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ATTACHMENT V

CARCINOGENIC RISK ESTIMATION FOR CHLOROFORM: AN ALTERNATIVE TO EPA'S PROCEDURES

Sir,—The Environmental Protection Agency (EPA) has proposed an amendment to the National Interim Primary Drinking Water Regulations as part of the Safe Drinking Water Act. The amendment proposed by EPA would establish a Maximum Contaminant Level for chloroform (and other trihalomethanes) of 0·1 ppm. The Agency indicates in its proposal that this action is necessary because it is felt that the concentrations of chloroform known to exist in drinking-water constitute an appreciable cancer risk to man (*Federal Register* 1978, 43, 5755).

The magnitude of the cancer risk from chloroform has been estimated by the EPA from data generated at the National Cancer Institute (Report on the Carcinogenesis Bioassay of Chloroform, Bethesda, MD, 1976). In this study, long-term administration of chloroform at very high doses was found to produce tumours in laboratory rodents. However, there is reason to believe that the risk extrapolation performed by EPA seriously overestimates the actual potential of chloroform to induce cancer in man. There are two major reasons why this particular risk estimate is probably inaccurate. First, the EPA has failed to consider important data concerning the mechanisms through which chloroform exerts its toxicity. This has led to a major error in making species-species extrapolations of carcinogenic risk. Secondly, the NCI study was carried out with very high doses of chloroform. At slightly lower doses, the relative carcinogenicity of chloroform falls sharply. This suggests that there may be detoxification mechanisms which effectively protect the animals until they are overwhelmed by very large doses. Detailed discussion of these two points follows. In view of the enormous economic impact of regulations such as these, it is imperative that the risk estimation be performed as accurately as possible so that the risk–benefit ratio can be properly considered.

Species-species extrapolation for chloroform toxicity

The Carcinogen Assessment Group of EPA performs risk extrapolations under the assumption that the carcinogenic process does not have a threshold. Since it is impossible to prove or disprove this point experimentally, further argument is futile and we will proceed without resolving this question. The risk extrapolation from animals to man involves two steps.

(A) First, the excess cancer incidence (if statistically different from the control) is empirically fitted to a mathematical model relating dose and response. The most commonly used models are:

$$\text{(i) Linear } P = \beta d$$
$$\text{(ii) 'One hit' } P = 1 - e^{-\beta d}$$

where P is the fractional incidence of excess tumours, d is the average daily dose in mg/kg, and β is a dimensionless parameter to be estimated from the experimental data.

(B) Next a species correction factor is applied to β. This has been chosen to be the cube root of the ratio of the body weights (i.e. adjustment on a dose unit surface area). Assuming an average weight of 30 g for mice, 500 g for rats and 70 kg for man, this translates as a 13·3-fold increase for a mouse-to-man extrapolation and a 5·19-fold increase for a rat-to-man extrapolation. Finally the risk to man is calculated by substituting the human dose into the equation. The net result is that man is always considered to be more sensitive to a carcinogen than the small animal species.

This type of species-species extrapolation was developed by Rall (*Envir. Res.* 1960, 2, 360) for application to a group of 18 antineoplastic agents. These chemicals produce *direct* cytotoxicity and, according to the author "... are generally not involved in the variable drug metabolizing systems ..."

In the case of chloroform, however, metabolism plays a different role in toxicity. This is because the toxicity of chloroform is apparently not due to the parent molecule itself, but rather to the production of reactive metabolite(s) which bind covalently to tissue proteins (Brodie *et al. Proc. natn. Acad. Sci.* 1971, 68, 160; Ilett *et al. Expl mol. Path.* 1973, 19, 215; Reid *Proc. Fifth Int. Congr. Pharmac., San Francisco, CA*, 1973; Reid & Krishna, *Expl mol. Path.* 1973, 18, 80; Reid *et al. Am. Rev. resp. Dis.* 1973, 107, 539). Chloroform thus appears to belong to a class of chemicals that require metabolic activation for toxicity. Consequently, any species variation in the capacity to metabolize chloroform should dramatically affect the oncogenicity of this compound.

In order to assess the relative importance of these factors, we have attempted to predict the carcinogenicity of chloroform to rats on the basis of the tumour incidence observed in mice (NCI, *loc. cit.*). Since rats were also tested in the NCI study of chloroform, the observed incidence of rat tumours can serve to indicate the validity of the predictions.

First, we used the interspecies extrapolation procedure of the EPA (procedure A). The rats in the NCI study had a mean body weight of approximately 500 g, while the mice had a mean body weight of approximately 30 g. The cube root of the body-weight ratio (500/30) is 2·55. The rats should be *more* sensitive to chloroform by this factor. That is, a dose of chloroform that produced 10% tumours in a group of mice should produce 25·5% tumours in a group of rats.

Secondly, we have attempted to predict carcinogenicity from the relative rates of production of the toxic chloroform metabolite (procedure B). Several investigators have studied the metabolism of chloroform in rats and mice and concluded that mice metabolize chloroform more rapidly than do rats. The most complete studies are those of Brown *et al.* (*Xenobiotica* 1974, 4, 151), who adminis-

tered a 60-mg/kg dose of chloroform orally to rats, squirrel monkeys and three strains of mice. They recovered very little unmetabolized chloroform from the mice (Table 1). In the rat, three times as much unmetabolized chloroform was recovered, while in the monkey, 13 times as much unaltered chloroform was exhaled. Fry *et al.* (*Archs int. Pharmacodyn. Ther.* 1972, 196, 98) administered chloroform to human volunteers and found that 17–66% of the material was exhaled unchanged. However, the human data may be somewhat misleading. The dose of chloroform used in man (? mg/kg) was much lower than that given to the animals. Consequently, the relative amount of chloroform that would be metabolized at higher doses may have been overestimated.

Table 1. *Interspecies comparisons: chloroform*

Species	Oral dose (mg/kg)	$CHCl_3$ excreted unchanged (% of dose)
Mouse	60	6*
Rat	60	20*
Monkey	60	78*
Man	c.?	17–66†

*Brown *et al. Xenobiotica* 1974, 4, 151.
†Fry *et al. Archs int. Pharmacodyn. Ther.* 1972, 196, 98.

Although it is obviously difficult to make quantitative predictions from this type of metabolic data, it is clear that procedure B (relative metabolic capacity) leads to the opposite prediction from that given by the EPA procedure (A). Since the mouse metabolizes chloroform most rapidly, and presumably produces more of the toxic metabolite, it should be more sensitive to chloroform than the rat. Therefore, the interspecies correction factor is used in reverse in this case.

The results of these predictions may be summarized as follows:

(1) Predicted from EPA's surface area model (Rall, *loc. cit.*)	Rat > Mouse (2·55-fold)
(2) Predicted by metabolic rates (Brown *et al. loc. cit.*)	Mouse > Rat (2·55-fold)
(3) Observed in NCI bioassay (NCI, *loc. cit.*)	Mouse > Rat (4·2-fold)

For the purpose of evaluating the actual carcinogenicity in the two species, the β parameter is estimated for the most sensitive sex in the NCI bioassay according to the one hit model. The relative sensitivity is then approximated for low doses as the ratio of the two β values. Clearly procedure B is a better predictor than procedure A, for the actual tumour incidence is 4·2-fold higher in the mouse at equivalent doses of chloroform. Thus the application of the EPA technique for interspecies extrapolation has produced an error of 10·7-fold in estimating the sensitivity of the rat from the mouse data.

It is also noteworthy that extrapolation of these data to man with these two procedures gives very different results. The EPA procedure (procedure A) produces human risk estimates that are inconsistent. The mouse data predict a human risk 11-fold higher than the rat-data prediction. In contrast, with procedure B, the two risk estimations agree very well. Risk predicted from the mouse data is only 1·6-fold higher than risk predicted from the rat data.

Based on these considerations we would consider man to be the least sensitive of the three species to the carcinogenic action of chloroform. This estimate is based on the data of Fry *et al.* (*loc. cit.*) as well as on the general principle that man normally metabolizes materials much more slowly than the small laboratory animals (Weiss *et al. Int. J. clin. Pharmac.* 1977, 15, 572). Although the absolute magnitude of the risk is dependent upon the particular mathematical model employed in step A, incorrect application of the 'species correction factor' causes an increase of more than two orders of magnitude in the estimated risk to man. Instead of an excess cancer risk "...on the order of 10^{-4}–10^{-5} ..." (EPA, Statement of the Basis and Purpose for an Amendment to the National Interim Primary Drinking Water Standards on Trihalomethanes. Office of Water Supply Criteria and Standards Division, Washington, DC, 1978), we feel that the risk cannot be higher than about 1% of this figure.

In addition to chloroform, there are many other instances where the relative carcinogenicity of chemicals correlates well with their rate of activation to a reactive species. For instance, vinylidene chloride (VDC) is metabolized more extensively in the mouse than in the rat (McKenna *et al. Envir. Hlth Perspect.* 1977, 21, 99). Tumours have been observed in mice exposed to VDC (Maltoni, *ibid* 1977, 21, 1) while studies in rats have failed to reveal a discernible oncogenic response (Maltoni, *loc. cit.*; Rampy *et al. Envir. Hlth Perspect.* 1977, 21, 33; Viola & Caputo, Cancerogenicity Studies on Vinylidene Chloride: paper presented at NIEHS Conference on Comparative Metabolism and

Toxicity of Vinyl Chloride Related Compounds, Bethesda, MD, 2–4 May 1977). Similarly 2-acetaminofluorene is metabolized to a reactive electrophile and the carcinogenicity of this material correlates well with the relative abundance of enzymes to carry out this activation in various species (Miller, Report to Conference on Environmental Carcinogenesis, Michigan State University, East Lansing, MI, 22–23 May 1978). Trichloroethylene is carcinogenic in B6C3F1 mice but not in Osborne–Mendel rats (NCI, Report on the Carcinogenesis Bioassay of Trichloroethylene, Bethesda, MD, 1976). This correlates well with the greater capacity of mouse microsomes to catalyse incorporation of trichloroethylene metabolites into DNA (Banerjee & Van Duuren, *Cancer Res.* 1978, 38, 776).

High dose/low dose extrapolations

For most risk estimations it is customary to extrapolate from high doses, where experimentally observable rates of tumour induction exist, to doses in the environment several orders of magnitude lower. For instance, the doses of chloroform used in the NCI study (Report on the Carcinogenesis Bioassay of Chloroform, Bethesda, MD, 1976) are about 100,000 fold higher than the Maximum Contaminant Level proposed by the EPA. This raises important questions about the validity of such extrapolations.

There are many instances where toxicity (and presumably also carcinogenicity) increases disproportionately once the normal detoxification mechanisms are overwhelmed. An example of this type of behaviour is the work recently reported by McKenna *et al. (loc. cit.)* for VDC. It has been postulated that VDC is metabolized in the body to a reactive electrophile which reacts with either endogenous glutathione (detoxification) or various macromolecules (toxic action). In the rat, glutathione is depleted by exposure to high levels of VDC (Jaeger *et al. Expl mol. Path.* 1974, 20, 187). Furthermore, fasting the rats prior to VDC exposure further depleted the glutathione and dramatically increased the hepatotoxicity of VDC. More importantly, McKenna *et al. (loc. cit.)* noted a sudden disproportionate increase in macromolecular binding of VDC metabolites as glutathione was depleted by more than 30%. These observations are particularly important in view of the fact that chloroform is also known to deplete glutathione, and that chloroform toxicity can be antagonized by administration of cysteine, a glutathione precursor (Docks & Krishna, *Expl mol. Path.* 1976, 24, 13).

The validity of using high doses of chloroform to predict the effects of low doses may be tested by examining the data of the NCI (Report on the Carcinogenesis Bioassay of Chloroform, Bethesda, MD, 1976) and of Roe (Preliminary Report of Long Term Tests of Chloroform in Rats, Mice and Dogs, personal communication, 1976). Data from the highest dose group will be used to estimate the parameters for the one hit model and the linear model. Tumour incidence for lower doses will then be predicted and compared to observed incidence rates. Because of the different spontaneous tumour rates for the various studies, the most logical way to compare results from the studies is through excess tumours calculated from Abbott's correction

$$P_{\text{excess}} = \frac{P_{\text{treatment}} - P_{\text{control}}}{1 - P_{\text{control}}}$$

Thus our models will be:

$$P_{\text{excess}} = \beta x \text{ (linear model)}$$
$$P_{\text{excess}} = 1 - e^{-\beta x} \text{ (one hit model)}$$

Upper 95% confidence limits are also calculated for the observed number of tumours. These are exact confidence intervals when there is no spontaneous background rate (i.e. when Abbott's correction is not necessary) or when the observed frequency rate in the treated group is zero. For non-zero frequencies with non-zero spontaneous rates the confidence interval is calculated using the normal approximation to the binomial distribution and using the appropriate standard deviation for a ratio from the Taylor series expansion of the ratio.

The predictions, as well as the actually observed incidences, are listed in Table 2. Neither the linear model nor the one hit model adequately predict the tumour incidence below 200 mg chloroform/kg. In most cases, even the upper 95% confidence interval for the observed tumour rates is below the predicted values. For instance, the NCI male mice given 138 mg/kg would be predicted to have 2.4 times more tumours than were actually observed. The tumour incidences observed by Roe *(loc. cit.)* in mice exposed to 60 mg/kg or less are even further from the predicted values (Table 2). Since Roe utilized four different strains of mice, and none of these results are well described by the model, the failure of the extrapolation is probably due to a deficiency in the model rather than to strain differences. However the only definitive procedure to establish the dose-dependency of the carcinogenic effect of chloroform is for the NCI to repeat the carcinogen bioassay over a wider range of doses. Until this has been done, there will be a great deal of uncertainty about the lower end of the dose-response curve.

Experience with human exposure to chloroform also appears to be inconsistent with EPA's risk assessment. For example, a study of British workers in a confectionery factory indicated chronic exposure (up to 10 years) to concentrations of 50–125 ppm chloroform vapours (Challen *et al. Br. J. ind. Med.* 1958, 15, 243). If we assume that the two routes of exposure (oral and inhalation)

Table 2. *Predicted and observed incidences of excess liver tumours in chloroform-treated mice*

Strain	Dose (mg/kg)	Predicted tumours (%)		Observed tumours	
		Linear*	One hit†	Incidence (%)	Upper 95% confidence interval
		Males			
B6C3F1	277	—	—	92	—
	138	46	72	30	47
C57B1	60	20	42	0	6
CBA	60	20	42	0	2
CF1	60	20	42	2 (NS)	13
ICI	60	20	42	7 (NS)‡	18
	17	6	14	8 (NS)	20
		Females			
B6C3F1	477	—	—	95	—
	238	47	78	80	91
ICI	60	12	31	0	8
	17	3	10	0	8

NS = Observed tumour incidence not significantly different from control incidence
* $P = 1 - e^{-(0.00012 \times \text{dose})}$ for males and $1 - e^{-(0.00028 \times \text{dose})}$ for females
† $P = 0.00332 \times$ dose for males and $0.00199 \times$ dose for females
‡ A 22% incidence of excess *kidney* tumours seen in this strain was significantly different from that in the controls

are essentially equivalent, these workers would be predicted to have a lifetime cancer risk of 20–40% in the group exposed for a mean of 5·4 years (EPA one hit model, NCI female mouse data with surface area correction). These exposures were documented 20 years ago, so that even with a long latency period, increased cancer incidence should be demonstrable if the EPA risk estimation is valid. Similar reports exist from the chemical industry (Bomski *et al. Arch. Gewerbepath. Gewerbehyg.* 1967, 24, 127; Lehman & Schmidt-Kehl, *Arch. Hyg.* 1936, 116, 131). However, to date, no epidemiology study convincingly links industrial exposure to chloroform with an increased cancer risk.

In summary, there are several reasons for believing that the current EPA risk estimation for chloroform is seriously in error. We feel that definitive studies of the metabolism of chloroform in various species must be carried out in order to allow a rational species/species extrapolation to be performed. Furthermore, there is evidence of a sudden change in the shape of the chloroform dose-response curve below 200 mg/kg. A complete experiment to evaluate the carcinogenicity of chloroform at lower doses must be carried out before high dose/low dose extrapolations can be performed. A study of the pharmacokinetics of chloroform in the selected species would be valuable in selecting appropriate doses for this study.

There is good evidence that there are two classes of carcinogens. One class appears to produce its effect directly (e.g. β-propiolactone) and for these agents, at least when given orally, it may be appropriate to apply surface-area correction factors (procedure A). Compounds in the second class require metabolic activation before they become carcinogenic (e.g. 2-acetylaminofluorene). In this case, the relative rate of metabolic activation appears to be the most reliable means of making interspecies extrapolation of carcinogenic risk. Consequently, we strongly suggest that the NCI develop an appropriate technique for distinguishing between these two classes of carcinogens and that EPA then apply appropriate species/species extrapolation techniques before proposing any regulations based on risk assessment.

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