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Time-to-tumour risk assessment for 1,3-butadiene based on exposure of mice to low doses by inhalation

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Summary. The excess risk for cancer due to lifetime occupational exposure to 1,3-butadiene at the proposed US Occupational Safety and Health Administration standard of 2 ppm was estimated on the basis of a quantitative risk assessment. The risk assessment was based on a recent study by the US National Toxicology Program of the carcinogenicity of butadiene in B6C3F1 mice, using exposure concentrations ranging from 6.25 to 625 ppm butadiene and controls. Cancer risks were estimated using a multistage Weibull time-to-tumour model; the dose was based on the external butadiene concentration, owing to the low-dose linearity of butadiene metabolism. The parameters of the time-to-tumour model were estimated for seven tumour sites in male mice and nine in female mice. The risk estimates were extrapolated from mice to humans on the basis of body weight raised to the three-fourths power, and the median lifespan of mice was equated to a human lifespan of 74 years. Estimates of excess risk for lifetime occupational exposure (8 h/day, 5 days/week, 50 weeks/year, for 45 years) to 2 ppm butadiene ranged from 0.2 per 10 000 workers, based on female mouse heart haemangiosarcomas, to 600 per 10 000 workers, based on female mouse lung tumours. An analysis was performed to assess the effects of varying the modelling assumptions (incidental *versus* fatal tumours, inclusion or exclusion of the 625-ppm dose group, and basis for interspecies scaling) on the risk estimates from the female mouse lung tumour model. Depending on the assumptions, estimates of lifetime excess risk derived from the female mouse lung model ranged from 60 per 10 000 to 1600 per 10 000. These results suggest that exposures to butadiene in the work place should be reduced to the lowest feasible level.

Introduction

1,3-Butadiene is an inflammable, chemically reactive gas, which is used extensively in the synthesis of styrene–butadiene and polybutadiene rubbers. Approximately 2.9 thousand

million pounds (1.3 million tonnes) of butadiene were produced in the USA in 1991 (Anon., 1992). The US National Institute for Occupational Safety and Health (1989) estimated that about 9500 US workers are occupationally exposed to butadiene. In 1984, the Institute recommended that butadiene be regarded as a potential occupational carcinogen, and that exposures to the compound be reduced to the lowest feasible concentration. The US Occupational Safety and Health Administration (1990) proposed that the existing 8-h time-weighted average permissible exposure level be lowered from 1000 to 2 ppm.

The results of epidemiological studies provide some evidence of increased mortality from lymphatic and haematopoietic neoplasms among exposed workers (Divine, 1990; Lemen *et al.*, 1990; Matanoski *et al.*, 1990). In each case, the association was limited to subgroups within the study. A strong association between exposure to butadiene and the occurrence of lymphohaematopoietic cancers (odds ratio, 9.36; 95% confidence interval, 2.05–22.9) was reported recently from a nested-case–control study of butadiene-exposed workers (Santos-Burgoa *et al.*, 1992).

The results of studies in which animals were exposed by inhalation demonstrate that butadiene is a carcinogen at many sites. Male and female Sprague-Dawley rats were exposed to butadiene at 1000 or 8000 ppm for two years: statistically significant increases in the incidences of pancreatic and testicular (Leydig cell) tumours were seen in males and of uterine, Zymbal gland, mammary and thyroid tumours in females (Owen *et al.*, 1987). Both male and female B6C3F1 mice exposed to 625 or 1250 ppm butadiene for 60–61 weeks, in the first of two bioassays by the US National Toxicology Program (1984), developed statistically significant increased incidences of lymphomas, heart haemangiosarcomas, lung tumours and forestomach tumours. In addition, female mice had significantly increased incidences of mammary, ovarian and liver tumours. This study, and that with Sprague-Dawley rats, were the bases of most previous risk assessments of butadiene, including that which serves as the basis for the proposed 2 ppm standard (reviewed by US Occupational Safety and Health Administration, 1990).

In a second bioassay of butadiene by the US National Toxicology Program (Melnick *et al.*, 1990), male and female B6C3F1 mice were exposed by inhalation to concentrations ranging from 6.25 to 625 ppm butadiene for two years. Both males and females had dose-related increases in the incidences of lymphomas, heart haemangiosarcomas and tumours of the lung, liver, forestomach and Harderian gland. The female mice also had dose-related increases in the incidence of mammary and ovarian tumours, and the males developed tumours of the preputial gland .

This paper presents a quantitative risk assessment based on the second US National Toxicology Program bioassay of butadiene in mice. A multistage Weibull time-to-tumour model was fitted to the bioassay data and was used, with various mouse-to-human scaling factors, to prepare risk estimates for workers exposed occupationally to butadiene. The sensitivity of the risk estimates to variations in several different assumptions for statistical modelling and mouse-to-human extrapolation is also explored.

Materials and methods

Data set

The bioassay that served as the basis for this risk assessment was described by Melnick *et al.* (1990). Groups of 70 or 90 (high-concentration group only) male and female mice were

exposed by inhalation for 6 h/day, 5 days/week to 0, 6.25, 20, 62.5, 200 or 625 ppm butadiene for up to 104 weeks. Two interim sacrifices of 10 animals of each sex from each exposure group were scheduled at 40 and 65 weeks. The data used in this analysis consist of the exposure concentration, time of death and tumour status of each mouse in the two-year study, including those killed at 40 and 65 weeks. The data are available from the US Occupational Safety and Health Administration (Docket No. H-041). We used only data on tumours for which the diagnosis was based on complete gross and microscopic evaluation.

Statistical model

The tumour onset models evaluated were the 1-, 2-, 3-, 4- and 5-stage Weibull time-to-tumour models, as implemented in the TOX RISK software program, version 2.1 (Clement Associates Inc., Ruston, LA, USA). For example, a two-stage model can be represented as:

$$P(t,d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2)(t^Z)],$$

where t is time, d is dose, $q_0 \ldots q_2$ are non-negative constants, and $1 \leq Z \leq 10$.

The models for each tumour site were ordered according to their likelihoods, and models differing by less than 3.84 in (–2 * log) likelihood were considered to be statistically comparable. The least complex (lowest number of 'stages') of the comparable models was considered preferable for use in risk extrapolation, on the grounds of parsimony.

The method for fitting these Weibull time-to-tumour models required that each tumour be described as either incidental to the survival of the animal or rapidly fatal. Lymphomas and heart haemangiosarcomas were classified as rapidly fatal tumours, and tumours of the forestomach, Harderian gland, liver, lung, mammary and ovary were classified as incidental, on the basis of an analysis of tumours occurring in historical controls in the US National Toxicology Program (Portier *et al.*, 1986). Histiocytic sarcomas have historically been classified as a type of lymphoma and were thus classified as rapidly fatal tumours. A comparison was made of the risk estimates for all tumour types under both the fatal and incidental tumour assumptions, as part of our sensitivity analysis.

Dose

The dose metric used for model fitting in this analysis was the external exposure concentration in parts per million. This choice was suggested by the observation that, at low concentrations, the rate of metabolism of butadiene by B6C3F1 mice is a linear function of exposure concentration (Laib *et al.*, 1988). Our interpretation of those data is that metabolism is linear up to approximately 500 ppm but nonlinear above that dose. In order to minimize the complications associated with nonlinear metabolism and nonlinear tumour responses, we determined *a priori* that our preferred analyses would not include the 625 ppm dose group. Models that did include that group were used only for sensitivity analyses.

Animal-to-human extrapolation

For the purposes of extrapolation, 900-day-old mice were considered to be equivalent to 74-year-old humans. This age was chosen by equating a human lifespan of 74 years to the estimated 900-day median lifespan of the B6C3F1 mice used as controls in the studies of the US National Toxicology Program (Portier *et al.*, 1986). Exposures of mice to butadiene were extrapolated to humans on the basis of body weight to the three-fourths power. In other words,

it was assumed that the equivalent human dose (in milligrams) for any given dose of butadiene in mice is larger by a factor of (human weight/mouse weight)$^{3/4}$, on the basis of analyses by O'Flaherty (1989) and Travis *et al.* (1990). The average weight of mice (males and females combined) was 39.8 g, and the average inhaled dose of butadiene was estimated to be 0.0219 mg/day per ppm, using a relationship described by Adolph (1949). Scaling from a mouse to a 70-kg human on the basis of body weight to the three-fourths power gives a mouse-equivalent human dose of butadiene associated with lifetime exposure of 5.95 mg/day per ppm.

Since we used the butadiene concentration in parts per million to represent dose in our models, a scaling factor based on respiration rate and body weight to the three-fourths power was used to modify the parts per million concentration for the purposes of interspecies scaling. The occupational dose of butadiene can be calculated by assuming exposure for 250 work days/year for 45/74 years and a respiratory volume of 9.6 m^3/8-h work day. The resulting lifetime average inhaled dose of butadiene is 8.85 mg/day per ppm (D.A. Dankovic, R.J. Smith, J. Seltzer, A.J. Bailer and L.T. Stayner, unpublished data). If this calculated occupational dose is compared with the mouse-equivalent human dose of 5.95 mg/day per ppm, the occupational dose of butadiene can be predicted to be 8.85/5.95 = 1.49 times greater than the mouse-equivalent dose. For example, the lifetime excess risk from occupational exposure to 2 ppm butadiene was modelled as equivalent to the excess risk estimated at 900 days of age for mice exposed to 2.98 ppm butadiene, under the conditions of the bioassay.

Sensitivity analysis

The sensitivity of the risk estimates to the various modelling assumptions was assessed by varying the assumptions with regard to tumour lethality (incidental/fatal), number of stages (1, 2,... 5), mouse-to-human age equivalence and inclusion of the 625-ppm dose group. In addition, the effect of alternative methods of extrapolating dose from mouse to human was explored by preparing risk estimates on the basis of dose equivalence in milligrams per kilogram per day and metabolized dose of butadiene. The estimates of metabolized dose were prepared using the data on metabolism *in vitro* of Csanády *et al.* (1992), as re-analysed by Kohn and Melnick (1993). The latter authors estimated that the ratio of metabolic activation to detoxification is about 2.8-fold greater in B6C3F1 mice than in humans.

Results

Extrapolated maximum-likelihood estimates of excess risk for lifetime occupational exposure to 0.5–5 ppm butadiene, based on the preferred male and female mouse models, are given in Table 1. The lowest risk estimate for a 2-ppm occupational exposure is 0.2 per 10 000, based on female mouse heart haemangiosarcomas. For exposures to low butadiene concentrations, the largest estimates of human risk are those based on tumours of the female mouse lung; using this model, the estimated excess risk for a lifetime occupational exposure to 2 ppm butadiene is 600 per 10 000 workers. Models based on tumours occurring at several other sites in male and female mice yield estimates of excess risk within a factor of three of those derived from the female mouse lung model (Table 1).

The sensitivity of the estimates for the risk of female mice for lung tumours to variations in the modelling assumptions about incidental versus fatal tumours, and the inclusion versus

Table 1. Maximum likelihood estimates of lifetime excess risk[a] due to 45-year occupational exposure to 1,3-butadiene, using selected models

Neoplasm	Model[a]	8-h Time-weighted average concentration			
		0.5 ppm	1 ppm	2 ppm	5 ppm
Male mice					
Forestomach tumour	I, W2	0.08	0.3	1	8
Harderian gland tumour	I, W1	70	100	300	700
Heart haemangiosarcoma	F, W2	1	5	20	100
Histiocytic sarcoma	F, W1	40	80	200	400
Liver tumour	I, W1	20	40	70	200
Lymphocytic lymphoma	F, W1	4	9	20	40
Lung tumour	I, W1	60	100	200	600
Female mice					
Forestomach tumour	I, W1	30	50	100	200
Harderian gland tumour	I, W1	40	70	100	300
Heart haemangiosarcoma	F, W3	0.004	0.03	0.2	4
Histiocytic sarcoma	F, W1	60	100	200	600
Liver tumour	I, W1	30	70	100	300
Lymphocytic lymphoma	F, W1	40	90	200	400
Lung tumour	I, W1	200	300	600	1000
Mammary gland tumour	I, W1	20	40	70	200
Ovarian tumour	I, W1	40	90	200	400

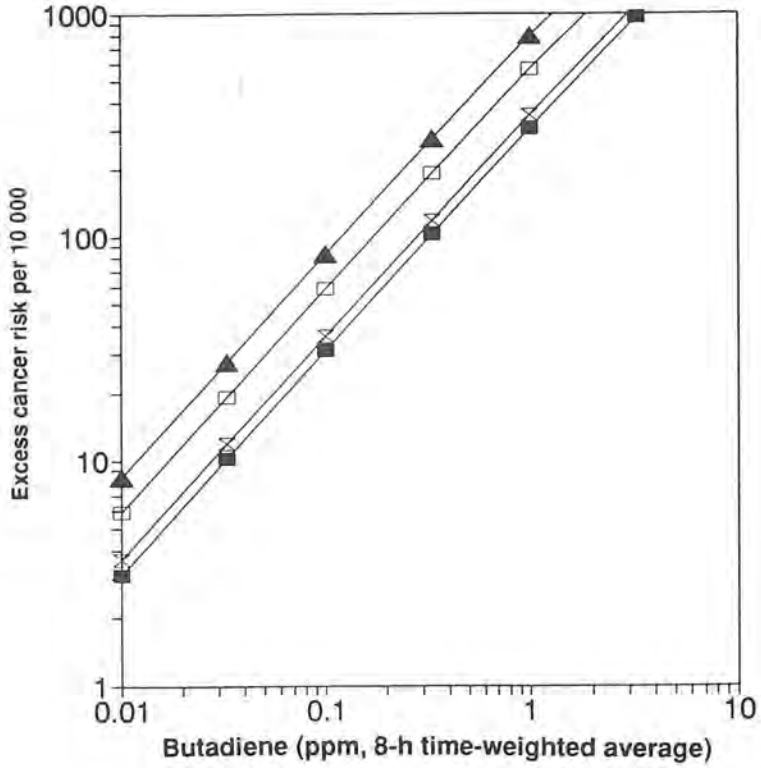
[a] Estimated lifetime excess risk for cancer per 10 000 workers; risk estimates rounded to one significant figure

[b] Model description: I, tumours assumed to be incidental to survival; F, tumours assumed to be rapidly fatal; W1, Weibull 1-stage; W2, Weibull 2-stage; W3, Weibull 3-stage

exclusion of the 625-ppm dose group, are shown in Figure 1. Changing the assumption that lung tumours are incidental to the survival of the animal to an assumption that lung tumours are rapidly fatal more than doubles the risk estimate at 2 ppm for models that do not include the 625-ppm dose group. Under the incidental tumour assumption, the risk estimates at 2 ppm are not greatly affected by the inclusion or exclusion of the 625-ppm dose group; inclusion of the 625-ppm group increases the estimate by approximately 16%. The extimates of risk for female mouse lung tumours are not affected by varying the number of stages in the dose term from one to five, as the estimated coefficients for the higher-order dose terms are equal to zero (data not shown). The 95% upper confidence limit on the risk estimate is 1.5 times the point estimate, at 2 ppm.

The risk estimates in Table 1 and Figure 1 were prepared under the assumption of scaling body weight to the three-fourths power from mouse to human. The effects of assuming dose equivalence in terms of either milligrams per kilogram or body weight to the three-fourths power adjusted according to the data on metabolism *in vitro* of Csanády *et al.* (1992), are shown in Figure 2. Scaling from mouse to human on the basis of milligrams per kilogram would reduce the risk estimates by a factor of 6.5 when compared with scaling on the basis of body weight to the three-fourths power. Adjustment of the latter scaling method

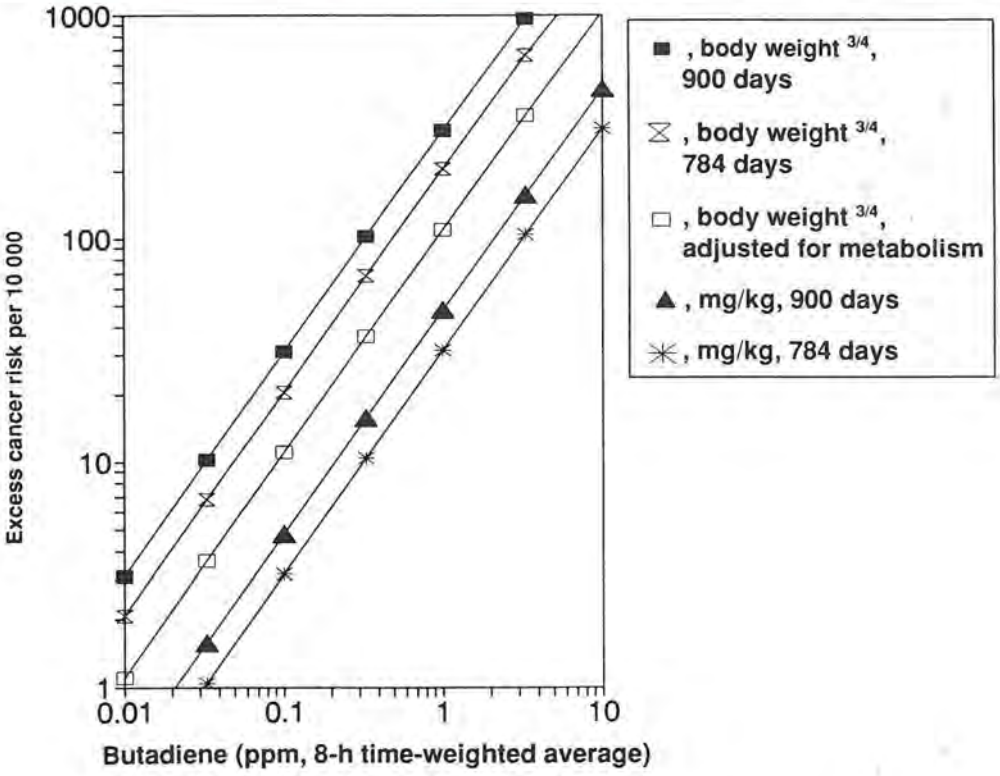
Figure 1. Lifetime excess occupational risk for cancer, estimated on the basis of female mouse lung tumours; effects of varying assumptions about tumour lethality and in of the 625-ppm dose group



Filled rectangles, tumours assumed to be incidental to survival, with exclusion of 625-ppm dose group; filled triangles, tumours assumed to be rapidly fatal, with exclusion of 625-ppm dose group; hourglass, tumours assumed to be incidental to survival, with inclusion of 625-ppm dose group; blank rectangles, tumours assumed to be rapidly fatal, with inclusion of 625-ppm dose group

according to the data on metabolism *in vitro* yields risk estimates approximately three times lower than those based on unmodified scaling but about two times greater than those based on milligrams per kilogram scaling. Figure 2 also illustrates the impact of varying our assumption that a human lifespan of 74 years is equivalent to a mouse median lifespan of 900 days to one which equates the human lifespan to the age of a mouse at the end of a two-year study (approximately 784 days of age). Varying this assumption has the effect of reducing the risk estimates by approximately one-third.

Figure 2. Lifetime excess occupational risk of cancer, estimated on the basis of female mouse lung tumours; effects of varying the mouse-to-human scaling factor from body weight to the three-fourths power to milligrams per kilogram or to body weight to the three-fourths power adjusted according to the data on metabolism *in vitro* of Csanády *et al.* (1992)



Mouse age assumed to be equivalent to human age of 74 years was also varied, from 900 to 784 days. The metabolism-adjusted risk estimates were prepared assuming that a 900-day-old mouse is equivalent to a 74-year-old human.

Discussion

Factors that introduce uncertainty into this risk assessment include: the choice of the data set for analysis; the choice of types of tumours for statistical analysis; the choice of statistical models; the procedure used to select the most preferable model; the treatment of dose in the models; and the method of extrapolating from mouse to human. The choice of data sets would seem to be clear, as the study of Melnick *et al.* (1990) is the only long-term bioassay of butadiene in which concentrations in the range of actual human exposure were used. Nevertheless, it must be recognized that data from other bioassays do exist, and that risk estimates based on those data would most likely differ from the results reported here.

This analysis has focused on tumours of the female mouse lung, primarily because the highest excess risk estimates at low butadiene concentrations were seen for that site. Clearly, the risk estimates are strongly dependent on the choice of tumour site for extrapolation; however, several sites yield risk estimates within a factor of 3 of the estimates based on the female mouse lung (Table 1).

Our decision to use a multistage Weibull time-to-tumour model was based primarily on considerations of competing risks. In the bioassay that served as the basis for this risk assessment, significant increases in tumour incidence were observed at multiple sites. As discussed by Melnick *et al.* (1990), the early occurrence of rapidly lethal tumours reduced the number of animals at risk for developing other neoplasms. No animal in the 625-ppm dose group and no female and only four males in the 200-ppm dose group survived to terminal sacrifice. Time-to-tumour methods take the competing risk distribution into account and so should be preferable to quantal approaches when there are substantial dose-related competing risks.

The choice of which Weibull time-to-tumour model (1-, 2-, 3-, 4- or 5-stage) is preferable for each tissue, the necessity for treating the various tumour types as either 'incidental' or 'rapidly fatal' and our decision to drop the 625-ppm dose group from the analysis also introduce uncertainty. The quantitative impact of the various alternatives on the risk estimates, based on female mouse lung tumours, is illustrated in Figure 1.

We chose the exposure concentration in parts per million as the dose metric for our analysis on the basis of the observation that the rate of butadiene metabolism in B6C3F1 mice is directly proportional to the butadiene concentration, for concentrations up to approximately 500 ppm (Laib *et al.*, 1988). We attempted to incorporate the species-specific data on metabolism *in vitro* of Csanády *et al.* (1992) into our risk estimates (Figure 2), but the choice of parts per million to express dose does not facilitate use of the tissue-specific differences in metabolism that were observed by those authors. The development of metabolic models that incorporate data on species-specific and tissue-specific metabolism would no doubt generate one or more alternative estimates of the biologically effective dose for each tissue.

Finally, some basis must be chosen for extrapolation from animals to humans. We assumed interspecies dose equivalence on the basis of body weight to the three-fourths power, as proposed by O'Flaherty (1989) and Travis *et al.* (1990). An assumption of dose equivalence on the basis of milligrams per kilogram basis rather than body weight to the three-fourths power would reduce the estimates of excess risk by a factor of about 6.5 for all sites with strictly linear models. The effect of scaling by milligrams per kilogram on the risk estimates for the female mouse lung model is shown in Figure 2.

Conclusions

On the basis of our point estimate for the most sensitive site (the female mouse lung), the excess risk for cancer resulting from lifetime occupational exposure to butadiene at the proposed 2 ppm standard would be about 600 per 10 000 workers. Adjusting body weight to the three-fourths power scaling factor according to interspecies differences in butadiene monoepoxide formation and detoxification would reduce the risk estimate to approximately 200 per 10 000. The use of milligram per kilogram scaling for interspecies extrapolation, rather than body weight to the three-fourths power, would reduce this risk estimate to about

100 per 10 000. Although the female mouse lung is the most sensitive site for butadiene-induced tumours, several other combinations of sex and tissue yield estimates of excess risk that are within a factor of 3 of the estimates based on the female lung (Table 1). These results suggest that prolonged occupational exposure to 2 ppm butadiene may result in a substantial excess risk for cancer and that significant health benefits may accrue from reducing the standard further. Such a reduction would be consistent with the previous statement by the US National Institute for Occupational Safety and Health (1984) that butadiene is a potential occupational carcinogen and that occupational exposure to this compound should be reduced to the lowest feasible level.

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