and Jenssen (32), respectively (D. Jenssen, personal communication). The study of Hussain et al. (personal communication, unpublished data) showed that

Statistics

\[Q_{\text{mncb}}: \text{frequencies of micronucleated binucleates per 1000 binucleates.}\]

Dose MNCB (mean $\pm SD$)$^b$

<table>
<thead>
<tr>
<th>Dose</th>
<th>MNCB (mean $\pm SD$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.4 $\pm$ 2.1</td>
</tr>
<tr>
<td>Styrene 7,8-oxide</td>
<td>8.0 $\pm$ 3.1</td>
</tr>
<tr>
<td>0.20 mMh</td>
<td>8.7 $\pm$ 5.3</td>
</tr>
<tr>
<td>0.30 mMh</td>
<td>14.2 $\pm$ 8.8</td>
</tr>
<tr>
<td>$\gamma$ radiation</td>
<td>54.8 $\pm$ 24.3</td>
</tr>
<tr>
<td>1 gray (=100 rad)</td>
<td>135.1 $\pm$ 50.0</td>
</tr>
<tr>
<td>2 gray (=200 rad)</td>
<td>200.5 $\pm$ 88.2</td>
</tr>
</tbody>
</table>

$^a$Repeated measures ANOVA: significant influence ($P < 0.05$) of dose on MNCB in the styrene 7,8-oxide.

$^b$MNCB: frequencies of micronucleated binucleates per 1000 binucleates.

Cancer risk estimation

We applied the rad-equivalence approach in order to calculate the lifetime risk for fatal lympho-hematopoietic cancer of styrene-exposed workers (8 h/day, 5 days/week) (4). From $D_a$, an annual dose received during working hours ($D_{ann}$) was calculated taking into account 45 workweeks per year and 40 working hours per workweek (see Appendix). Cancer risk per ppm per year ($P$) was then calculated by multiplying $D_{ann}$ with the estimate of the lympho-hematopoietic cancer mortality risk for $\gamma$ radiation ($= 0.04 \times 10^{-4}$/rad) and the genotoxicity potency factor $Q$ obtained from the in vitro study and literature (11). A detailed outline of the procedure, a description of the applied formula’s and values can be found in the appendix.

\[Q_{\text{mncb: frequencies of micronucleated binucleates per 1000 binucleates.}}\]
Increased during exposure and reached an average maximum physical exercise. Styrene 7,8-oxide blood concentration exposed to 50 ppm during 2 h while carrying out a light physical exercise. Styrene 7,8-oxide blood concentration in blood of four individuals exposed to 50 ppm during 2 h while carrying out a light physical exercise. Styrene 7,8-oxide blood concentration in blood of four individuals exposed to 50 ppm during 2 h while carrying out a light

Exposure studies in volunteers. Results of exposure studies in volunteers, data from biomonitoring studies in workers or the toxicokinetic model (Table II).

Blood concentration of styrene 7,8-oxide after 1 ppm styrene exposure in occupational settings has been determined using an indirect method for styrene 7,8-oxide determination as in Wigaeus et al. (39) exposed eight male subjects for 2 h to ~70 ppm styrene during light physical exercise. Styrene 7,8-oxide levels of 50 ± 30 × 10⁻⁶ mM in venous blood were found in samples collected after 5–30 min of exposure to 70 ppm styrene. The styrene 7,8-oxide concentration (0.71 × 10⁻⁶ mM) corresponding to 1 ppm styrene was calculated by dividing 50 ± 30 × 10⁻⁶ mM by 70. Although styrene 7,8-oxide was determined in this study shortly after the start of the exposure, the styrene 7,8-oxide concentrations proved 5.5 times higher than in Johanson et al. (24), possibly because of differences in the analytical methods. Johanson et al. (24) determined styrene 7,8-oxide directly after extraction with n-hexane, while Wigaeus et al. (39) converted extracted styrene 7,8-oxide to styrene glycol before analysis.

Field studies in workers exposed to styrene. Korn et al. (22) carried out a field study in 13 workers exposed to styrene concentrations between 10 and 73 ppm. Styrene 7,8-oxide blood concentrations ranged between 0.9 and 4.1 µg/literblood (equivalent to 7.5 and 34.1 × 10⁻⁶ mM). The authors used a regression model to estimate a steady-state level of 1 µg/L styrene 7,8-oxide/literblood (=8.3 × 10⁻⁶ mM) associated with an exposure to 20 ppm styrene. Christakopoulos et al. (40) studied 17 laminators (16 referents) exposed to ~75 ppm (0–130 ppm) based on mandelic acid concentration in urine and styrene glycol in blood. An average styrene 7,8-oxide level of 90 × 10⁻⁶ mM (range 40–130 × 10⁻⁶ mM) was measured in blood from seven workers.

Dividing the styrene 7,8-oxide concentrations of both studies (8.3 × 10⁻⁶ mM and 90 × 10⁻⁶ mM) by the respective average styrene exposure (20 and 75 ppm) gives us estimated styrene 7,8-oxide concentrations for 1 ppm styrene of 0.42 × 10⁻⁶ mM and 1.20 × 10⁻⁶ mM, respectively. Christakopoulos et al. (40) used also an indirect method for styrene 7,8-oxide determination as in Wigaeus et al. (39) and found a three times higher value as compared with Korn et al. (22). Torniero-Velez et al. (42) measured styrene and styrene 7,8-oxide directly in pentane extracts of blood from 35 reinforced plastics workers exposed to 4.7–97 ppm styrene. The authors predicted a much lower end of shift concentration of 0.2 µg/literblood (equivalent to 1.7 × 10⁻⁶ mM) for a styrene exposure of 50 ppm. At exposure levels of 1 ppm styrene, a corresponding styrene 7,8-oxide blood concentration of 0.03 × 10⁻⁶ mM is to be expected.

Christakopoulos et al. (40) also measured Hb adduct levels. Hb adduct levels represent a measure of biologically effective dose and are considered to provide good estimates of target dose (4). Based on the slope of the regression equation, we can estimate that exposure to 1 ppm styrene would lead to an increased Hb adduct level of 0.21 pmol/g Hb. In a previous study, we determined N-terminal valine adducts in 44 workers (44 referents) exposed to styrene at exposure levels ranging between 0–37 ppm (mean 10 ppm) (41). We found lower increases of Hb adducts (0.10 pmol/g Hb per ppm styrene).

From this, an estimate of blood styrene 7,8-oxide equal to 0.03 × 10⁻⁶ mM was obtained as explained in the methods and as illustrated in the Appendix. The formation rate constant of phenylhydroxyethyl adduct to Hb was set at 9.5 × 10⁻⁶ literblood/h/g Hb (27). In both studies, styrene air concentrations

1 mMh styrene 7,8-oxide gave 4.2 mutants per 10⁸ survivors in E. coli SD4 (slope regression equation = 3.291/mMh) and 10 gray (=1000 rad) gave 12 mutants per 10⁸ survivors (slope regression equation = 0.01583/rad). The respective Q value for styrene 7,8-oxide was 208 rad/mMh. A median value for Q of 37 rad/mMh has been used in the cancer risk calculation of the appendix.

Determination of blood dose

Blood concentration of styrene 7,8-oxide after 1 ppm styrene exposure in occupational settings has been determined using results of exposure studies in volunteers, data from biomonitoring studies in workers or the toxicokinetic model (Table II).

Exposure studies in volunteers. Johanson et al. (24) measured styrene 7,8-oxide concentration in blood of four individuals exposed to 50 ppm during 2 h while carrying out a light physical exercise. Styrene 7,8-oxide blood concentration increased during exposure and reached an average maximum of 6.7 × 10⁻⁶ mM 2 h after the start of the exposure, where after styrene 7,8-oxide concentration gradually decreased to pre-exposure levels. We used this value as an estimate of the average styrene 7,8-oxide level because the time course of blood styrene 7,8-oxide in our toxicokinetic model simulations gave styrene 7,8-oxide levels after 2 h to be approximately equal to the average styrene 7,8-oxide concentration during a typical 8 h exposure period (Figure 3). This indicates that the timing of blood sampling is important when studying concentrations in blood. Dividing 6.7 × 10⁻⁶ mM by 50 (ppm) yields an average blood styrene 7,8-oxide concentration of 0.13 × 10⁻⁶ mM corresponding to 1 ppm styrene exposure (Table II).

Wigaeus et al. (39) exposed eight male subjects for 2 h to ~70 ppm styrene during light physical exercise. Styrene 7,8-oxide levels of 50 ± 30 × 10⁻⁶ mM in venous blood were found in samples collected after 5–30 min of exposure to 70 ppm styrene. The styrene 7,8-oxide concentration (0.71 × 10⁻⁶ mM) corresponding to 1 ppm styrene was calculated by dividing 50 ± 30 × 10⁻⁶ mM by 70. Although styrene 7,8-oxide was determined in this study shortly after the start of the exposure, the styrene 7,8-oxide concentrations proved 5.5 times higher than in Johanson et al. (24), possibly because of differences in the analytical methods. Johanson et al. (24) determined styrene 7,8-oxide directly after extraction with n-hexane, while Wigaeus et al. (39) converted extracted styrene 7,8-oxide to styrene glycol before analysis.

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were inferred from data on glycol concentrations in blood (40) and mandelic acid concentrations in urine (40,41).

Toxicokinetic modeling. The model of Csánády et al. (27) was used to predict $D_h$ after occupational exposure to 1 ppm styrene. Modeling was done using the human physiological, physicochemical and biochemical parameters and absorption rate constants as described in Csánády et al. (27). Occupational exposure was programmed as follows: styrene exposure during 8 h/day, 5 days/week. The formation rate constant in humans of styrene 7,8-oxide adducts to Hb was set at $9.5 \times 10^{-6}$ liter blood/h (27,28). The venous blood styrene 7,8-oxide concentration during occupational exposure to 1 ppm was on average $0.23 \times 10^{-6}$ mM.

Tornero-Velez and Rappaport (28) also carried out simulations with a modified model of Csánády et al. (26) to assess relative contributions of styrene 7,8-oxide derived from direct inhalation and from styrene metabolism to the blood dose in humans. They postulated that styrene 7,8-oxide enters the blood from hepatic metabolism of styrene at a lower rate than from direct absorption following inhalation. As a consequence, they calculated a much lower styrene 7,8-oxide blood concentration after exposure to 1 ppm styrene of 0.01 μg/liter blood (equivalent to $0.08 \times 10^{-6}$ mM) (28) compared to the results of the Csánády model (27).

Cancer risk estimate associated with occupational exposure to 1 ppm styrene

The lympho-hematopoietic cancer mortality risk for γ radiation has been estimated at $0.04 \times 10^{-4}$ rad (11). In Table III, an overview of the cancer risk estimations is given per blood dose received during working hours and $Q$ for styrene 7,8-oxide derived in the present study. We did not take into account blood styrene 7,8-oxide concentrations derived from studies measuring styrene 7,8-oxide indirectly, since there are indications that styrene 7,8-oxide blood concentrations are overestimated. The median corresponding lifetime risk of acquiring a fatal lympho-hematopoietic cancer after exposure to 1 ppm styrene during 1 year is 0.21 in 10$^6$ workers and varies between $0.05 \times 10^{-6}$ (= $0.03 \times 10^{-6}$ $\times 22$ $\times 45$ $\times 40$ $\times 0.04$ $\times 10^{-3}$; $D_h = 0.03 \times 10^{-4}$ mM, $Q = 22$ rad/Mh) and 6.2 $\times 10^{-6}$ (= $0.42 \times 10^{-6}$ $\times 208$ $\times 45$ $\times 40$ $\times 0.04$ $\times 10^{-3}$; $D_h = 0.42 \times 10^{-6}$ = $0.42 \times 10^{-6}$ mM, $Q = 208$ rad/Mh).

Discussion

Cancer risk estimations based on the rad-equivalence method start with the determination of the blood dose in humans exposed to the carcinogen under study (4–8). Our estimations, based on different sources, gave an average styrene 7,8-oxide blood concentration, during exposure to 1 ppm styrene ranging between $0.03 \times 10^{-6}$ and $1.2 \times 10^{-6}$ mM. The upper limit of the range was obtained from studies measuring styrene 7,8-oxide in blood using an indirect analytical method (39,40). Both studies revealed a three times higher blood concentration than observed in studies determining styrene 7,8-oxide directly in blood.
and were considered to overestimate the styrene 7,8-oxide concentration (24). The lower styrene 7,8-oxide concentrations were derived from studies of Tornero-Velez (28,42) and N-terminal valine adduct levels in workers exposed to styrene (40,41). The resulting styrene 7,8-oxide concentrations were a factor 2–10 lower than the styrene 7,8-oxide blood concentration of other studies measuring styrene 7,8-oxide directly in venous blood (22,24).

Tornero-Velez (28,42) postulated that styrene 7,8-oxide enters the blood from hepatic metabolism of styrene at a lower rate than from direct absorption following inhalation. Since styrene 7,8-oxide co-exposure could have varied among workers and within workers over time, styrene 7,8-oxide blood concentrations after exposure to 1 ppm styrene (Table II) could be biased high in some studies, due to unmeasured co-exposures to styrene 7,8-oxide. This could also be important for cancer risk, since direct exposure to styrene 7,8-oxide is likely to be 500–1000 times more potent than styrene at producing styrene 7,8-oxide in blood. However, this could not be assessed since most of the published studies did not carry out styrene 7,8-oxide and styrene measurements in the air.

A second element in the rad-equivalence method is the determination of the dose of a carcinogen that produces the same level of genotoxic response per γ radiation dose. The Q factors obtained from micronucleus and mutagenicity data ranged between 22 and 208 rad/mMh. Bacterial cells seemed more sensitive to styrene 7,8-oxide-induced DNA damage than mammalian cells, as a consequence, the actual Q value lays probably close to the median value of 37 rad/mMh. More studies will be necessary to confirm this. Although available for styrene 7,8-oxide and γ radiation, we did not consider in vitro-induced genotoxicity assessed with single-cell electrophoresis and sister chromatid exchange assays because of mechanistic considerations (30,33). Both parameters are rather markers of exposure than of effect. Interestingly, both studies indicated that styrene 7,8-oxide in the applied dose ranges is able to induce a relative high amount of DNA strand breaks and a high incidence of sister chromatid exchanges but relatively few 6-thioguanine-resistant mutations. In contrast, γ radiation tends to induce 6-thioguanine-resistant mutations much more than sister chromatid exchanges and DNA strand breaks.

According to our calculations, an exposure to 1 ppm styrene for 1 year would lead to a median estimate of 0.21 extra lympho-hematopoietic cancers in 10^6 exposed individuals (range 0.05 × 10^-6 and 6.22 × 10^-6). Taking into account a professional career of 40 years and an exposure to 20 ppm styrene gives a lifetime cancer risk median estimate of 170 lympho-hematopoietic cancers in 10^6 workers (range 37 × 10^-6 and 4970 × 10^-6). Greim (43) estimated cancer risk associated with a 40 years exposure during work time to styrene (20 ppm) to range between 17 and 75 per 10^6 exposed persons. This value is ~2 to 10 times lower than our median lifetime cancer risk estimate. We consider our worst-case cancer risk of 4970 lympho-hematopoietic cancers in 10^6 workers as an overestimation (see also Discussion concerning Q value derived from bacterial cells), since it is not supported by epidemiological research, finding little or no increase of lympho-hematopoietic cancers in workers exposed to styrene (1,44).

Cancer risk assessments always need to deal with assumptions and uncertainties, which induce variations in the final results. The estimates of Greim (43) were based on experimental long-term studies with animals, and consequently need to deal with interspecies differences and extrapolations from high to low dose, of which the uncertainty is basically impos-
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References


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