HSIA HALOGENATED SOLVENTS INDUSTRY ALLIANCE

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FILING INSTRUCTIONS:
H/H/T
Trichloroethylene
EPA HAD Addendum
HSIA Comments to SAB
KEYWORDS:

August 12, 1987

TO: HEALTH AND SCIENCE COMMITTEE

FINAL SAB STATEMENTS

Attached are HSIA's statements submitted to the Science Advisory Board for the August 11-14 meetings on mouse liver and rat kidney tumors, methylene chloride, and trichloroethylene. A bound copy with tabs of the appropriate statements was sent directly to each member of the Halogenated Organics Subcommittee and the Environmental Health Committee. All documents listed in the attachments were included with the copies sent to the SAB.

Daniel M. Byrd III
Director of Scientific Affairs

Attachments

SL 037848

HSIA HALOGENATED SOLVENTS INDUSTRY ALLIANCE

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August 7, 1987

<Title> <First Name> <Last Name>
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Dear <Title> <Last Name>:

We have assembled the enclosed information to assist your review of EPA's draft Addendum to the Health Assessment Document for Trichloroethylene. Most of the information in this package consists of photocopies of research reports and papers from scientific literature (some of which are difficult to obtain). Overall, HSIA does not agree with the qualitative or quantitative conclusions regarding possible carcinogenic effects of trichloroethylene. The draft Addendum lacks a serious effort to evaluate epidemiological data and, for this reason alone, merits revision and re-review.

Some of the information concerning trichloroethylene is included in our comments regarding the workshop on mouse liver tumors and rat kidney tumors on August 12, 1987.

If you should have questions about any of the enclosed materials, please call us at (202) 223-5890.

Sincerely,

Paul A. Cammer, Ph.D.
President

Enclosures

SL 037849

HSIA HALOGENATED SOLVENTS INDUSTRY
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STATEMENT BEFORE THE SCIENCE ADVISORY BOARD ON THE DRAFT ADDENDUM TO THE HEALTH ASSESSMENT DOCUMENT FOR TRICHLOROETHYLENE

AUGUST 7, 1987

SL 037850

STATEMENT BEFORE THE SCIENCE ADVISORY BOARD ON THE DRAFT ADDENDUM TO THE HEALTH ASSESSMENT DOCUMENT FOR TRICHLOROETHYLENE

Our overall response to EPA's assessment of the carcinogenicity of trichloroethylene is summarized immediately below. Detailed evaluations, data, and supporting information can be found in the indicated attachments. Our five major points are:

(1) EPA has not carried out an adequate evaluation of a new epidemiology study by Shindell and Ulrich, which has a sufficiently large cohort to detect a risk double that of background. Neither the Shindell and Ulrich study nor the six other epidemiology studies support the conclusion that human exposure to trichloroethylene increases cancer risk. (Attachment A).

(2) Conclusions in the draft Addendum regarding the carcinogenic pathway in rodents are in error. The preponderant evidence from pharmacokinetic, whole animal bioassay, and cell culture experiments is that trichloroacetic acid is the proximal carcinogen by virtue of its ability to induce peroxisomes. However, cell culture experiments show that trichloroacetic acid is not likely to be carcinogenic in man. Even if it is assumed that trichloroacetic acid exerts carcinogenic effects in man, pharmacokinetic experiments indicate that rodent models greatly overstate this risk. (Attachment B)

(3) Animal evidence is "limited" under EPA's guidelines for carcinogen risk assessment because of metabolic evidence that mouse liver tumors are not indicative of human risk. This conclusion is supported by studies of carcinogenic mechanisms of mouse liver and rat kidney tumors. This subject is summarized in our comments for the Workshop on August 12 and only a few points will be repeated here. (Attachment C)

(4) Trichloroethylene lacks genotoxicity for mammals and other higher animals. (Attachment D)

(5) Recent oncogene studies indicate that the B6C3F1 mouse may be genetically predisposed to liver tumors, making it an inappropriate model for direct comparison to the human. (Attachment E)

EPA's draft Addendum does not take into account the available human epidemiology evidence. The best scientific evidence addressing the question of human carcinogenicity of any substance is provided by epidemiology studies. Such studies are often confounded by factors such as cohort size, extent of exposure, latency considerations, and comparison to appropriate control cohorts, which frequently result in the inability to

prove an absence of hazard. Thus, the hazard evaluation process relies on the results of animal toxicity studies as well as on human experience. In the case of trichloroethylene, the epidemiological studies carried out to date, including the most recently completed study by Shindell and Ulrich, do not demonstrate an increase in cancer in a large cohort with trichloroethylene exposure. While all of the epidemiology studies are confounded to varying degrees by the previously mentioned factors common to most epidemiological studies, they are consistent with extensive workplace experience with this solvent. Any assessment of risk, or lack thereof, from trichloroethylene must take these human data into account. EPA also has not attempted an integration of the available human studies.

EPA has calculated the risk to humans as if trichloroethylene were a human carcinogen by applying a linear, non-threshold, mathematical extrapolation model to several sets of animal tumor data for trichloroethylene. The calculated risk is less than likely could be observed in any hypothetical epidemiological study, no matter how extensive, given worst case estimates of exposure conditions and available cohort sizes.

Our views as to the relevance of recent work concerning peroxisome proliferation and oncogenes also lead to an evaluation of the potential carcinogencity of trichloroethylene in humans that is different than EPA's. Trichloroethylene induces rodent tumors through metabolism to trichloroacetic acid, the proximal rodent carcinogen. Human tissue does not induce the biochemical activities associated with a carcinogenic response to trichloroacetic acid in rodents.

Thus, much of the rodent bioassay evidence on trichloroethylene is not relevant to man. Hepatocarcinogenic effects similar to those seen in the mouse have not been observed in the rat, which responds differently to trichloroethylene. Because the rat and the mouse are much more closely related to each other than either is to the human, the lack of replication of the mouse findings in the rat indicates that automatic extrapolation of the B6C3F1 liver tumor response to man is not appropriate.

EPA should describe the animal data as limited instead of adequate. Although many of the effects in animals have been replicated, they are of uncertain biological significance and were obtained at doses that were too high to allow meaningful hazard assessment. EPA needs to integrate the mechanism data which have been developed for trichloroethylene with the bioassay data, in order to develop a plausible hypothesis as to the sensitivity of the mouse relative to the rat and human.

Pharmacokinetic and other evidence suggests that trichloroethylene is not a proximal carcinogen in the mouse. A metabolite, trichloroacetic acid, is the likely cause of the

liver tumor response obs rved in the bioassays. As recently reported by Herren-Freund and coworkers, trichloroacetic acid causes hepatocellular carcinoma in B6C3F1 mice.

Following exposure to trichloroethylene, blood levels of trichloroacetic acid are 7-fold greater in mice than in rats. In cell culture experiments Elcombe has shown that mouse hepatocytes produce 30-fold more trichloroacetic acid that rat hepatocytes, which in turn produce 3-fold more trichloroacetic acid than human hepatocytes. The rat undergoes biochemical responses to trichloroacetic acid in vitro that are similar to those observed in the mouse, when isolated hepatocytes from the two species are exposed in cell culture experiments. The absence of a carcinogenic effect in the intact rat would appear to be a function of its lower rate of production of trichloroacetic acid. Trichloroacetic acid levels vary between species due to pharmacokinetic differences. This can account for species variation in the induction of tumors in rodents.

Trichloroacetic acid is a peroxisomal inducer for rodent liver, both in the intact animal and in cell culture. Peroxisomal induction by many substances has been associated with rodent liver tumors (Attachment B).

Elcombe has shown that peroxisome proliferation does not occur in human hepatocytes following in vitro exposure to trichloroacetic acid, whereas it does occur in rodent hepatocytes. Thus, humans are unlikely to show a carcinogenic response to trichloroethylene, presumably because they fail to respond biochemically by the formation of peroxisomes. Humans also do not respond to a variety of other substances which induce peroxisomes in rodent cells and induce liver tumors in the mouse.

EPA's treatment of the pharmacokinetics of trichloroethylene (also referred to in the section on "oncodynamics") is in error. The evidence strongly favors trichloroacetic acid as the proximal carcinogen, and the pharmacokinetics of this substance are understood.

Some scientists believe that liver tumor findings in the B6C3F1 mouse, in the absence of demonstrable direct genotoxic activity, but in the presence of other epigenetic (promotional) events, are an anomaly and are not always indicative of true carcinogenic risk for man. The high spontaneous incidence of these tumors complicates both the statistical and biological evaluation of the weight of the evidence. Two recent papers (Attachment E) show that DNA from liver tumor tissue taken from B6C3F1 mice that had spontaneously developed the tumors expressed the activated H-ras oncogene. These studies indicate that the B6C3F1 mouse may be genetically predisposed to liver tumors, making it an inappropriate model for direct comparison to the human.

Induction of peroxisomes is not thought to be a linear function of trichloroacetic acid concentration in the liver (i.e., not thought to be a one-hit phenomenon), and in the case of trichloroethylene, is accompanied by the induction of DNA synthesis in the B6C3F1 mouse liver. The data suggest that peroxisomal proliferation and induction of hepatic DNA synthesis, along with the presence of the H-ras oncogene in the B6C3F1 mouse liver, cause the B6C3F1 mouse to be an ultrasensitive indicator of tumor enhancing potential. Genetic predisposition is a critical factor. Henschler has reported that, after oral exposure to purified, amine-stabilized trichloroethylene, Swiss mice do not develop hepatic tumors, whereas NTP has shown that B6C3F1 mice do, although both strains are thought to metabolize trichloroethylene in a similar fashion quantitatively. These data, taken together, support the concept that trichloroethylen enhances spontaneous liver tumors in B6C3F1 mice by a secondary (perhaps promotional) mechanism, which conceptually embodies the principle of a practical threshold.

EPA's summary of the earlier Health Assessment Document (HAD) and its review by the Science Advisory Board is misstated. The HAD described trichloroethylene as belonging in either IARC Group 2B or 3. SAB reviewed this classification and disagreed with the Agency, recommending instead that trichloroethylene belonged in Group 3. IARC Group 3 is not consistent with classification of trichloroethylene in Category B2 under the EPA Guidelines. The statement in the HAD that mouse liver tumors alone constitute sufficient evidence to place trichloroethylene into Category B2 was not reviewed by SAB. It is flatly inconsistent with the Board's position in its recent review of an addendum to the Health Assessment Document for perchloroethylene.

Finally, the draft Addendum states (at 4-5) that Goldsworthy and Popp found that trichloroethylene is more potent than trichloroacetic acid as a peroxisomal inducer. This is not accurate. Goldsworthy and Popp reported that 1000 mg/kg of trichloroethylene increases peroxisome levels 625% in male B6C3F1 mice and 360% in female mice, whereas 500 mg/kg of trichloroacetic acid increased male peroxisome levels by 280% and female by 305%.

EPA has also mischaracterized the results of Bergman, who found DNA adducts only in in vitro experiments. The draft Addendum interprets Bergman's in vitro experiments as if they were whole animal adduct studies. This experimental situation differs vastly from one in which animals exposed to trichloroethylene are shown to have DNA adducts. In fact, Bergman did such experiments and found no DNA adducts. Further, EPA has not analyzed the experiments of Stott, Quast, and Watanabe correctly. Those experiments specified a very low upper bound to possible DNA adducts, based on detection limit, not actual DNA adducts, as described in HAD.

HSIA member companies have conducted (and desire to continue to support) research to provide additional insight into the pharmacokinetics and mechanism of toxicity of trichloroethylene. In addition, the Chemical Industry Institute of Toxicology has studies in progress on hepatic peroxisomal proliferation in mice and renal alpha-2-microglobulin induction in rats.

ATTACHEMENT A

- Text of Comments
- Shindell, S., and Ulrich, S., A Cohort Study of Employees of a Manufacturing Plant Using Trichloroethylene, J. Occup. Med. 27:577-579 (1985).

EPIDEMIOLOGY STUDIES

The draft Addendum indicates that EPA considers the epidemiologic data base inadequate to evaluate the carcinogenic potential of trichloroethylene. Epidemiology studies cannot generally disprove a cancer risk estimate based on animal studies, because the exposures will be lower and because experimental conditions cannot be controlled. Nevertheless, the 1985 Health Assessment Document (HAD) summarizes six such studies on trichloroethylene, none of which indicated elevated levels of cancer deaths. These studies have been criticized by EPA, but none is consistent with or supports the hypothesis that trichloroethylene is a human carcinogen, as the 1985 HAD states (p. 8-128):

> The epidemiologic studies on TCI [trichloroethylene] provide no evidence for its carcinogenicity...

There is, moreover, a subsequent significant addition to the epidemiologic data base for trichloroethylene: a study by Shindell and Ulrich of a cohort of over 2,600 employees who worked three months or more during the period from January 1957 to July 1983 in a manufacturing plant that used trichloroethylene as a degreasing agent. Ninety-eight percent of the cohort was traced, accounting for over 16,000 man-years of employment (i.e., an average of over 6 years per employee) and over 38,000 man-years of follow-up. This study found no increased risk of cancer in the exposed population. Rather, the study found a deficit of cancer-related deaths compared to the number expected in exposed white male workers, and this result was statistically significant ($p < 0.05$). This study offers a far more extensive period of follow-up and a larger cohort than previous studies, and thus adds substantially to the scientific data base concerning human health effects. The occupational exposures in the study, particularly in the past, were orders of magnitude higher than ambient exposures.

The review of the Shindell and Ulrich study in the draft Addendum does not adequately assess the utility of these data to assist in a determination of carcinogenic risk of trichloroethylene exposure to humans. The draft Addendum simply repeats the words of the study authors as to cohort definition, potential for exposure, vital status ascertainment, and mortality experience with little interpretation of the significance of the results. The draft Addendum seems more concerned with the presentation of the data in the Shindell and Ulrich article (e.g., failure to report the distribution of the follow-up and the duration of exposure) than with the actual conduct of the study itself.

In fact, the study follows rather standard cohort analysis procedures. An apparent shortcoming of the study is its inability to determine the exposures which the cohort would have received historically, although the article reports that monitoring of current employees suggests that exposures were within the OSHA Permissible Exposure Limit of 100 ppm. More detail on exposure would be desirable, but this does not preclude a reasonable assessment of the mortality experience of the cohort in order to determine if there is an increased risk of cancer in this population.

In short, the evaluation of the Shindell and Ulrich study in the draft Addendum is inadequate. The draft Addendum tends to focus on the presentation rather than on the strength of the study to detect an effect. There should be an evaluation of the power of the study, i.e., its ability to detect an increased risk of cause-specific mortality when one exists in the cohort, before the study is determined to be inadequate to assess human cancer risk. Power calculations for various detectable risks in the Shindell and Ulrich study are presented below for all cancers, nonrespiratory cancer, and respiratory cancer.

Power

Relative Risk	All Cancers	Nonrespiratory Cancer	Respiratory Cancer
1.5	80.2%	62.0%	44.6%
2.0	99.8%	97.6%	86.9%
3.0	100.0%	100.0%	99.9%

These calculations suggest that this study cohort was of a sufficient size and the age and follow-up were adequate to detect a 50% increased risk of death due to cancer in this working population if in fact one existed. As the Agency tends not to require interspecies site concurrence for cancer, the "all cancers numbers" is the most useful. It demonstrates that this study has very good sensitivity for an epidemiology study. The power of the study to detect increased risk of nonrespiratory cancer and respiratory cancer is also quite good, demonstrating that a two-fold risk could have been detected 90% of the time.

Given the power of the Shindell and Ulrich, the failure of the draft Addendum to use it to calculate an upper bound on risk is a major shortcoming in the document. It is not appropriate to continue to rely on the upper bound computed in the 1985 HAD from the Axelson study, which was far less comprehensive.

A Cohort Study of Employees of a Manufacturing Plant Using Trichlorethylene

Sidney Shindell, M.D., LL.B., and Slack Ulrich, B.Sc.Ch.E.

A prospective study was conducted of 2,646 employees who worked three months or more during the period January, 1957, through July, 1983, in a manufacturing plant that used trichlorethylene as a degreasing agent throughout the study period. Ninety-eight percent of the study cohort were traced: they accounted for 16,338 person-years of employment and 38,052 person-years of follow-up. Mortality experience was found to be generally more favorable than that of the comparable segment of the U.S. population over the same period of time. For the white male cohort there were fewer deaths than expected from heart disease, cancer, and trauma (standard mortality rate for all causes =0.79, p<.01). Reports by current and former employees of health problems requiring medical treatment showed that there were only one third as many persons with heart disease or hypertension as were reported in a comparable reference population studied over the past five years.

In the summer of 1983 a study was initiated at a manufacturing plant in northern Illinois where trichlorethylene (TCE) had been used as a degreasing agent almost exclusively for the whole of the plant's existence. The purpose of the study was to determine whether employees at the facility had experienced any patterns of illness or death different from those observed among comparable segments of the populations of the United States at large and/or of the local geographic area.

During the course of a water survey conducted in the area by the Illinois Department of Energy and Natural Resources,[1] ground water in the area in which the plant was located was found to have an elevated nitrate content, and tests for selected volatile organic compounds showed small amounts of a variety of chemicals in some of the wells of the area as well. The wells supplying water to the plant itself were also found to contain small amounts of TCE. Since TCE is used extensively as a degreasing agent following both the machining and stamping operations at the plant and since all employees drank water containing trace amounts of TCE, concern regarding the long-term health effects of exposure to this chemical was expressed.

While its use in the plant is under controlled conditions, i.e., in degreasing machines that are designed to control the vapors, small amounts of this highly volatile substance tend to escape into the atmosphere. During the reclamation process and while maintenance activities were being performed on these machines, additional possibilities existed for exposure of workers engaged in these activities. Quantification of the amount of the exposure for the entire period of the plant operation was not possible. Monitoring of the levels to which current employees have been exposed revealed conformance to Occupational Safety and Health Administration standards for the work environment. The wells contained amounts comparable with those found in the public water supplies in which the chemical has been detected (43 ppb).

Prior studies of workers exposed to TCE in the work environment have not reflected any long-term hazard. Axelson and co-workers[2] in Sweden studied 518 men exposed to TCE in the 1950s and 1960s and found both the overall mortality experience and the cancer mortality experience to be less than expected (risk ratio of 0.8) during a follow-up of 20 years. Tola and co-workers[3] in Finland examined the mortality experience of 2,117 workers exposed from 1963 to 1976 and found similar results. Two studies in Czechoslovakia similarly reported negative results in a retrospective study of persons with liver cancer[4] and among dry cleaners using TCE[5].

Because these studies either covered a limited number of workers or provided a limited period of follow-up, it was felt that a study dealing with a cohort of more than 2,600 employees and covering an extensive period of em-

From the Department of Preventive Medicine, Medical College of Wisconsin, P.O. Box 26509, Milwaukee, WI 53226 (Dr. Shindell); and Ergotopology, Inc., Milwaukee (Mr. Ulrich).

The study was commissioned by the Warner Electric Brake & Clutch Company and was conducted in its plant in Roscoe, Ill.

ATTACHMENT B

- Prout, M. S., Provan, M., and Green, T., Species Differences in Response to Trichloroethylene, Toxicol. Appl. Pharmacol. 79:389-400 (1985).

- Green, T., and Prout, M. S., Species Differences in Response to Trichloroethylene, Toxicol. Appl. Pharmacol. 79:401-411 (1985).

- Herren-Freund, S. L., Pereira, M. A., Olsen, G. and DeAngelo, A. B., The Carcinogenicity of Trichloroethylene (TCE) and Its Metabolites, Trichloroacetic Acid (TCA), and Dichloroacetic Acid (DCA) in Mouse Liver, Proceedings of AACR, Vol. 27, (1986).

- Daniel, J., The Metabolism of Cl-Labelled Trichloroethylene and Tetrachloroethylene in the Rat, Biochem. Pharmacol. 12:795-802 (1963).

ATTACHMENT C

- Text of Comments

- Henschler, D., Elasser, H., Romen, W., and Elder, E., Carcinogenicity Study of Trichloroethylene, With and Without Expoxide Stabilizers, in Mice, J. Cancer Res. Cln. Oncol. 107:149-156 (1984).

- Kimbrough, R. D., Mitchell, F. L., and Houk, V. N., Trichloroethylene: An Update, J. Toxicol. Environ. Health 15:369-383 (1985).

- Elcombe, C. R., Rose, M. S., and Pratt, I. S., Biochemical, Histological, and Ultrastructural Changes in Rat and Mouse Liver following the Administration of Trichloroethylene, Toxicol. Appl. Pharmacol. 79:365-376 (1985).

- Michalopoulos, G. K., Eckl, P. M., Cruise, J. L., Novicki, D. L., and Jirtle, R. L., Mechanisms of Rodent Liver Carcinogenesis, Toxicol. Ind. Health Vol. 3, No. 1, (1987).

- Butterworth, B. E., Loury, D. J., Smith-Oliver, T., and Cattley, R. C., The Potential Role of Chemically Induced Hyperplasia in the Carcinogenic Activity of the Hypolipidemic Carcinogens, Toxicol. Ind. Health Vol. 3, No. 1, (1987).

OVERALL WEIGHT OF THE EVIDENCE

The overall weight of the evidence supports classification of trichloroethylene as a possible human carcinogen in Group C under the EPA Guidelines. The only unequivocally positive results in the bioassays are mouse liver tumors. These should constitute limited evidence of animal carcinogenicity, in light of the available information showing that trichloroethylene is not genotoxic and that the liver tumors result from peroxisome proliferation. A number of bioassays show mixed results in the mouse lung. The only positive results reported in the rats are either benign, equivocal, or not likely to be related to trichloroethylene administration. In addition, the available human epidemiology data do not support classification of trichloroethylene as a probable human carcinogen.

The animal bioassay results are summarized below. Data on epidemiology, mechanism, and genotoxicity are addressed in Attachments A, B, and D.

I. Mouse Studies

A. 1976 NCI Study

This gavage study showed an increased incidence of liver tumors in male and female $B_6C_3F_1$ mice. Science Advisory Board members and others have offered a number of criticisms of

the study, including the use of massive doses in large amounts of corn oil and improper housing of the test animals. Interpretation of the study is confounded by the presence of stabilizers, including epichlorohydrin, in the test material.

B. 1982 NTP Study

This gavage study also showed an increased incidence of liver tumors in $B_6C_3F_1$ mice. While the test material was purified, the same gavage dosing technique was used as in the 1976 study, resulting in atypical metabolic effects.

C. 1980 Henschler Study

NMRI mice were exposed to purified trichloroethylene by inhalation. No increase in cancer was reported in male mice. An increase in the incidence of lymphomas was observed in females, but the authors did not ascribe this effect to trichloroethylene exposure.

D. 1984 Henschler Study

This corn oil gavage study showed no significant increases in tumors in either sex of Swiss mice gavaged with purified trichloroethylene. The authors concluded that "there is no indication of a tumorigenic potential of pure, amine based-stablized TRI [trichloroethylene]" and "this study does not support the suggestion that trichloroethylene itself is carcinogenic under realistic exposure conditions."

E. 1983 Fukuda Study

ICR mice were treated by inhalation with trichloroethylene. No increased incidence of tumors was

observed in the males. Female mice had an increased incidence of lung tumors at the two highest doses. If adenomas and carcinomas are combined, no increase in tumors was observed. Instead, there was a high incidence of benign adenomas in untreated controls, some of which appeared to convert to adenocarcinomas after exposure to trichloroethylene.

F. Maltoni Study

This inhalation study showed an increased incidence of lung tumors in male Swiss mice and female $B_6C_3F_1$ mice. Only benign adenomas were found, not carcinomas. Hepatomas also were observed in male Swiss mice and both sexes of $B_6C_3F_1$ mice. These results have not been peer-reviewed.

G. Van Duuren Study

This series of studies in female Swiss mice involved administration of trichloroethylene by topical, subcutaneous, and gavage routes, and included initiation-promotion studies. No carcinogenic response was reported.

H. Herren-Freund Study

No statistically significant increase in tumors was found in male $B_6C_3F_1$ mice exposed to trichloroethylene by drinking water in this study. Treatment by trichloroethylene metabolites increased the incidence of liver carcinomas.

II. Rat Studies

A. 1976 NCI Study

This gavage study showed no increased incidence of cancer in Osborne-Mendel rats.

B. 1982 NTP Study

This gavage stady in Fischer 344 rats was considered inadequate to evaluate the presence or absence of a carcinogenic response.

C. 1987 NTP Studies

These gavage studies in four strains of rats were considered inadequate to evaluate the presence or absence of a carcinogenic response.

D. 1980 Henschler Study

This inhalation study showed no increased incidence of cancer in Wistar rats.

E. 1983 Fukuda Study

This inhalation study showed no increased incidence of cancer in female Sprague-Dawley rats.

F. 1986 Maltoni Studies

These studies in Sprague-Dawley rats, both by gavage and inhalation, reportedly showed an increased trend or incidence of leukemias, adenocarcinomas and Leydig cell tumors in males. No increased incidence of cancer was observed in the females. These results have not been peer-reviewed.

III. Other Studies

A. 1980 Henschler Study

This inhalation study showed no increased incidence of cancer in Syrian hamsters.

B. 1978 MCA Study

An audit of this inhalation study in Charles River rats and $B_6C_3F_1$ mice concluded that it was inadequate to evaluate the presence or absence of a carcinogenic response.

IV. Other Data

As discussed in Attachment A, seven epidemiology studies of workers exposed to trichloroethylene show no elevated risk of cancer in the exposed populations. The most recent of these studies, involving a cohort of over 2,600 employees, found a statistically significant deficit of cancer-related deaths in the largest employee group.

As discussed in Attachment B, extensive research has demonstrated that trichloroethylene induces hepatic peroxisome proliferation in mice, but not in rats. Much more extensive metabolism of trichloroethylene occurs in mice than in rats, resulting in much higher blood levels of the metabolite trichloroacetic acid. Man metabolizes significantly less trichloroethylene than mice or rats, resulting in significantly lower levels of trichloroacetic acid in man than in rodents. Moreover, research has established that trichloroacetic acid does not induce peroxisome proliferation in human hepatocytes in vitro.

As discussed in Attachment D, stablized trichloroethylene has the potential to cause marginal increases in genotoxic effects in higher order animals. Purified trichloroethylene appears to be nongenotoxic.

V. Application of the Guidelines

Analysis of the animal bioassay data on trichloroethylene is difficult because of the existence of conflicting results. Two studies have shown an increased incidence of liver tumors in $B_6C_3F_1$ mice. The Science Advisory Board has previously indicated, in the case of tetrachloroethylene, that such results even where replicated do not constitute sufficient evidence under the EPA Guidelines.

Three inhalation studies show conflicting results in four different strains of mice. An increase in lung tumors was reported in male Swiss mice and female $B_6C_3F_1$, mice, and in female ICR mice. No increase in lung tumors was reported in either sex of NMRI mice, male $B_6C_3F_1$, mice, female Swiss mice, or male ICR mice. The mechanism by which lung tumors have been produced is not understood, nor is the reason for the conflicting results in various strains. In these circumstances, pending a determination of the biological significance of the varying mouse lung results, the positive studies should be considered limited, not sufficient, evidence of carcinogenicity in animals.

The draft Addendum emphasizes the Fukuda study as supportive of a finding of sufficient evidence. The Addendum states that the concentration of epichlorohydrin as stabilizer was 0.019%. It does not mention that a number of other putative carcinogenic substances were present as well in the reagent grade of trichloroethylene that Fukuda used.

Scientists from the Centers for Disease Control offered the following critique of the Fukuda study in a recent article*/:

> The incidence of total lung tumors -- that is, adenomas and adenocarcinomas -- was not significantly increased if controls were compared to exposed mice. In addition, the incidence at the higher dose was about the same as that at the lower dose.

In addition, metastases of mammary tumors (in both controls and dosed groups) were observed to spread to the lung. Based on the report it is not possible to tell whether metastatic tumors were included in the tumor count. However, there was a 12% tumor incidence in the lungs of control mice. At most, there might have been an enhancement of spontaneously occurring lung tumors in dosed animals.

Results in rat bioassays have been negative, with the exception of recent gavage and inhalation studies by Maltoni. Maltoni reported no increase in tumors in female rats. There was an increased incidence of Leydig cell tumors in male rats. Maltoni regarded the Leydig cell tumors as benign, as Leydig

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*/ Kimbrough, R.D., Mitchell, F.L., Houk, V.N., Trichloroethylene: An Update, J. Tox. Env. Health 15: 369-383 (1985). This article also points out that the report of the Fukuda study does not indicate whether the slides were examined in a blind fashion.

cells are microscopically benign in character. Historical data would be useful to determine if there is any evidence that these tumors progress to malignancy. Under the EPA Guidelines, benign tumors are considered limited evidence of carcinogenicity unless they have the potential to progress to an associated malignancy of the same histogenic organ.

There was a slight elevation in renal adenocarcinomas and of leukemias in one exposure group in the male rats in the Maltoni study. These findings are also equivocal, however, and it is by no means clear that they are either meaningful or related to trichloroethylene exposure.

In considering the Maltoni results and their relevance for assessing human risk, it must be emphasized that those studies have never received peer review as we know it in the United States. Maltoni publishes his work privately. The other studies in the draft Addendum have generally received scientific scrutiny prior to being reviewed by EPA.

Maltoni breeds his own animals and the particular sensitivity of these breeds and their historical tumor incidence rates need to be understood to place his most recent results in proper perspective. Moveover, Maltoni follows a different procedure from the current "state of the art" method for conducting carcinogenicity bioassays. After exposure for 52 weeks, the animals typically are allowed to live until spontaneous death. This may make it difficult to interpret

the relevance of late-occurring tumors that would not have been detected at terminal sacrifice taking place at weeks 105 through 107. This may result in counting metastatic tumors rather than tumors which arise de novo in an organ, and thus reporting greater potency than is appropriate.

A workshop was held in Washington, D.C. in December 1985 to discuss Maltoni's recent work. Only a few slides were available for review. The workshop resulted in recommendations for

- a pathology review of a 10-20% sample of tumors for a quality assurance evaluation, and
- a detailed statistical analysis of all tumor responses, particularly with mortality correction (this was not done in either the private publication or in the draft Addendum).

A delegation was to have visited Maltoni's laboratory to conduct the pathology review. Since this has never taken place, the quality of the data is subject to question.

While the draft Addendum attempts to correct for mortality, more data are needed to adjust for mortality effects. Similarly, a comparison with historical control data is necessary to understand the nature of the Leydig cell tumors in these studies relative to Maltoni's rat colony.

In conclusion, the animal bioassay data are largely conflicting and equivocal. The mouse lung results are

divided. Tne unequivocally positive mouse liver results have been shown to be caused by induction of peroxisomal proliferation by trichloroethylene metabolites, an effect that would not be seen in humans. The benign Leydig cell tumor increase does not meet the criteria for sufficient evidence. Other assertedly positive responses in male rats reported by Maltoni are too equivocal to be significant. Trichloroethylene should be considered a possible, but not a probable, human carcinogen on the basis of this limited animal evidence.

J Cancer Res Clin Oncol (1984) 107: 149–156

Cancer Research
Clinical Oncology

TCE

Original Papers

Carcinogenicity Study of Trichloroethylene, With and Without Epoxide Stabilizers, in Mice

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Summary. Previous analytical studies of industrial samples of trichloroethylene (TRI) have revealed the presence of mutagenic and carcinogenic epoxides which, it was proposed, might be responsible for the carcinogenicity of such samples, as demonstrated with mice in other laboratories. To test this hypothesis, Swiss mice (ICR/HA) of both sexes, bred and kept in SPF conditions, were dosed daily with TRI in corn oil by gavage (males: 2.4 g/kg, females: 1.8 g/kg) with or without the addition of epichlorohydrin (EPC, 0.8%, w/w), 1,2-epoxybutane (BO, 0.8%), or EPC + BO (0.25% + 0.25%) for 18 months. The ensuing observation period terminated at 106 weeks (from start of experiment). Gross and microscopic examination of all organs revealed a statistically significant increase in the incidence of forestomach papillomas and carcinomas after EPC-, BO-, and (EPC + BO)-stabilized samples of TRI, but not after pure, amine base-stabilized TRI. This type of tumor is believed to be induced by the direct alkylating epoxides epichlorohydrin and epoxybutane, whose industrial use in stabilizing chlorinated aliphatic hydrocarbons should be discontinued. No other significant increase in tumor incidences was found. Again, this study does not support the suggestion that trichloroethylene itself is carcinogenic under realistic exposure conditions.

Key words: Trichloroethylene – Epichlorohydrin – 1,2-Epoxybutane – Carcinogenicity.

Introduction

The assumed carcinogenic potential of trichloroethylene (TRI) (NIOSH 1976) is still the subject of debate. Findings on its mutagenicity in vitro are controversial (Greim et al. 1975; Shahin and von Borstel 1977; Bartsch et al. 1979). A long-term inhalation study in rats, mice, and Syrian hamsters, with 0, 100, and 500 ppm of highly purified trichloroethylene stabilized with 0.0015% triethanolamine, gave no indication of a tumorigenic potential of TRI; a dose-related increase in lymphoma rate in female mice was regarded as a possibly nonspecific effect, or at least not as proof of a carcinogenic potential (Henschler et al. 1980a).

A bioassay with industrial-grade TRI at maximum tolerated doses resulted in a dose-related increase in malignant liver tumors in both male and female $B_6C_3F_1$ mice, but not in rats (NIOSH, 1976). The industrial TRI sample was stabilized with epichlorohydrin (EPC) and 1,2-epoxybutane (BO), both of which are mutagenic and suspected carcinogenic substances which might cause, or contribute to, the carcinogenic effect (Henschler et al. 1977). To test this hypothesis and to elucidate the significance of the observed liver carcinomas in a strain of mice in one parent strain of which, C_3H, this kind of tumor reputedly has a high sponaneous incidence (Deringer 1959; Heston et al. 1960; Sabine et al. 1973), we performed a comparative long-term carcinogenicity study by gavage at similarly high doses of TRI alone and of TRI with added epichlorohydrin and/or 1,2-epoxybutane in a strain of mice with no or minimally few spontaneous liver tumors (Eaton et al. 1980).

Materials and Methods

Experimental Animals

In all, 600 SPF-bred ICR/HA-Swiss mice (Ivanovas GmbH, Kisslegg/Allgäu) aged 5 weeks were randomized into 12 groups of 50 animals each. Body weights varied over ranges of 19–28 g (males, 6 groups) and 18–26 g (females, 6 groups). The mice were kept in transparent plastic cages (Makrolon) covered with stainless steel wire, in subgroups of 5 each (females) or singly (males). A diet of standardized pellets (Altromin no. 1324) and tap water, autoclaved and acidified to pH 3 with HCl, was available ad libitum 24 h/day.

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TRICHLOROETHYLENE: AN UPDATE

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The toxicity of tricholoroethylene (TCE) has been summarized in a number of reviews. In this particular update, only the more recent studies that deal with metabolism and carcinogenicity have been examined. In reviewing the more recent publications on metabolism of TCE, we determined that differences exist in its metabolism if low doses are compared with high doses in animals. There may also be a difference in the metabolism of TCE between different species—namely mice, rats, and humans. TCE has not been shown to be a potent carcinogen in rats and it only seems to be a potent carcinogen in one specific strain of mice, namely the B6C3F1 mouse. Epidemiology studies have been rather limited. The number of persons examined so far for chronic toxic effects is small, compared with the enormous size of the work force that is exposed to TCE over prolonged periods. On an empirical basis, the occupational experience with TCE does not suggest that this compound is a potent carcinogen. The risk associated with exposure to trace amount (ppb) concentrations of TCE in water appear to be minimal or perhaps negligible.

Because there are differences in metabolism of TCE, it is important that theoretical risks attributed to TCE in the past be reexamined. It is highly possible that in humans, the metabolic pathway leading to the formation of the proximate carcinogen is not activated at low doses, where TCE is excreted by first-order kinetics.

INTRODUCTION

Tricholoroethylene (TCE) is a solvent used extensively for vapor degreasing of fabricated metal parts. To a minor degree, it is used as a solvent in the textile industry, as a solvent for adhesives, as a lubricant, and as a low-temperature heat transfer fluid. It is a component in several consumer products such as spot removers and cleaning fluids for rugs. A pharmaceutical grade of TCE is used as a general anesthetic in surgical, dental, and obstetrical procedures, and as an analgesic in the treatment of trigeminal neurolgia. It has also been used as a disinfectant and detergent for skin, minor wounds, and surgical instruments. It has also been used on a variety of animals as a volatile anesthetic (IARC, 1979).

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health and Human Services.

Requests for reprints should be sent to Renate D. Kimbrough, Center for Environmental Health, Centers for Disease Control, Public Health Service, U. S. Department of Health and Human Services, Atlanta, Georgia, 30333.

Journal of Toxicology and Environmental Health, 15:369-383, 1985

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TOXICOLOGY AND APPLIED PHARMACOLOGY 79, 365–376 (1985)

Biochemical, Histological, and Ultrastructural Changes in Rat and Mouse Liver following the Administration of Trichloroethylene: Possible Relevance to Species Differences in Hepatocarcinogenicity

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Received January 21, 1984; accepted February 6, 1985

Biochemical, Histological, and Ultrastructural Changes in Rat and Mouse Liver following the Administration of Trichloroethylene: Possible Relevance to Species Differences in Hepatocarcinogenicity. ELCOMBE, C. R., ROSE, M. S., AND PRATT, I. S. (1985). *Toxicol. Appl. Pharmacol.* 79, 365–376. Trichloroethylene (TRI), administered by gavage for 10 consecutive days, at doses of 500 to 1500 mg/kg body wt increased liver weight (175% of control), decreased hepatic DNA concentration (66% of control), and increased the synthesis of DNA (500% of control; as measured by [^{3}H]dT incorporation) in B6C3F$_1$ mice and Alderley Park mice. Similar treatment of Osborne–Mendel rats or Alderley Park rats resulted in smaller increases in liver weight (130% of control) and decreases in DNA concentration (83% of control). No effect of TRI on DNA synthesis was seen in rats. The increased DNA synthesis in the mouse was not apparently due to regenerative hyperplasia since no signs of necrosis were seen. Furthermore the increased [^{3}H]dT incorporation probably represented semiconservative replication of DNA and not repair, since a parallel increase of mitotic figures was observed. Hence, the liver growth noted after TRI administration appears to be due to liver cell enlargement (hypertrophy) in the rat, but both hypertrophy and hyperplasia (cell proliferation) in the mouse. An important observation has been that TRI induced the peroxisomal enzyme activities, catalase, and cyanide-insensitive palmitoyl-CoA oxidation (147 and 786% of control, respectively), in mice but not in rats. Furthermore, increases in peroxisome volume density (up to 1110% of control) were observed in mice receiving TRI. These observations lead us to suggest that the species difference in hepatocarcinogenicity of TRI, seen between the rat and mouse, is possibly due to a species difference in peroxisome proliferation and cell proliferation, the peroxisome proliferation leading to increased reactive oxygen species and DNA damage, and the cell proliferation then acting to promote this lesion.

Trichloroethylene (TRI) is a colorless, volatile liquid which has been used extensively as an industrial solvent. Its primary use is a fat solvent for degreasing metal parts prior to painting, anodizing, and electroplating. Worldwide production of TRI was estimated to be 2260 million pounds in 1973 (McConnell *et al.*, 1975).

Studies by the National Cancer Institute (1976) demonstrated an increased incidence of hepatocellular carcinoma in B6C3F$_1$ hybrid mice after oral administration of TRI at high doses for 78 weeks. In a parallel study no increased incidence of hepatocellular carcinoma was observed in Osborne–Mendel rats. The significance of these studies was questioned because the TRI utilized contained mutagenic epoxide stabilizers. However, more recent studies by the National Toxicology Program (1983) with non-epoxide stabilized material over a period of 103 weeks have

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TOXICOLOGY AND APPLIED PHARMACOLOGY 79, 389–400 (1985)

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Species Differences in Response to Trichloroethylene

I. Pharmacokinetics in Rats and Mice

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Received June 29, 1984; accepted February 28, 1985

Species Differences in Response to Trichloroethylene. I. Pharmacokinetics in Rats and Mice. PROUT, M. S., PROVAN, W. M., AND GREEN, T. (1985). *Toxicol. Appl. Pharmacol.* 79, 389–400. The elimination of radioactivity in two strains of rats and mice following a single po dose of trichloro[^{14}C]ethylene at dose levels from 10 to 2000 mg/kg has shown a marked dose dependence in rats but not in mice. The metabolism of trichloroethylene in the mouse was linear over the range of doses used, whereas in the rat it became constant and independent of dose at 1000 mg/kg and above. At the 10-mg/kg dosage, both species metabolized trichloroethylene almost completely, 60% of the dose being excreted in urine with only 1 to 4% being eliminated unchanged in expired air in the first 24 hr. At 2000 mg/kg, 78% of the dose was eliminated unchanged in the rat, but only 14% in the mouse. Consequently at high dosages, the mouse was exposed to significantly higher concentrations of trichloroethylene metabolites than the rat. Blood level kinetics of trichloroethylene and its metabolites confirmed a faster rate of metabolism in the mouse than in the rat. Peak concentrations of the metabolites were reached within 2 hr of dosing in the mouse compared to 10 to 12 hr in the rat. The concentrations of both trichloroethanol (4×) and trichloroacetic acid (7×) were significantly higher in the mouse than in the rat. Whereas trichloroethanol was rapidly eliminated from blood, the higher concentrations of trichloroacetic acid were maintained for over 30 hr. The high blood quantities of trichloroethylene-derived trichloroacetic acid are known to induce hepatic peroxisome proliferation in mice but are insufficient to induce this response in rats. These data suggest that trichloroacetic acid blood amounts, peroxisome proliferation, and the link between peroxisomes and liver cancer are the basis of species difference in response to trichloroethylene.

1,1,2-Trichloroethylene is a volatile, nonflammable liquid used extensively in vapor degreasing of fabricated metal parts. In recent years two carcinogenicity bioassays (NCI, 1976; NTP, 1983) have indicated that the daily administration of high oral doses of trichloroethylene leads to an increased incidence of hepatocellular carcinoma in B6C3F1 mice. In the same studies trichloroethylene did not cause hepatocellular carcinoma in Osborne–Mendel (NCI, 1976) or Fischer 344 (NTP, 1983) rats.

Several mechanisms have been suggested to explain trichloroethylene-induced hepatocarcinogenicity. These have included the interaction of reactive trichloroethylene metabolites with cellular macromolecules and in particular DNA (Greim *et al.*, 1975; Simmon *et al.*, 1977) which may result in carcinogenicity by somatic mutation. It has also been suggested that cytotoxicity and the resulting cell division caused by chemicals of this type enhance the high background incidence of hepatocellular carcinoma found in B6C3F1 mice (Schumann *et al.*, 1980; Elashoff *et al.*, 1979). These two mechanisms are those most

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TOXICOLOGY AND APPLIED PHARMACOLOGY 79, 401–411 (1985)

Species Differences in Response to Trichloroethylene

II. Biotransformation in Rats and Mice

T. GREEN[1] AND M. S. PROUT

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Received June 29, 1984; accepted February 28, 1985

Species Differences in Response to Trichloroethylene. II. Biotransformation in Rats and Mice. GREEN, T., AND PROUT, M. S. (1985). *Toxicol. Appl. Pharmacol.* 79, 401–411. Detailed analysis of urine from two strains of rats and mice dosed po with trichloroethylene at four doses from 10 to 2000 mg/kg failed to detect any major species or strain differences in the metabolism of trichloroethylene. Although a greater proportion of the dose was metabolized in mice than in rats, the relative proportions of the major metabolites were very similar in both strains and were unaffected by the dose amount. Analysis of the same urine samples for minor metabolites failed to establish a major species difference. Small amounts of dichloroacetic acid (<1% of the dose) were present in both rat and mouse urine and were not considered significant. Monochloroacetic acid accounted for <0.1% of the dose. Daily dosing of trichloroethylene (1000 mg/kg po) for 180 days did not induce the overall metabolism of trichloroethylene but did double the urinary excretion of trichloroacetic acid. This finding was accompanied by an equivalent percentage decrease in the concentration of trichloroethanol. CO_2 has been shown to be a major metabolite of trichloroacetic acid, suggesting that this is the source of trichloroethylene-derived CO_2. Trichloroacetic acid was also excreted in bile in both rats and mice suggesting possible conjugation of this metabolite in the liver. Very little evidence was found for the formation of chemically reactive species from trichloroethylene in either rats or mice and none that could be the basis of a major species difference. The increased rate of metabolism in the mouse, the resulting high blood concentrations of trichloroacetic acid, and stimulation of hepatic peroxisome proliferation in this species appears to be the major species difference possibly related to tumor formation in the liver. The conjugation of trichloroacetic acid and its metabolism to CO_2 may be related to peroxisome proliferation.

It was first suggested by Powell (1945) and again later by Byington and Leibman (1965) that trichloroethylene is metabolized via an epoxide to chloral and chloral hydrate. During the 1970s many studies suggested that monochloroethylene (vinyl chloride) is an animal and human carcinogen as a result of metabolism to a reactive electrophilic epoxide intermediate (IARC, 1979a). This work implied that the other chloroethylenes, including trichloroethylene, could present a similar risk. Subsequent bioassays of vinylidene chloride (IARC, 1979b), trichloroethylene (NCI, 1976; NTP, 1983), and perchloroethylene (NCI, 1977) have demonstrated each of them to be carcinogens in laboratory animals and it is generally assumed that epoxides play a role in their carcinogenicity (Henschler *et al.*, 1976). There is no evidence, however, to suggest that these other chloroethylenes are human carcinogens; their potency in laboratory animals is considerably less than that of

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The Carcinogenicity of Trichloroethylene (TCE) and Its Metabolites, Trichloroacetic Acid (TCA) and Dichloroacetic Acid (DCA), in Mouse Liver. [1]S.L. Herren-Freund, [1]M.A. Pereira, [2]G. Olson, and [1]A.B. DeAngelo. [1]U.S. EPA, Cincinnati, OH 45268 and [2]Pathology Associates, Inc., Cincinnati, OH 45229 (Introduced by T.V. Reddy)

TCE, which is used as an industrial solvent, has been shown to be carcinogenic in mouse liver. The metabolism of TCE results, in part, in the formation of chlorinated acetic acids: TCA and DCA. These chlorinated acetic acids have been shown to be non-genotoxic and to induce peroxisome proliferation. Therefore, we determined the ability of TCE, TCA and DCA to act as tumor promoters in mouse liver. Male, B6C3F1 mice were administered i.p. 2.5 ug/g ethylnitrosourea (ENU) on day 15 of age. At 28 days of age, the mice were placed on drinking water containing TCE (5 and 50 mg/l), TCA (2 and 5 g/l), or DCA (2 and 5 g/l). All drinking water had a final pH of 7.0. The animals were killed after approximately 61 weeks of exposure (65 weeks of age). Both DCA and TCA at a dose of 5 g/l were carcinogenic without prior initiation with ENU and resulted in 86% and 50%, respectively, of the animals with tumors. Exposure to TCE at 50 mg/l without prior ENU initiation resulted in 19% of the animals with tumors. Pretreatment with ENU did not increase the tumor incidence in the TCE, TCA or DCA treated mice. ENU treatment by itself yielded tumors in 11% of the animals. Only 3% of the untreated animals had tumors. All three of these compounds induced peroxisome proliferation in mouse liver with TCA being the strongest induced followed by DCA and then TCE. Thus the carcinogenic potency of these compounds appeared not to parallel their relative ability to induce peroxisomes. This abstract does not necessarily reflect EPA policy.

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CELL CYCLE-DEPENDENT INITIATION OF HEPATOCARCINOGENESIS BY (±)BENZO(a)PYRENE DIOL-EPOXIDE I (BPDE). RJ Rahija, WK Kaufmann, DG Kaufman. Department of Pathology and Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC 27514

The sensitivity of proliferating cells to carcinogenesis may be influenced by the phase of the cell cycle at which carcinogen-induced damage is incurred, the ability of cells to repair DNA damage, and replication of damaged DNA. To investigate the inter-relationships of these factors during the initiation of hepatocarcinogenesis, hepatocyte proliferation was induced in F344 rats by a two-thirds partial hepatectomy (PH) and rats received one dose of BPDE (0.02 umole/kg) via the portal vein when hepatocytes were in early G_1 (4h) or early S (15h). Carcinogenesis was promoted with dietary phenobarbital and neoplasms were enumerated 45 weeks post-treatment. Repair of DNA damage and the effect of BPDE on DNA synthesis were also evaluated by utilizing an ELISA for BPDE-DNA adducts and by measuring the specific activity of ^{3}H-thymidine incorporation into DNA. Rats treated when hepatocytes were in early S had a significantly higher incidence of hepatocytic neoplasms (80%) than did rats treated in early G_1 (42%). The peak of DNA synthesis after PH was delayed by 22 hrs in livers treated when hepatocytes were in early G_1. In the same livers, adducts were reduced by 50% at the time of this peak of DNA synthesis. These preliminary results suggest that hepatocyte susceptibility to initiation of carcinogenesis by BPDE is cell cycle-dependent, with hepatocytes in early S being more sensitive than hepatocytes in early G_1. For G_1 phase hepatocytes the delay in DNA synthesis following BPDE treatment may have reduced the risk by providing additional time for the repair of damaged DNA. Supported by NIH grants CA31238 and ES0717.

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PHASE II TRIAL OF N-METHYLFORMAMIDE (NMF) IN HEAD AND NECK CANCER. W.C. Vogel, A.A. Fora sugi, R.B. Natale, G. Schnur. The University and Ann Arbor VA Medical Centers, Ann Arbor,

NMF is a polar compound which demonstrate activity and cell differentiation in pre-cli In Phase I studies dose-limiting toxicity wa observed primarily with weekly doses exceedi Nausea and vomiting and cardiac toxicity als higher doses. In this trial, the initial do 1000 mg/M^2 IV bolus, days 1-5 (3 pts) and, 3 pts). In the absence of toxicity dose was es mg/M^2/cycle (max. 1000 mg/M^2). Dose was red severe (gr 3) nausea and vomiting. The media (range 46-69) and performance status 80 (ran pt had no prior therapy, 4-radiotherapy (RT) apy (CT), 5-surgery and RT, 6-surgery + RT + had adequate bone marrow reserve, serum crea normal SGOT, SGPT, serum bilirubin, alkaline (AP). Pts with heart disease were not eligib uable pts (2 too early) 15 had progression o treated with 800 mg/M^2 was withdrawn from st severe hepatotoxicity. Nausea and vomiting pts and was severe in 4. Hepatotoxicity def. liver function test (LFT) elevated 3 X basel 9/20 cycles: elevated SGOT in 9/20 cycles, S AP-3/20 cycles. One patient was removed from elevation >100 X baseline. Two pts had pers abnormalities which delayed retreatment. One tant mild LFT abnormalities 4 months after t penia occurred in 2 pts (WBC 3,600 and 3,200 thrombocytopenia in 2 (platelets 62,000 and mm^3). No cardiotoxicity was observed. These cate that NMF as used in this trial is not a in the treatment of squamous cell head and n patients.

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SPECTRUM OF N-ACETOXY-2-ACETYLAMINOFLUORENE-I MUTATIONS IN THE MAMMALIAN DHFR LOCUS. A.M. R.W. Steigerwalt, G. Urlaub, L. Chasin, and Grunberger, Columbia University, Institute of Research and Department of Biological Sciences NY 10032.

We are examining the spectrum of mutatio by the carcinogen, N-acetoxy-2-acetylaminoflu at the dihydrofolate reductase locus of Chine ovary cells. We have obtained 29 independent starting with a DHFR$^-$ hemizygote cell line. the locus is detected by Southern blot analys tant and parental DNAs digested with BstXI a a mixed probe of 10 genomic and cDNA fragment mutants showed a banding pattern identical to strain and are likely to carry point mutation point mutations were localized by restriction lymorphism; in one, a TaqI site in exon 1 and a SacI site in exon 3 were lost. In a third, transversion creating a new BstXI site in ex curred. Furthermore, six deletion mutants wer In two other deletion mutants, however, the r ments of the gene are no longer colinear sugg translocation, inversion, or duplication. We by HPLC the ratio of deacetylated (88%) to ac (12%) dG adducts formed in the parental cells ment with AAAF. The ratio of point mutations gross structural changes (28%) is similar to dG-AF and dG-AAF adducts formed indicating a correlation between the type of conformationa duced by the adducts and the mutational spect work was supported by NIH grants CA 39537 and

ATTACHMENT D

- Text of Comments
- Bergman, K., Interactions of Trichloroethylene with DNA in vitro and with RNA and DNA of Various Mouse Tissues in vivo, Arch. Toxicol. 54: 181-193 (1983).
- Stott, W. T., Quast, J. F., and Watanabe, P. G., The Pharmacokinetics and Macromolecular Interactions of Trichloroethylene in Mice and Rats, Toxicol. Appl. Pharmacol. 62:137-151 (1982).

GENOTOXICITY

EPA's conclusion concerning the genotoxicity of trichloroethylene in the 1985 Health Assessment Document (HAD) was apparently carried forward into the draft Addendum. The 1985 HAD states (p.1-3):

> A conclusion about the mutagenic potential of pure TCI [trichloroethylene] cannot be made. If TCI is mutagenic, the available data suggest that it would be a very weak indirect mutagen.

This conclusion was based on (1) overinterpretation of a few marginally positive results at very high doses, (2) misinterpretation of the DNA binding study of Stott and coworkers, in which a detection limit was taken as suggesting that "TCI [trichloroethylene] or its metabolites are capable of binding to DNA but only to a limited extent," and (3) speculation about the role of trichloroethylene oxide, a labile, postulated intermediate in the metabolism of trichloroethylene. In fact, trichloroethylene has been tested extensively in many different biological systems, including man. In the 1985 HAD, 19 studies were considered negative, 10 were thought positive and the results of the remaining 17 were not interpretable. The only large increases came in a mouse assay for which there is an alternative interpretation based on peroxisomal induction. (Peroxisomal induction leads to increased levels of intracellular hydrogen peroxide, and hydrogen peroxide is mutagenic.) The draft Addendum summarizes new data since the 1985 HAD, but does not evaluate these studies or integrate this information with the previous evaluation. Six out of seven of the new assays are negative.

Trichloroethylene is generally not considered to be genotoxic. While the existing data are difficult to interpret because much testing of trichloroethylene has been done on commercial grades containing stabilizers and impurities, there is a consensus that trichloroethylene is either not mutagenic, or is at most a very weak, indirect mutagen when stabilized. According to Elcombe:

> TRI (trichloroethylene) has been extensively examined for mutagenic potential, but many studies were bedeviled by the presence of mutagenic epoxide stabilizers. However, in general, TRI has been found to be only "marginally" mutagenic or non-mutagenic.

Similarly, the 1985 HAD concludes (p. 7-36):

> The available data provide suggestive evidence that commercial-grade TCI (trichloroethylene) is a weakly active, indirect mutagen. Based on this, commercial TCI may have the potential to cause weak or borderline increases above the spontaneous level of mutagenic effects in exposed human tissue. The data on pure TCI do not allow a conclusion to be drawn about its mutagenic potential. However, mutagenic potential cannot be ruled out. If it is mutagenic the available data suggest TCI would be a very weak, indirect mutagen.

This distinction between stabilized and pure trichloroethylene is of considerable importance and should be emphasized in the Addendum.

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Elcombe, C.R. "Species Differences in Carcinogenicity and Proxisome Proliferation Due to Trichloroethylene: A Biochemical Human Hazard Assessment," Arch. Toxicol. Suppl. 8: 6-17 (1985).

Arch Toxicol (1983) 54: 181–193

Archives of TOXICOLOGY

Interactions of Trichloroethylene with DNA in vitro and with RNA and DNA of Various Mouse Tissues in vivo

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Abstract. The covalent binding of ^{14}C-1,1,2-trichloroethylene (^{14}C-TRI) metabolites to calf thymus DNA in vitro and to RNA and DNA of mouse brain, lung, liver, kidney, spleen, pancreas, and testis after repeated i.p. injections has been studied. Hydrolysates of DNA reacted with ^{14}C-TRI in vitro and hydrolysates of RNA and DNA from selected organs were separated on Aminex A6 for quantitation of alkylation products. The presence of 3,N^4-etheno(deoxy)cytidine, 1,N^6-etheno(deoxy)adenosine and 1,N^6-ethenoadenine was investigated.

No radioactivity could be registered in DNA incubated with ^{14}C-TRI in the absence of liver microsomes. Covalent binding of ^{14}C-TRI to DNA took place in the presence of liver microsomes from control mice. The binding was enhanced by 50% if liver microsomes from phenobarbital pretreated mice were used. The radioactivity in DNA reacted with ^{14}C-TRI and microsomes from control mice was eluted in early fractions and together with thymidine. The same two peaks appeared on chromatography of DNA incubated with ^{14}C-TRI and liver microsomes from phenobarbital pretreated mice. In addition, radioactivity was eluted together with 1,N^6-ethenoadenine.

Radioactivity was registered in RNA and DNA from all of the studied organs after i.p. injections of ^{14}C-TRI. The radioactivity in RNA increased in the order brain < testis < pancreas < kidney < liver < lung < spleen. The radioactivity in DNA increased in the order brain < kidney < testis < lung < pancreas < liver < spleen.

Aminex A6 chromatography revealed that the entire radioactivity in RNA from liver and kidney and in DNA from kidney, testis, lung, pancreas, and spleen was due to metabolic incorporation, particularly into guanine and adenine. This finding indicates that the C-C bond in TRI is split, with the formation of C_1-fragments, during biotransformation in vivo. In liver DNA,

Send offprint requests to: K. Bergman's present address: Toxicology Laboratory, National Food Administration, Box 622, S-751 26 Uppsala, Sweden

ATTACHMENT E

- Fox, T. R., and Watanabe, P. G., Detection of a Cellular Oncogene in Spontaneous Liver Tumors in B6C3F1 Mice, Science 228:596-97 (1985).

- Fox, T. R., Schumann, A. M., and Watanabe, P. G., Activation of a Cellular Proto-Oncogene in Spontaneous Liver Tumor Tissue of the B6C3F1 Mouse, Arch. Toxicol. 10:217-227 (1987).

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