Health Effects of Organics: Risk and Hazard Assessment of Ingested Chloroform

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In the July 1977 JOURNAL, H.E. Stokinger presented a thesis critical of the methodology that stigmatizes certain impurities in water as cancer-inducing agents. This article is meant to put the total situation in better perspective by explaining the rationale of conservative analysis used to set limits on suspected carcinogens such as chloroform.

Chloroform occurs widely in drinking water at levels higher than most contaminants. This compound has demonstrated, for some experiments, carcinogenic properties. Consequently, evaluation of its possible risks and hazards to the human population is warranted.

Establishing Chloroform as an Animal Carcinogen

Although the evaluation of carcinogenic properties is a highly complex and difficult procedure requiring informed professional judgment, a few fundamental criteria can be identified.

Pathologists must apply their skills, knowledge, and experience to the identification of lesions (in this case, benign and malignant tumors) in the various organs of the experimental animal models. Often diagnoses that a lesion is in fact cancer must be confirmed by more than one pathologist.

Analysis of the data from controlled animal experiments must demonstrate a statistically significant increase in neoplasia in treated animals over the spontaneous incidence in control animals. Perhaps more important, a dose-related increase in the incidence of cancer should be observed to ensure reliability in the conclusion that the lesions are caused by the test agent.

Depending upon the design and execution of the study, time-to-tumor incidence data, which should demonstrate a decrease in latency with increasing dose levels, can occasionally be obtained. Such a relationship adds weight to the interpretation of the cause and effect relationship. If assays for carcinogenesis are only qualitative in nature—that is, without dose-response structure—and if the experimental cancer incidence is relatively low (less than 10 per cent), reproducibility of the bioassays becomes highly critical to the interpretation of experimental conclusions. Reproducibility not only within the same strain and species but also within other species is crucial to the interpretation that the agent in question is responsible for the cancerous lesions.

A review of animal studies. To date, the effects of chloroform have been studied using three species of animals and by two routes of exposure (Table 1). In five of these studies, chloroform has elicited a tumorigenic response, whereas in four studies no tumors were observed.

In 1971 the International Agency for Research on Cancer Working Group on the Carcinogenic Risk of Chemicals to Man evaluated the animal studies on the carcinogenicity of chloroform. Three studies were reported, two involving oral administration to mice and one subcutaneous injection in mice. In the study by Eschenbrenner and Miller, five different strains of mice of each sex received oral doses, each dose of 0.1, 0.2, 0.4, 0.8, and 1.6 ml chloroform/kg body weight in olive oil at four-day intervals. Of the five females that survived dosages of either 0.8 or 0.4 ml/kg of body weight, all had nonmetastasizing hepatomas and cirrhosis. No hepatomas were observed at the two lowest dose levels or in the controls. No tumors were observed in organs other than the liver.

Rudali administered chloroform at a dose level of 0.1 ml (40 per cent solution in oil) by intubation twice weekly for six months to 24 mice. This regimen produced three hepatomas in five survivors.

Roe et al administered chloroform by subcutaneous injection (single dose of 200 µg of chloroform in arachis oil to 24 hr old mice, or eight daily doses of 200 µg for the first week of life) and obtained no evidence of carcinogenicity when animals were sacrificed at 80 weeks.

However, animals fed 17 mg/kg/day of chloroform showed no incidence of renal carcinoma. Some renal tumors were also seen in control animals in a later study.

Negative results were observed in the dog experiment, which can be explained on the basis of insufficient exposure time and other reasons. There were negative results for the rat study as well. These too can be rationalized. Based on data from the NCI study, one would expect that the lower dose level used by Roe was below the limit of detection.

NCI recently completed a carcinogenesis bioassay of USP-grade chloroform using Osborne-Mendel rats and B6C3F1 mice. Table 2 outlines the experimental design of the study. The compound was administered in corn oil by gavage to 50 animals of each sex at two dose levels five times per week for 78 weeks. Male rats were given 90 or 180 mg/kg of body weight; female rats were treated with 100 or 200 mg/kg of body weight. The dosage levels for the mice were 138 or 277 mg/kg for males and 238 or 477 mg/kg for females.

The results of the NCI study on rats and mice describing target organs and tumor types are listed in Table 3. In the male rats, kidney epithelial tumors were observed, whereas in the female rats benign thyroid tumors were diagnosed. The results for the female rats were considered not biologically significant by the pathologist. In both sexes of mice hepatocellular carcinoma was diagnosed with a high frequency. Nodular hyperplasia of the liver was observed in many low-dose male mice that had not developed hepatocellular carcinoma.

Quantitative analysis of the incidence of malignancy vs dosage levels yielded the results summarized in Table 4. For malignancies in the kidney of the male rats and in the livers of the male and female mice, a dose-related incidence of cancer was observed. The increased incidences of tumors at the low dose levels as compared with controls were statistically significant. The incidence of tumor development also showed a dose-related increase. Of greater biological significance was the development in the treated rats of a relatively rare type of cancer in the kidney—one seldom observed in the controls and not seen in the matched controls.

When the data were analyzed with respect to latency, defined as "time-to-
tumor at death," the results also indicated a dose-related response (Table 5). In the female mouse, both incidence and latency seemed to have reached a plateau at the low and high dose levels of exposure.

The previously described data indicate that the basic criteria for establishing chloroform as a carcinogen have been met. The lesions described and confirmed by the pathologist were malignant and metastatic in at least one study. In addition, the compound produced tumors in more than one species (rat and mouse) and in more than one strain of mouse. Equally important, malignancies were produced in a dose-response fashion in both rat and mouse, giving reasonable assurance that the effect was indeed compound-related and not merely an artifact. If, for example, the only data available were those from Roe, there would be substantial reservations as to the appropriateness and validity of the conclusion.

**Extrapolating Data From Experimental Animals to Man**

Once the carcinogenic property of a compound has been reasonably established, two critical issues remain to be addressed: the extrapolation of experimental findings to man and the extrapolation from the necessarily high doses employed in the bioassay to the substantially lower doses existing in man's exposure media.

The extrapolation of toxicity data, including data on carcinogenic activity, from experimental animals to man is unquestionably complex and difficult. The hazard assessment for human exposure to chloroform must deal with this problem, however, in order to make relevant recommendations concerning human safety.

In the past, both mouse and rat have served reasonably well in confirming the carcinogenic activity of agents known to affect man. Examples of these chemical agents, which are relatively few in number, include bis-chloromethyl ether and vinyl chloride. (One anomaly to this general observation is arsenic.)

**Influence of metabolic factors.** Even compounds shown to be carcinogenic in animal models as well as in man produce differences both among species and among various strains within species. Some animals are hypersensitive whereas others are refractory to the same chemical carcinogen. In some cases, differences in site specificity can be observed among various strains and species. Many of these differences can be related to metabolic factors: that is, the compounds are metabolized through pathways that generate an ultimate toxin or carcinogen. This metabolic activity is focused in specific organs, thereby increasing the probability of a toxic response with that organ.

Within the framework of metabolism, the rates of biotransformation are quite critical in that the relative rates of activation vs inactivation are important in determining the duration of exposure of target molecules to the carcinogenic substance. Systemic distribution also plays a vital role, since it is possible that the ultimate toxin is generated in one organ and redistributed to another to exert its toxic effects.

**Repair mechanisms and their rates play a similarly important role in the ultimate manifestations of the lesions. If the rate of repair is relatively fast, far more agent can be expected to be necessary to produce irreversible biochemical lesions that lead to clinical manifestations. Conversely, with slow rates of repair relatively small quantities of an agent may elicit a toxic syndrome such as cancer. Routes of excretion and rates of elimination also are significant in**
removing the toxin or carcinogen from the locus in which it can combine with the target receptors.

Qualitatively, man and mouse appear to metabolize chloroform by similar pathways. However, data on biotransformation are not as complete as they might be for in-depth comparison. There is no evidence to demonstrate differences in sensitivity in so far as humans compare to rodents. The rate of DNA repair in man appears to be somewhat higher than in the rodent.

Although limited, some epidemiologic data exist on chloroform. Daily ingestion for ten years of cough suppressant containing chloroform-codeine at dose levels estimated at 23–27 mg/kg/day for a 70-kg person produced only reversible hepatotoxicity. In a separate study with a dentifrice and a mouthwash, chloroform consumption calculated at 0.34–0.96 mg/kg daily over a one- to five-year period did not produce hepatotoxicity based on liver function tests. Hepatotoxicity therefore appears to occur in humans as a result of oral doses greater than 1 mg/kg/day but less than 25 mg/kg/day.

There is no evidence to date to document chloroform carcinogenicity in humans either through occupational or environmental exposures although studies employing occupational cohorts are currently being planned.

From the limited data, one can conjecture that man may respond as the rodent in terms of hepatotoxicity and carcinogenesis. The mouse frequently elicits a target organ response to a chemical carcinogen that is different from man's. One can speculate that a likely target organ for chloroform in man might be the kidney, as reflected in the Osborne–Mendel rat data.

**Extrapolating From High to Low Dose Levels**

Many factors must be taken into consideration in extrapolating from high to low dose levels. These factors include the multiplicity of pathways and the dosage levels at which individual pathways become activated; the ratio of active compound to number of susceptible cells and target molecules; the distribution rate within the organism, leading possibly to longer exposure to susceptible components or longer residency time for detoxification; the nature and duration of contact with metabolic enzymes; the integrity of the cellular cycle, including cycle time and frequency of cell cycling; and integrity of the repair systems, including the rates of repair mechanisms.

The mechanism by which a compound induces its toxic effects is also critical in evaluating the anticipated toxicity of lower dose levels. In the case of chloroform, account should be taken of the possibility that the compound may have elicited positive response in experimental animals by virtue of extensive tissue damage generated in the target organs exposed to very high concentrations of the test agent or its metabolites: the carcinogenic response may be secondary to extensive damage of the kidney or liver. If this is correct (as believed for at least one other chemical carcinogen), then the carcinogenic response in man or animals should not occur at levels below which tissue damage is induced. Additional mechanistic considerations include the possibility of interactions with other stresses, either physical or chemical, to generate a synergistic or antagonistic action.

Unfortunately, little clinical information is available for input into the evaluation of the risk to chloroform exposures. Consequently, the evaluation is performed on the basis of largely mechanical (that is, statistical) systems using several assumptions weighted on the side of conservatism and safety.

**Estimating Risk**

Several different methods or models can be used to determine the maximum risk from chloroform ingestion via tap water. Four such models have been used to evaluate the chloroform toxicity data: (a) margin of safety, (b) probit-log, (c) linear or one-hit, and (d) two-step. The results of these evaluations are listed in Table 6.

**Margin of safety method.** For many years, the margin of safety model has been applied to direct and indirect food additives as well as to compounds used for other purposes. Classically, if appropriate and reliable human data exist, a margin of safety of ten can be applied to the maximum no-effect dose to obtain a maximum "safe" level. The maximum no-effect level for liver damage from the chronic ingestion of chloroform is in the range of 0.3–0.9 mg/kg/day. Applying a margin of safety of ten to the lower end of the curve yields a value of 0.03 mg/kg/day.

Weil has suggested that when a carcinogenic response is involved and when the study has been appropriately designed, a margin of safety of 5000 applied to the minimum-effect dose is appropriate. If the data on kidney carcinoma from the NCI study are used as a basis and 90 mg/kg/day is judged to be minimum effective dose, then the application of such a margin of safety leads to a maximum daily dose of 0.02 mg/kg/day. Utilizing this model, the calculated maximum daily dosages are assumed to represent either no risk or negligible risk of toxicity, including carcinogenesis, to the general population.

**Statistical models.** The other models (probit-log, linear, and two-step) are statistical in nature and are based on the assumption that the incidence of cancer decreases in the population with decreasing exposure levels such that some calculable risk always exists regardless of the magnitude of the dose.
The probit-log model can be applied in either one of two ways: using the slope of one, as recommended by Mantel and Bryan, or using the actual slope derived from the experimental data if sufficient information exists to do so. A comparison of the two approaches is presented in Table 6.

By one's using a slope of one to derive the estimated maximum risk from chloroform ingestion, the conclusion is reached that at a maximum daily dose of 0.01 mg/kg/day the risk falls somewhere between 0.016 and 0.683 cancers per million population per year, depending upon which experimental basis is used as the starting point for the calculations. By one's using the identical data and the identical statistical model, but the actual slope of the dose-response curve as opposed to the slope of one in the previous calculation, the results obtained are a maximum risk estimate of less than one tumor per billion population per lifetime at a maximum exposure level of 0.01 mg/kg/day.

In developing their statistical model, Mantel and Bryan concluded that most dose-response curves for carcinogens have a slope greater than one, and consequently they selected the slope of one for a conservative estimate of risk. A few exceptions to this precept exist: the slopes for cigarette smoking and for diethylstilbesterol (DES) are less than one.

The linear or one-hit model is considered by many to be the most conservative extrapolation model because at very low doses the line tends to diverge away from zero as opposed to the probit-log model for which the line converges upon zero. Using the NCI data from the rat and the mouse, one obtains a risk estimate between 0.42 and 0.84 cancers per million population per year at a maximum dosage level of 0.01 mg/kg/day.

The two-step model, which appears to be currently favored by NCI biometricians, falls between the probit-log model used in the slope of one and the linear model when applied to the NCI data for rat kidney carcinoma and mouse liver carcinoma. With the two-step model, an estimated maximum risk between 0.267 and 0.283 cancers per million population per year is calculated at a maximum dose level of 0.01 mg/kg/day.

Defining Acceptable Risk

The maximum daily dosage expressed as mg/kg/day can be translated to maximum concentrations in tap water. Although in many cases such calculations have been based on adult body weights and adult water consumption, the author believes that such a procedure is too liberal for a substance that may be a carcinogenic risk. Since exposure begins most likely in utero and in many cases immediately after birth and since the youngest members of the population are perhaps at greatest risk when exposed to carcinogens, it appears most appropriate to consider maximum protection for the most sensitive subset of the population: that is, infants. If the assumption is made that a 7-10-kg infant consumes one liter of water per day (as frequently happens when powdered milk formulas are used), the maximum allowable concentrations in tap water should be no greater than 70 µg/l (ppb) to ensure that the maximum allowable concentration does not exceed 0.01 mg/kg/day.

An additional consideration is the possibility that other haloforms (e.g., bromodichloromethane, chlorodibromomethane, and bromoform) have the same potential toxicity potency. Thus it may be more reasonable to limit the total haloform concentration to 70 µg/l.

The previously derived risk estimates should have some meaning in the real world. The population of the US exhibits a baseline level of liver cancer and kidney cancer as reported by the NCI10 with which the estimated or calculated maximum cancer risk from chloroform in tap water can be compared. This comparison is summarized in Table 7.

The cancer rate for both males and females has been averaged for each site (liver and kidney), giving an overall rate for liver carcinoma of 52.5 per million per year and an overall rate for kidney carcinoma of 29.2 per million per year.

Three estimated cancer-risk levels have been selected for comparison: most conservative, median conservative, and least conservative. The assumption has been made that the maximum dose level will be 0.01 mg/kg/day. The results of this evaluation indicate that chloroform in drinking water may be responsible for as much as 1.6 per cent of the current yearly liver cancer incidence in this country (168 liver-cancer deaths per year) and for as much as 1.44 per cent of the yearly kidney cancer incidence (64 kidney-cancer deaths per year). Of approximately 300,000 cancer deaths per year, an estimated maximum of 252 may be attributable to chloroform in tap water.

Conclusions

The reality of the cancer incidence situation attributable to chloroform lies somewhere between no cancer risk to kidneys and liver and a maximum cancer risk of 1.6 per million population per year. No experimental tools at present can define the true rate between those two points with accuracy. A joint societal and regulatory decision is required to appraise the risk from the consumption of chloroform via drinking water. Such a decision must be counter-balanced against a substantial number of issues such as the cost of regulation, burdens from alternate diseases related to the removal of chlorine as a disinfectant, and the burden from alternate diseases that may be related to byproducts of alternate disinfectants. Clearly, such decisions can be made only with insight focused sharply into the current risks from exposure to chloroform via drinking water.

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