REVIEW

Is human exposure to styrene a cause of cytogenetic damage? A re-analysis of the available evidence

Stefano Bonassi, Fabio Montanaro, Marcello Ceppi and Angelo Abbondandolo

The possibility that occupational exposure to styrene causes genotoxic effects in humans has been the focus of many biomonitoring studies based on classic cytogenetic biomarkers. Contrasting results have been reported, positive studies being counterbalanced by a number of negative findings. The strength of the conclusions of single studies, either positive or negative, was often weakened by factors such as limited sample size, inadequate exposure assessment, poor epidemiological design, or inappropriate statistical analysis. We have undertaken a meta-analysis of 25 biomonitoring studies of occupational exposure to styrene, in the attempt to discover whether, regardless of the limitations of the individual studies, a general trend could be evinced from a quantitative review of the available evidence on this topic. Essentially, our approach involved a dichotomic classification of all studies according to the median environmental exposure level to styrene, i.e. 125 mg m^{-3} (30 ppm), and a quantitative evaluation of the biological effects comparable among the studies considered, i.e. frequency ratio (*FR*). In order to provide combined estimates of effect across all studies, a weight was attributed to each study depending on its sample variance, and weighted frequency ratios (*wFR*) were calculated for the endpoints considered, i.e. chromosome aberrations (CA), sister-chromatid exchanges (SCE), and micronuclei (MN). A significant increase of the *wFR* was found for CA from the studies performed on workers with 'high level' exposure to styrene (*wFR* = 2.18; 95%.CI = 1.52–3.13), while inconclusive data were obtained for SCE and MN.

Keywords: *styrene, meta-analysis, chromosome aberrations, sister-chromatid exchanges, micronuclei.*

Introduction

Styrene (C.A.S. name: ethenylbenzene; C.A.S. No.: 100-42-5) was first isolated a century and a half ago from storax, an oriental balsam obtained from the trunk of *Liquidambar orientalis* (Windholz and Budavari 1983). Once marginally important in the old pharmacopoeia, styrene has now acquired outstanding prominence among synthetic chemicals of current industrial use.

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The toxicological properties of styrene have been the subject of many studies. The genotoxic profile of styrene includes positive results in a number of different test systems (IARC 1987, Sorsa *et al.* 1993). The genotoxic activity of styrene is dependent on its metabolic activation, and direct genotoxicity of its major metabolite, styrene-7,8-oxide, is well substantiated (Barale 1991). As far as cytogenetic damage is concerned, both styrene and styrene-7,8-oxide have proven capable of inducing this effect in a variety of *in vitro* and *in vivo* systems (Barale 1991). However, reservations have been advanced about the positivity of styrene and styrene-7,8-oxide in *in vivo* rodent assays and about the genotoxicity of styrene in humans (Preston 1990a, b, Scott and Preston 1994). Induction of chromosome aberrations (CA) and sister-chromatid exchanges (SCE) has been observed in human lymphocytes upon addition of styrene to whole blood *in vitro* cultures (Linnainmaa *et al.* 1978a, b, Norppa *et al.* 1980, 1983, Norppa and Vainio 1983, Norppa and Tursi 1984, Jantunen *et al.* 1986, Bonatti *et al.* 1994). This is a relevant point for the following since these endpoints, as well as micronuclei (MN), are currently used as an outcome in human biomonitoring studies. Concern for the consequences of human exposure to styrene stems mainly from the manufacture of fibreglass-reinforced plastic products, which entails the highest potential human exposure to this chemical (Sorsa *et al.* 1991). Increasing knowledge of the potential health hazards associated with styrene exposure has led to a parallel stiffening of pertinent occupational standards in some countries: in 1989 in the US, from a permissible 8-h time-weighted average (TWA) exposure of 100 ppm to 50 ppm (Brenner *et al.* 1991), and in Finland, from 100 ppm to 50 ppm in 1985 and to 20 ppm in 1987 (Sorsa *et al.* 1991).

The first report on a cytogenetic investigation on workers exposed to styrene in a boat factory was published by Meretoja and coworkers (1977). Since then, several similar studies have been published, many of them showing increased cytogenetic effects in exposed persons. In spite of this, consensus on the genotoxicity of styrene to humans has never been reached. A crucial point with population cytogenetic biomonitoring is whether or not a quantitative association between exposure and cytogenetic effects can be demonstrated. Indeed, it has recently been claimed that a dose–effect relationship is not revealed by the available data (*The SIRC Review* 1993, Scott and Preston 1994) and that factors other than exposure to styrene may be responsible for the increased cytogenetic effects observed in some occupational settings (Ratpan *et al.* 1993, Tates *et al.* 1994). Several authors have reviewed the genotoxicity of styrene in the last few years (Norppa *et al.* 1988, Bond 1989, Preston 1990a, b, Barale 1991, ECETOC 1992, Scott and Preston 1994). We will refer to these exhaustive reviews and to the pertinent IARC Monograph (1987) for information on the mutagenicity of styrene and styrene-7,8-oxide. In the present study we have undertaken a meta-analysis of published data from 1977 to the present time in the attempt to evaluate by means of a quantitative approach the weight of evidence in favour of or against (i) an association between human exposure to styrene and cytogenetic effects, and (ii) the presence of higher risks of cytogenetic damage in the groups of workers with the higher exposure to styrene.

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Literature review

In order to set up the database for the meta-analysis, a detailed review of the biomonitoring studies on occupational exposure to styrene was carried out. In the evaluation of this material, we followed some simple rules: (i) only original papers providing quantitative measurements of the exposure were considered; (ii) only findings on cytogenetic effects, i.e. CA, SCE, and MN were examined; (iii) repeated studies on the same group of workers were not included in the database; (iv) groups of workers clearly distinct by exposure level and included in a given study were considered separately; (v) a reference group of unexposed subjects had to be present. Taken as a whole, the data evaluated come from 25 reports and refer to 35 different groups of exposed workers. A summary of these data is shown in Table 1, as is the basic information for each endpoint, i.e. the author and the year of publication, the level of exposure to styrene, the size of the study group, and the extent of the cytogenetic damage. The most intricate aspect in the layout of this table was the comparison of the exposure levels among various studies: some papers reported the concentration of styrene in ambient air; others, the level of its metabolite, i.e. mandelic acid (MA) in urine samples; others again reported both of these values. In addition, different units of measure were used. To reduce the extent of this variability, which prevented an efficient synthesis of the studies analysed, we used a semi-quantitative scale. Exposure reported in the cytogenetic studies was thus classified as of 'low' or 'high' level, by using the value of 125 mg m^{-3} (30 ppm) of air concentration of styrene to discriminate between the two categories. This threshold corresponds to the median value of environmental concentration of styrene in our database. When environmental data were not available or the classification was doubtful (such as when only the exposure range is reported), the threshold of 505 mg $g^{-1}_{creatinine}$ of urinary MA, which corresponds to an environmental exposure of 125 mg m^{-3} of styrene (Scott and Preston 1994), was used. An outline of the main features of the studies included in our review with respect to exposure assessment is reported in Table 2.

The study by Tates *et al.* (1994) was removed from the following steps of the analysis since the authors reported the presence in that occupational environment of 'an exceptionally high exposure to dichloromethane ... which frequently masked exposure to styrene', and admitted their incapability to know whether styrene or dichloromethane induced the high level of chromosome damage observed.

The biological effects of human exposure to styrene have been evaluated through other relevant biomarkers, including Single-Strand Breaks (SSB), cells with high frequencies of SCEs (HFCs), HPRT mutations, styrene–haemoglobin adducts NA–AAF binding (Brenner *et al.* 1991, Mäki-Paakkanen *et al.* 1991, Walles *et al.* 1993, Tates *et al.* 1994). Most of these biomarkers seem to demonstrate a promising capability to discriminate between exposed and reference groups. In particular, the SSB test revealed a clear dose–response relationship in a group of workers of a fibreglass-reinforced plastic industry (Walles *et al.* 1993), while in a multi-endpoint study in a group of workers exposed to styrene and dichloromethane, HFCs turned out to be the most sensitive biomarker (Tates *et al.* 1994). However, because of their small number, these studies were not included in our analysis.

Association between styrene exposure and cytogenetic effects

In order to provide a quantitative estimate of the association between occupational exposure to styrene and cytogenetic effects, we calculated for each study the ratio between the mean frequency of cytogenetic damage in exposed and control subjects. This measure of effect, usually called the frequency ratio (*FR*) (Warner *et al.* 1994), is independent of the original values of the cytogenetic endpoints reported in the original study and is comparable across the studies evaluated. Moreover, this statistic is straightforward, since it expresses the proportional increase of cytogenetic damage in the exposed group compared with the reference group.

The need for a quantitative approach instead of the common tally count, i.e. the crude comparison of positive studies versus negative or inconclusive studies, was emphasized in a classic work by Sander Greenland (1987). This approach attributes a weight to each study which differentiates the contribution of large and small studies.

A general picture of the findings from the 35 groups of workers exposed to styrene selected from the scientific literature is given in Figures 1, 2 and 3. The bars indicate *FR* values for each group for the three cytogenetic endpoints. Whenever an appropriate estimate of the variance in the exposed and control groups was reported 95% confidence intervals of *FR* values were estimated, for descriptive purposes only, assuming that ln *FR* follows a *t* distribution on $(n_{exp} + n_{contr} - 2)$ degrees of freedom. For all three endpoints, in nearly one-third of the original studies the exposed workers had significantly higher levels of chromosome damage, with a more marked evidence for CA, where 9 out of 30 studies reported a frequency of metaphases with chromosome aberrations in the exposed workers more than double when compared with controls.

To provide an overall measure of effect expressing the extent of the cytogenetic damage associated with styrene exposure, we estimated a weighted *FR* (*wFR*) throughout the studies for each of the three markers considered (Kleinbaum *et al.* 1982, DerSimonian and Laird 1986, Greenland 1987, Dickersin and Berlin 1992). No deviation from normality was revealed by the Kolmogorov–Smirnov goodness-of-fit test for any endpoint. Therefore, the calculation of the weighted estimates and their standard errors was carried out through a random-effects regression model, i.e.

$$Y_i = \alpha + \beta_i + \varepsilon_i$$

being Y_i the log of the ratio between the means of the exposed and the controls in each study, i.e. ln *FR*; α the overall effect of the exposure, i.e. ln *wFR*; β_i the random effect. This latter term, which allows the between-studies variability to be taken into account is assumed to be sampled from a normal distribution with mean zero and variance to be estimated from the data.

References	Group	Level of exposure	CA				SCE				MN			
			Exposed /Controls	Cells with CA (%) Exposed	Controls	FR	Exposed /Controls	Mean per cell Exposed	Controls	FR	Exposed /Controls	Cells with MN (‰) Exposed	Controls	FR
Meretoja *et al.*, 1977	a	High	10/5	16.3	1.6	10.2					10/5	8.8	0.8	11.00
Meretoja *et al.*, 1978	b	Low					11/3	5.3	4.4	1.20				
Fleig and Thiess, 1978	c1	Low	5/20	1.6	2.1	0.76								
Fleig and Thiess, 1978	c2	Low	12/20	1.9	2.1	0.91								
Fleig and Thiess, 1978	c3	High	14/20	5.3	2.1	2.52								
Högstedt *et al.*, 1979	d	Low	6/6	6.9	2.5	2.76								
Thiess *et al.*, 1980	e	Low	24/24	1.9	1.5	1.27								
Andersson *et al.*, 1980	f1	High	14/37	7.8	3.2	2.44	6/21	8.7	7.5	1.20				
Andersson *et al.*, 1980	f2	High	22/37	8.0	3.2	2.50	14/21	8.2	7.5	1.10				
Watanabe *et al.*, 1981[a]	g1		9/5	3.3	3.6	0.92	7/8	6.7	7.6	0.88				
Watanabe *et al.*, 1981	g2	High	7/8	3.6	2.9	1.24	9/5	7.8	7.6	1.03				
Högstedt *et al.*, 1983	h	Low									38/20	5.9	3.6	1.64
Watanabe *et al.*, 1983	i	High	18/6	1.1	1.1	1.05	18/6	8.9	8.5	1.05				
Camurri *et al.*, 1983	j	High	25/21	34.5	7.1	4.89	22/22	14.0	10.8	1.29				
Hansteen *et al.*, 1984	k	Low	18/9	1.2	1.7	0.71	18/9	6.6	6.5	1.02				
Nordenson and Beckman, 1984	l	Low	15/13	2.8	2.7	1.03					12/12	3.5	0.8	4.38
Camurri *et al.*, 1984	m	High	17/9	25.4	6.4	3.94	13/8	12.3	9.4	1.31				
Pohlová and Srám, 1985[a]	n1		36/19	1.4	1.3	1.11								
Pohlová and Srám, 1985	n2	High	22/22	2.3	1.6	1.40								
Mäki-Paakkanen, 1987	o	Low	21/21	3.0	3.7	0.81	21/21	7.6	7.4	1.03	21/21	15.0	16.0	0.94
Jablonická *et al.*, 1988	p	High	11/11	1.3	1.4	0.94								
Forni *et al.*, 1988[a]	q1		32/32	2.3	1.6	1.44								
Forni *et al.*, 1988[a]	q2		8/8	2.5	1.5	1.67								
Hagmar *et al.*, 1989	r	Low	11/14	1.2	1.5	0.80					20/22	4.3	4.4	0.98
Kelsey *et al.*, 1990	s	High					20/20	6.7	6.6	1.01				
Brenner *et al.*, 1991	t1	Low					6/9	9.4	10.1	0.93	6/9	10.0	6.5	1.54
Brenner *et al.*, 1991	t2	High					4/9	10	10.1	0.99	4/9	10.8	6.5	1.66
Sorsa *et al.*, 1991	u1	Low	25/54	1.8	1.6	1.12	19/31	7.4	7.0	1.06	11/37	7.3	8.0	0.91
Sorsa *et al.*, 1991	u2	High	50/54	1.9	1.6	1.13	25/31	7.5	7.0	1.07	28/37	6.5	8.0	0.81
Mäki-Paakkanen *et al.*, 1991	v	High	17/17	3.0	3.1	0.97	17/17	11.4	12.4	0.92	17/7	14.0	12.0	1.17
Tomanin *et al.*, 1992	w1	Low	7/7	1.6	1.4	1.10					7/7	8.7	10.2	0.86
Tomanin *et al.*, 1992	w2	High	11/11	3.8	0.8	4.67					10/10	12.6	8.5	1.49
Tates *et al.*, 1994[a]	x	Low	46/23	2.0	0.4	5.00	46/23	10.2	5.6	1.82	46/23	35.1	14.3	2.46
Artuso *et al.*, 1995	y1	Low	23/51	2.8	2.1	1.30	22/34	4.0	3.3	1.21				
Artuso *et al.*, 1995	y2	High	23/51	4.0	2.1	1.88	23/34	3.5	3.3	1.07				

Table 1. Chromosome aberration, sister-chromatid exchanges and micronuclei in peripheral blood lymphocytes of 35 groups of workers exposed to styrene.

[a] Not included in the meta-analysis.

Finally, ε_i is the sampling error in estimating the effect of the exposure occurring in each study. It is assumed to be independent normal with mean zero and variance:

$$\mathrm{Var}(\varepsilon_i) = \mathrm{Var}(me_i)/(me_i)^2 + \mathrm{Var}(mc_i)/(mc_i)^2$$

where me_i and mc_i are the mean frequency of the cytogenetic endpoint in the exposed and control group, respectively, and $\mathrm{Var}(me_i)$ and $\mathrm{Var}(mc_i)$ the corresponding variance (DerSimonian and Laird 1986). $\mathrm{Var}(\varepsilon_i)$ is an estimate of the within-studies variability, and its inverse represents the weight of each study. When the variance of the mean was not reported in the original study (10 groups out of 54 for all the endpoints, i.e. 19%), we surrogated the missing value with the median of the weights computed for that endpoint. Approximate 95% confidence limits of *wFR* were calculated using:

$$e^{\ln(wFR) \pm 1.96 * SE(\ln(wFR))}$$

Data have been fitted to the model using the procedure META of the Epilog Plus statistical package (Epicenter Software 1993). An important feature of these meta-analytic studies based on weighted means is the assumption of homogeneity.

	No. of studies	
Subjects (exposed and controls)		**Mean (min.-max.)**
CA	30	38.8 (12–104)
SCE	19	34.4 (13–69)
MN	13	34.5 (13–69)
Years of exposure	25	9.3 (3.2–21.6)
		Median[a] (min.-max.)
Styrene (mg m^{-3})	24	125.6 (2–1204)
MA (mg g^{-1} $_{creatinine}$)	14	479.5 (65–725)
mg l^{-1} urine	10	333.4 (29.5–1430.1)

Table 2. Main features of the studies on styrene workers.

[a] Studies providing exposure range only were not considered.

This states that the studies evaluated estimate the same effect, and that differences between the estimates are due to random error (Greenland 1987).

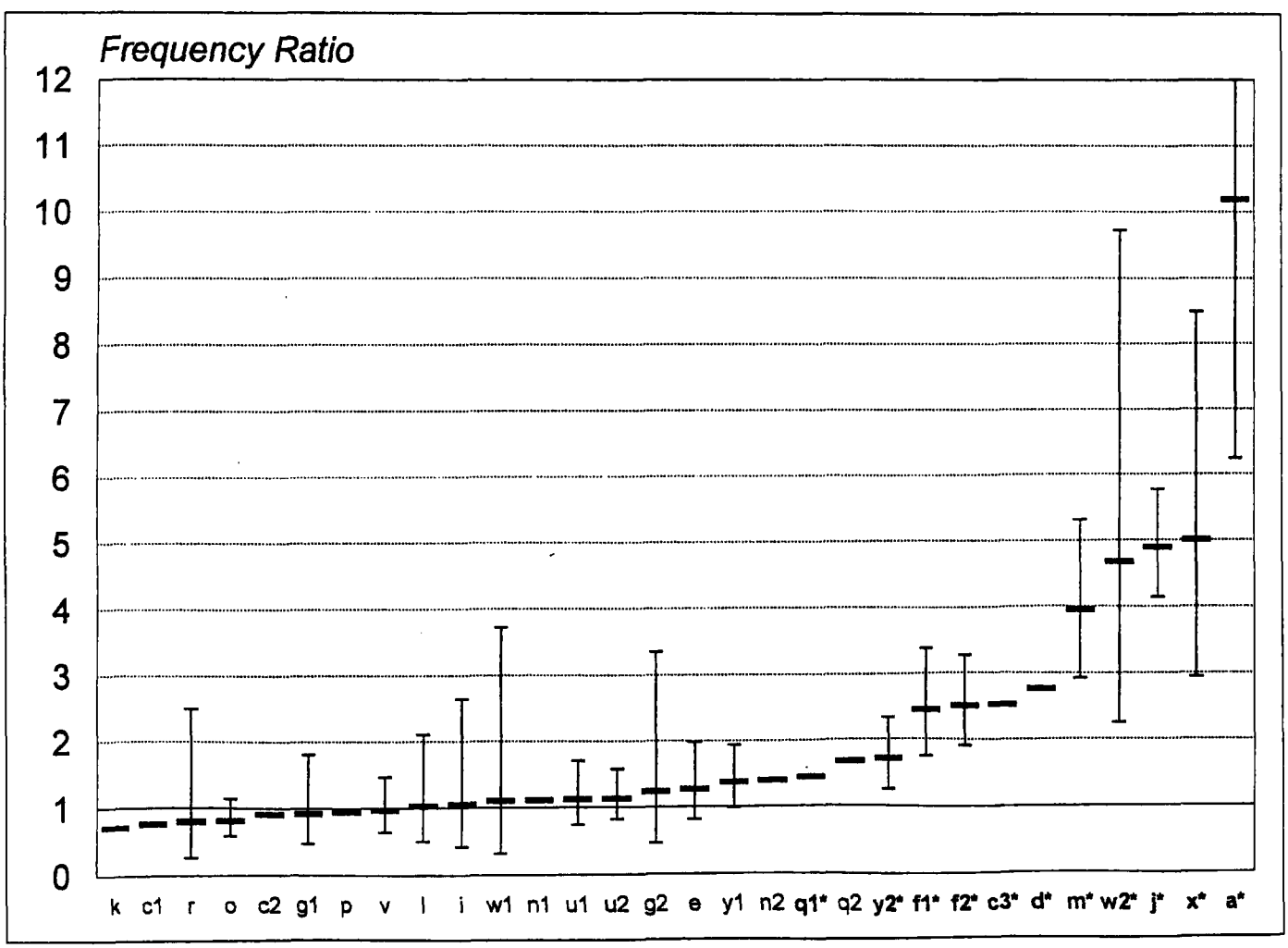

Figure 1. Frequency ratio (exposed/controls) of chromosomal aberrations in 30 studies of styrene workers. 95% confidence intervals are shown where available. Statistically significant results in the original report are shown in bold type and with an asterisk.

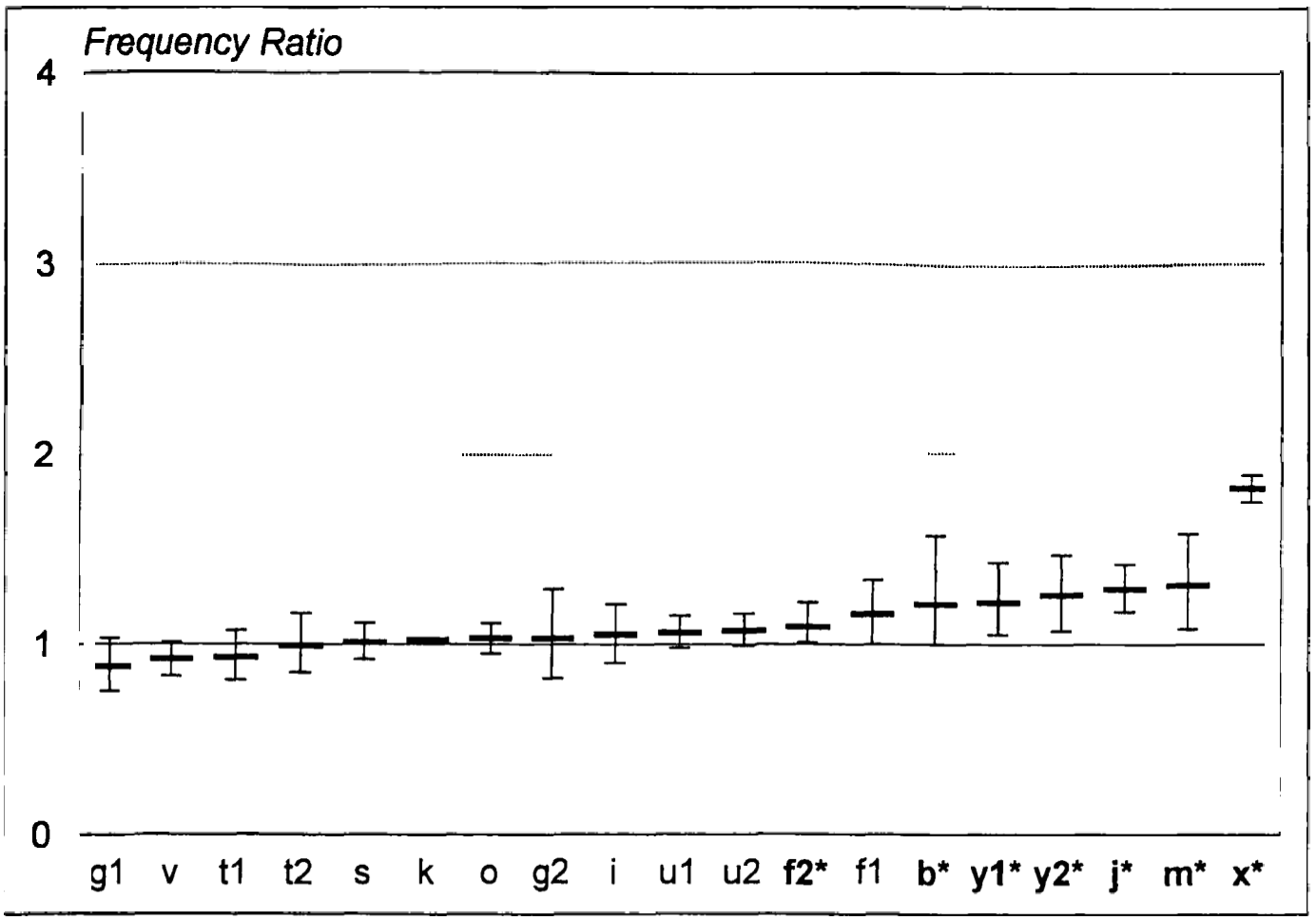

Figure 2. Frequency ratio (exposed/controls) of sister-chromatid exchanges in 19 studies of styrene workers. 95% confidence intervals are shown where available. Statistically significant results in the original report are shown in bold type and with an asterisk.

In our study an overall estimate of the effect of styrene exposure on each cytogenetic endpoint was not provided, since the effects estimated varied too widely. This heterogeneity was greatly reduced after breaking down the whole dataset by level of exposure, especially for CA, and therefore, the evaluation of the weighted estimates of effect was carried out separately in the studies classified at 'low' or 'high' level of exposure to styrene.

Four groups of workers in the CA dataset, i.e. g1, n1, q1, q2, and one in the SCE dataset, i.e. g1, were removed from the analysis at this point, since no quantitative data on styrene exposure were available.

As far as we know, a quality scoring system on human cytogenetic studies has not yet been formally assessed: we therefore deemed unsuitable the inclusion in the analysis of any measure of study quality.

Dose–effect relationship: general considerations and quantitative approach

The presence of a dose-related effect, paramount in a causality-assessing process, has been evaluated at the group level only in a few reports.

A dose-related increase of *FR* was observed in four CA studies out of eight in which groups of workers at different levels of exposure were considered (Fleig and Thiess 1978, Pohlová and Šrám 1985, Tomanin *et al.* 1992, Artuso *et al.* 1995, versus Andersson *et al.* 1980, Watanabe *et al.* 1981, Forni *et al.* 1988, Sorsa *et al.* 1991). None of the five studies in which SCE were evaluated showed an increasing trend (Andersson *et al.* 1980, Watanabe *et al.* 1981, Brenner *et al.* 1991, Sorsa *et al.* 1991, Artuso *et al.* 1995), although a further study by Yager *et al.*, (1993), not included in this re-analysis for the lack of an adequate reference group, showed an exposure-related increase of mean SCE in a group of 48 reinforced-plastic boat workers. Finally, of the three studies considering the presence of a dose-related effect of styrene on MN occurrence, one study was positive (Tomanin *et al.* 1992), one negative (Sorsa *et al.* 1991) and one inconclusive (Brenner *et al.* 1991).

Any comment on these findings should take into account some remarks concerning the quality of the studies reviewed. Firstly, the size of most studies was inadequate. The consequent low statistical power is a major problem, especially for endpoints like SCE, where the effect of exposure is smaller, i.e. less than 10%. The importance of this criticism is strengthened by the observation that in eight out

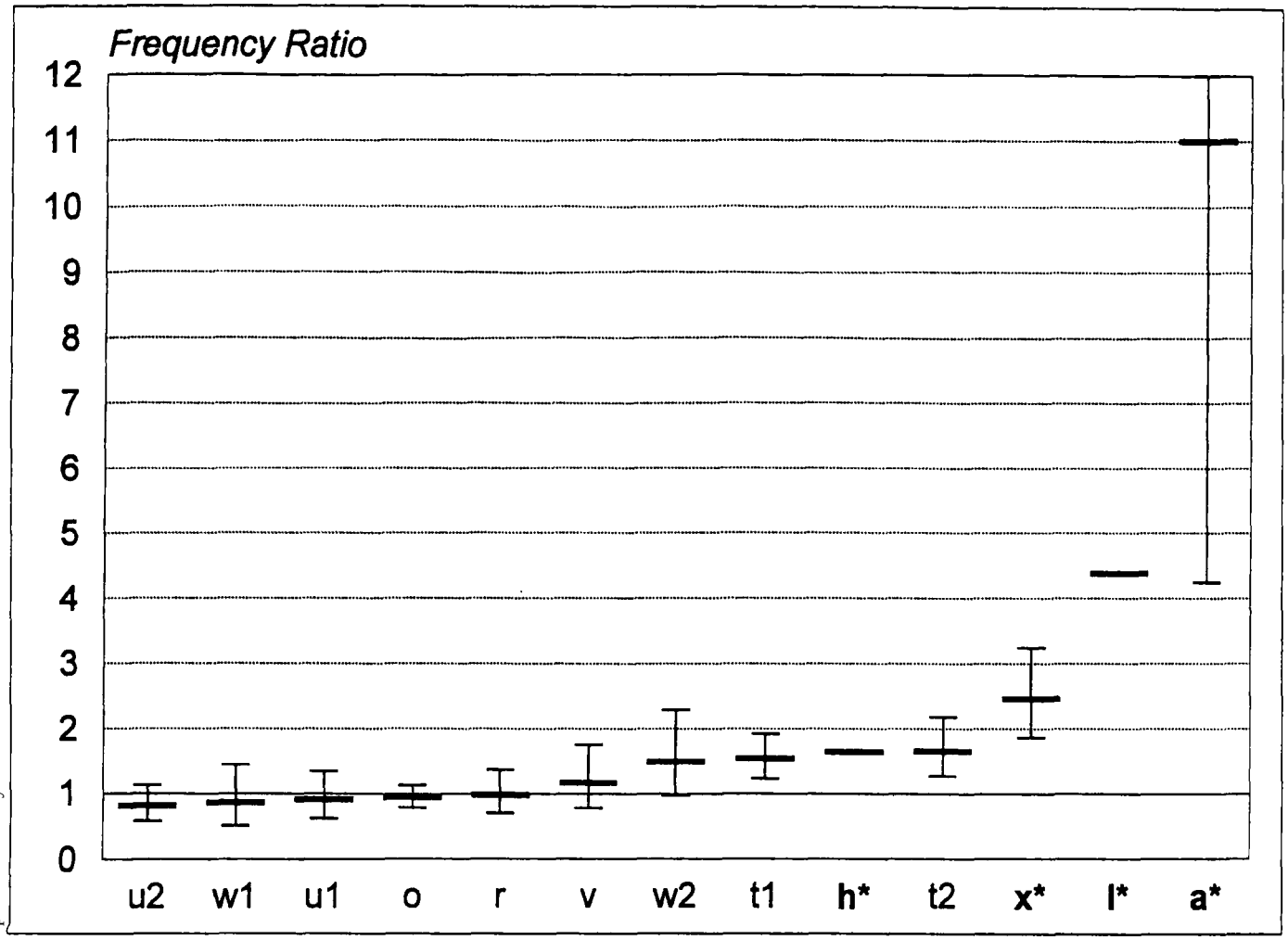

Figure 3. Frequency ratio (exposed/controls) of micronuclei in 13 studies of styrene workers. 95% confidence intervals are shown where available. Statistically significant results in the original report are shown in bold type and with an asterisk.

of the 12 studies evaluated as negative, an SCE increase in styrene-exposed workers was found, albeit too small to reach statistical significance. A further consideration is that most studies did not take into account the effect of confounding factors. This incomplete statistical evaluation of data could at times be seriously misleading due to the relevant difference between crude and adjusted risk estimates in the presence of actual confounding. The short duration of occupational exposure to styrene in most of the studies reviewed should also be taken into account; for instance in our review, only seven study groups reported a mean exposure longer than 10 years. This need is underscored by the findings of Forni *et al.* (1988), who found higher levels of CA in workers with low current exposure but that had experienced long-term exposure in the past with respect to workers with high levels of current exposure but low cumulative exposure. One final remark concerns the non-uniform dosimetric assessment of the exposure.

Endpoint	Level of exposure to styrene	No. of studies	Range of *FR* values	*wFR*	95% CI
CA	Low	11	(0.71–1.30)	1.07	(0.84–1.36)
	High	14	(0.94–10.2)	2.18	(1.52–3.13)
SCE	Low	6	(0.93–1.21)	1.04	(0.99–1.09)
	High	11	(0.92–1.31)	1.08	(1.00–1.15)
MN	Low	7	(0.86–4.38)	1.35	(0.91–1.99)
	High	5	(0.81–11.00)	1.50	(0.96–2.36)

Table 3. Evaluation of the effect of styrene exposure by cytogenetic endpoint and level of exposure.[a]

[a] Low level: 0–125 mg m^{-3} (or 0–30 ppm) of styrene concentration in air. High level: > 125 mg m^{-3} (or > 30 ppm) of styrene concentration in air.

All the limitations in the study design are likely to make a dose–effect relationship, if existing, difficult to demonstrate. In addition, a major intrinsic difficulty is that the broad differences observed among studies in the measurement of styrene exposure produce background noise that may well be high enough to hide a trend.

In this re-analysis, to reduce the extent of the above mentioned sources of variability and the extent of the heterogeneity as well, we classified the exposure to styrene as low or high, since

the use of this simple scale is likely to limit exposure misclassification. The results are reported in Table 3. A clear increase of *wFR* for CA was found in the 'high' level, but not in the 'low' level group, thus corroborating the existence of a dose-related effect due to the exposure to styrene. The analysis of studies on SCE and MN did not show similar evidence of exposure-related effect, although a higher *wFR* was estimated from studies classified as 'high level' exposure to styrene.

Concluding remarks

The presence of contrasting evidence in human studies is not surprising when weak mutagens are involved, especially if exposure assessment is troubling. This seems to be the case for styrene. As a matter of fact, even for well assessed causal associations there is a minority of reports which do not show the expected effect; a remarkable example is the case of cigarette smoking and SCE frequency, where more than one-third of the studies failed to reveal significant excesses in smokers (IARC 1986).

The present study indicates that, if a quantitative approach is applied to the review process, an association between working activities involving high exposure to styrene and cytogenetic damage, at least for CA, is evinced from published data.

The presence of a dose-related cytogenetic damage in persons exposed to styrene is a central issue in the current debate on the human genotoxicity of this compound. This topic has been considered in the context of some comprehensive reviews on styrene exposure, sometimes leading to contrasting conclusions. Barale (1991), after reviewing 19 published reports, concluded: 'It appears that damages to chromosomes were preferentially found in workers exposed to higher levels of styrene.' In a review based on a few more studies, Scott and Preston (1994) affirmed: 'The positive or negative outcome of the various investigations bears no relationship to the degree of exposure of the workers.' The results of this re-analysis are consistent with the hypothesis of a dose-related effect of styrene exposure on the frequency of CA. The extent of the chromosome damage in workers exposed to an air concentration of more than 125 mg m^{-3} (30 ppm) of styrene is remarkable, with a CA rate more than double when compared with controls. The frequency of SCE appears to be slightly increased in styrene workers and, even if there was a marginally significant higher *wFR* in highly exposed workers, the extent of this increase, i.e. 8%, seems too limited to attribute a conclusive value to these findings. Finally, MN data are rather heterogeneous, with a strong dichotomy between positive and negative studies. This heterogeneity was not completely attributable to the exposure intensity, since only a small difference in *wFR* was observed between the two levels of exposure. From a speculative point of view only, it should be mentioned that for this latter dataset the *wFR* of the 'low' level studies was dramatically increased by one study with an *FR* of 4.38 (Nordenson and Beckman 1984). Furthermore, this study was performed in a workplace with a mean environmental exposure of 24 ppm, just below the threshold limit used to discriminate the exposure level between studies.

The present findings did not reveal any relevant increase of risk for any endpoint among workers with an exposure below 125 mg m^{-3} (30 ppm) of styrene. This result does not mean *tout court* that below this limit there is no genotoxic risk. In fact, the above mentioned weakness in design and the limited statistical power of most studies covering this range of exposure prevent a reliable estimate of effect.

Possible concern about the choice of an arbitrary threshold value to classify a study group as 'low' or 'high' level exposure to styrene were addressed by performing a simulation with a wide range of possible threshold values. Different air concentrations of styrene were used to discriminate between the two levels of exposure, i.e. 75, 100, 125, 150, 175, 200 mg m^{-3}, and *wFR* values were estimated for all the groups generated by this procedure. The aim of this approach was to evaluate whether the results presented in this paper depended strictly on the threshold limit value of 125 mg m^{-3} or were independent of this parameter. The results of this analysis, which showed how the estimates of *wFR* in the simulated exposure groups were very close to, and often overlapped those chosen for the analysis, uphold the validity of our findings. The largest differences were observed for CA at the level of 200 mg m^{-3}, with a little smoothing of the difference between exposure levels, i.e. *wFR* = 1.57 (95% CI: 1.14–2.18) for 'low' level, and *wFR* = 1.87 (95% CI: 1.29–2.70) for 'high level' exposure.

Concern about the quality of cytogenetic studies on this topic has been expressed in a recent review (1994) by Scott and Preston, which discussed in detail the many limitations that reduce the validity of studies yielding positive results in styrene workers. Similar criticisms also apply to studies with negative results. The problem of measuring study quality, at least in the field of human biomonitoring, is difficult to solve, and therefore we have deliberately avoided considering the quality of the studies in our analysis. The minimum criterion of including only studies which had passed the screening of journal referees was adopted. The consideration that poorer quality studies tend to show greater effects than higher quality studies is not always supported by data. In fact, biases can also be toward the null, and studies with a less accurate design may indeed provide an attenuated estimate of the effects (Dickersin and Berlin 1992).

In many studies in which an increased incidence of chromosomal damage was observed, the presence of other chemicals in addition to styrene in the work environment was reported. These include solvents, especially acetone (Fleig and Thiess 1978, Högstedt *et al.* 1979, 1983, Nordenson and Beckman 1984, Brenner *et al.* 1991) and methylene chloride (Fleig and Thiess 1978, Högstedt *et al.* 1983, Brenner *et al.* 1991), polyester precursors, such as phthalic and maleic acid and anhydride, ethylene and propylene glycol (Högstedt *et al.* 1979, 1983), and peroxides, such as methyl ethyl ketone peroxide, cyclohexane and benzoyl peroxide (Högstedt *et al.* 1979, 1983). Some of these compounds produce chromosomal damage *in vitro* and/or in laboratory animals (Scott and Preston 1994). However, with the notable exception of the study by Tates and co-workers (1994), who concluded that exposure to dichloromethane was a more likely cause for the chromosomal damage observed in styrene workers, the role of

exposure to substances other than styrene could not be assessed. Styrene was held by most authors to be the agent responsible for the observed effect, a conclusion supported by the finding of a positive dose–response (Fleig and Thiess 1978, Andersson *et al.* 1980, Tomanin *et al.* 1992, Yager *et al.* 1993, Artuso *et al.* 1995) or by the consideration that styrene was quantitatively the most abundant pollutant in the air of the work places (Camurri *et al.* 1983, Högstedt *et al.* 1983, Nordenson and Beckman 1984, Brenner *et al.* 1991).

Final consensus on the presence and the extent of chromosome damage induced by occupational exposure to styrene in humans has yet to be reached. A more conservative assumption is that work processes in styrene industry involve exposure to genotoxic agents. This conclusion, above and beyond the controversy surrounding styrene, upholds the need for improvements in the surveillance and safety programmes in this occupational setting.

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