HALOGENATED SOLVENTS INDUSTRY ALLIANCE

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TO: HEALTH AND SCIENCE COMMITTEE

DRINKING WATER ARTICLES

Attached are two articles sent to me by Kathy Rhyne. I am forwarding a copy of each to you as I suspect there is a chance you may have missed them, considering the journal.

You will not be pleased with the conclusion of the first paper, while the results of the second are a little more cheering.

Paul A. Cammer
Executive Director

Attachment

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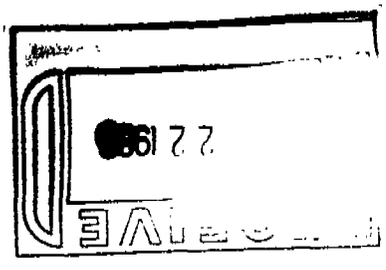

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CARCINOGENICITY STUDY IN RATS WITH A MIXTURE OF ELEVEN VOLATILE HALOGENATED HYDROCARBON DRINKING WATER CONTAMINANTS

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ABSTRACT

A lifetime carcinogenicity study was carried out in Wistar rats, with a mixture of the following halogenated hydrocarbons: trichloromethane, tetrachloromethane, monobromodichloromethane, trichloroethylene, tetrachloroethylene, 1,2,-dichlorobenzene, 1,3,-dichlorobenzene, 1,4,-dichlorobenzene, 1,2,3,-trichlorobenzene, 1,2,4,-trichlorobenzene, 1,3,5-trichlorobenzene. From this mixture 0.22, 2.2, or 22 mg was added per liter drinking water representing concentrations being three orders of magnitude higher than found in several water wells. Most of the changes found in body weight, hematology and pathology correlated with intercurrent diseases or were in accordance with background pathology. With respect to incidence and time of occurrence of tumors, no significant differences were found between the control and the high dose group when lifespan correction was applied. Thus it is concluded that in the present study no significant toxic or carcinogenic effects are induced by lifetime exposure of rats to a mixture of volatile halogenated hydrocarbons in the drinking water.

INTRODUCTION

From a study performed by the National Institute of Public Health and the National Institute of Water Supply it appeared that 20 out of 232 ground water wells in the Netherlands were contaminated by volatile halogenated hydrocarbons in concentrations over 1 µg/l (Zoeteman, 1979).

For the contamination of ground water several sources can be incriminated, the major sources being disposal of industrial wastes and industrial impoundments and solid waste disposal sites (Speth et al., 1981). Another cause of contamination of drinking water in certain areas is the chlorination of drinking water in order to destroy harmful bacteria, yielding trihalogenated methanes in particular (Deinzer, 1978, Zoeteman, 1979, Crump, 1982). In order to investigate the long term effects with emphasis on carcinogenic properties of these contaminants, a lifetime study has been carried out with a mixture of eleven volatile halogenated hydrocarbons; the choice of these compounds was based upon their occurrence in drinking water wells (Zoeteman, 1979).

EXPERIMENTAL

Animals and maintanance

For the experiment, SPF-derived outbred weanling Wistar rats, (Riv: Tox [M]) were obtained from the Institute's own breeding colony, and allotted to a control group and three dose groups, each group consisting of 60 ♀♀ and 60 ♂♂ rats. They were distributed littermate and aselect over the groups, and were kept under conventional conditions, two per cage according

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to sex. The animal rooms were controlled at a temperature of 22 ± 2°C, a 12 hr light-dark cycle and a relative humidity of 40-60%. The air in the animal rooms was continuously refreshed (ventilation fold: 8/hr). To prevent substantial exposure of control animals to the volatile test compounds via the inhalatory route, control and treatment groups were housed separately. The rats received a commercially available semi-synthetic diet (Muracon SSP-Tox standard, Trouw Ltd., Putten, The Netherlands) during the first 33 weeks of the experiment and thereafter a Laboratory Animal Diet (RMH-B Hope Farms, Woerden, The Netherlands). Food and drinking water were provided ad libitum. The diets were regularly analyzed for contaminants, essential elements and vitamins. During weeks 66 and 67, 4 ml Sulfadimidine Na. 33 (Aesculaap BV., Boxtel, The Netherlands) was added per liter drinking water for the treatment of a respiratory tract disease.

Chemicals

The following eleven halogenated hydrocarbons were selected for the mixture under investigation, and were obtained from Fluka unless otherwise stated: trichloromethane (Merck), tetrachloromethane, monobromodichloromethane, trichloroethylene, tetrachloroethylene, 1,2,-dichlorobenzene (Merck), 1,3,-dichlorobenzene, 1,4,-dichlorobenzene, 1,2,3,-trichlorobenzene, 1,2,4,-trichlorobenzene, 1,3,5-trichlorobenzene. Their purity was 97% or higher.

Dosages and route of administration

The rats received drinking water to which 0, 0.22, 2.2 or 22 mg of a mixture consisting of equal quantities of the halogenated hydrocarbons in 1 ml ethanol was added per liter, and which was prepared daily; control animals received 1 ml ethanol/l drinking water. The highest dose was based upon the maximum quantity of this mixture soluble in 1 ml ethanol. The concentrations of the individual halogenated hydrocarbons in the drinking water were measured regularly. From this it appeared that substantial losses had occurred, presumably during preparation procedures, yielding actual concentrations 15 - 50% lower than the intended ones. During a 24-hr period, losses were only minimal, except for tetrachloromethane in groups 2 and 3, and for tetrachloroethylene, 1,3,5- and 1,2,4-trichlorobenzene in group 4. In the tap water, used for drinking water preparation for all groups, minimal concentrations of trichloromethane were measured (0.001 > - 0.019 mg/l). Also, the air in the animal rooms was regularly analyzed, from which it was concluded that the exposure of the animals to chlorinated hydrocarbons via the inhalatory route can be neglected. In addition, the occupational risk for the technicians was considered negligible since the concentrations of those halogenated hydrocarbons of which MAC values are known were several orders of magnitude lower than these limits.

Observations

Health, behaviour and mortality of the animals were checked daily and animals, when moribund or showing large tumor masses, were killed and autopsied. Body weight and water consumption were recorded weekly during the first twelve weeks, and thereafter every four weeks; after one year, water consumption was recorded only every eight weeks.

Hematology was performed by Dr.P.W.Helleman, Unit Clinical Chemistry

and Hematology. Blood samples were collected under ether anesthesia from the retro-orbital plexus after 12 and 24 months of exposure, from 10 animals of each sex and group. The following parameters were determined: hemoglobin concentration , hematocrit value (packed cell volume), erytrocyte, total and differential leucocytes counts. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

After 25 months of exposure, the surviving animals were killed by bleeding from the abdominal aorta under ether anesthesia. All animals, both after scheduled and intercurrent death were subjected to detailed gross inspection. From all animals, the following tissues and organs were collected except when advanced autolysis had occurred: brain, heart, lungs, liver, spleen, kidneys, pituitary, thyroid, thymus, pancreas, adrenals, ovaries, testes, uterus, prostate, mesenteric lymph nodes, submandibular salivary glands, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon and rectum, urinary bladder, vertebral column, sciatic nerve, quadriceps muscle. In addition, all tumors and lesions suspected of being tumorous were collected. Samples were fixed, embedded in paraffin wax and 5 µm sections were cut and stained with hematoxylin and eosin (HE).

Histopathological examination for cancerous and possible precancerous lesions was carried out on all animals autopsied in the control and high dose group. Also from these groups, ten randomly selected male and female rats surviving two years were subjected to histopathological examination for non-neoplastic lesions.

Except for pathology, experimental data were analysed by the Student's t-test. Pathology data, if indicated, were subjected to statistical analysis according to the prevalence method (Peto et al., 1980).

RESULTS

After four months a high incidence of intercurrent deaths occurred caused by gastrointestinal obstruction from trichobezoars. This condition was almost exclusively observed in females and the incidence was higher in the treatment groups than in the control group. Changing the diet from Muracon SSP-Tox standard to RMH-B was followed by a drop in mortality due to gastro-intestinal obstructions. The latter diet has a higher crude fiber content, which might have exerted a stimulating effect upon gastrointestinal motility and function.

After 60 weeks of exposure, a high morbidity and mortality from a respiratory tract disease was observed in the treatment groups, from which predominantly males were affected. From the lungs of the diseased animals, frequently Bordetella bronchiseptica was isolated among several other microorganisms. Therefore a two-week treatment with sulfadimidine in the drinking water was started for control and treatment groups.

Towards the end of the study age-related clinical signs developed, such as weight loss, poor condition of the fur, dyspnoea, posterior paralysis, chronic peritarsitis. These features occurred equally among control and treatment groups. Except for scheduled sacrifices survival after 12 months was 100% for control males and 96% for males from the top-dose group. Control females showed 82% and top-dose females 74% survival after this period. After 24 months these data were 55% and 38% for males and 49% and 34% for females.

During the first six weeks a significant higher body weight gain

occurred in the treatment groups compared with the controls. This phenomenon was not dose-related. During the course of the experiment growth retardation occurred in females of groups 3 and 4 from week 16 onwards and in males from the treatment groups starting at week 60; this feature coincided with the underlying diseases, intestinal obstruction and respiratory infections, respectively.

During the first experimental month, treatment groups showed a higher water consumption than controls, whereas from week 9 a lower water consumption was observed; this dose-related decrease in water consumption persisted in females until the end of the experiment, and returned to the control level in the males after the first experimental year. An increase in water consumption in all groups occurred from week 32 - 36: this coincided with the change of the diet and with the period at which major intercurrent mortality declined.

In hematology after two years significant increases were found in treatment groups for hemoglobin concentration, packed cell volume, erythrocyte counts (females only), MCV and MCH (males only). In differential leucocyte counts, an increase in eosinophils was observed in females from the top dose group after 12 months, whereas this parameter was decreased after 24 months. In the treated males an increase in neutrophils was observed which appeared not to be dose-related. This is supposed to be related to intercurrent respiratory infections during the second year, which were most prominent in these groups.

Gross and histopathological examination of the non-neoplastic lesions showed that, except from the findings in the respiratory tract, these lesions were equally distributed amongst control and treatment groups, and are considered age- and strain related, caused by intercurrent disease or background pathology. The lesions in the respiratory tract included chronic suppurative bronchitis-peribronchitis, lobar bronchiectasis and atelectasis. This condition occurred more pronounced and more frequently in treated animals. A notable finding in two males out of the high dose group was the presence of nodular metaplastic bone structures in the lungs, which were not associated with inflammatory processes. Based upon morphology these lesions were not considered to be neoplastic in nature.

With respect to neoplastic lesions, the absolute number of tumor bearing rats, the total number of tumors, and the total number of tumors according to organ site e.g. pituitary, adrenals, appeared to be lower in the top dose group when compared with the control group. However, when statistical analysis allowing for longevity was applied, no significant differences between the groups was found. In addition, no unusual tumor types for this strain of rats were found after treatment.

DISCUSSION

The present study in which male and female Wistar rats were exposed to a mixture of halogenated hydrocarbons in the drinking water during their lifetime, is in a certain sense an unorthodox study, since not a single compound is under investigation, but a mixture which mimics in exaggerated form the natural exposure of a human population. The composition of this mixture is, in a qualitative sense, representative for the contamination of several ground wells used for the drinking water supply, sampled and analyzed at regular intervals during the period of 1976 - 1978 in the

Netherlands (Zoeteman, 1979). Similar findings are reported from the USA (Speth et al., 1981). Mutagenicity studies carried out with polluted surface or ground water (Loper, 1980; Kool, 1983), revealed mutagenic activity in several test systems; epidemiological studies indicate a slight increase in risk for colon, rectum and bladder cancer, associated with organic contaminants in drinking water (Williamson, 1981 Crump, 1982). However, mutagenicity studies performed at our Institute with this mixture of eleven compounds showed no mutagenic action in five different assays (Voogd et al., 1979), though the concentrations tested were relatively low because of cytotoxicity and poor solubility. Also negative results were obtained with these single components in Salmonella typhimurium TA 100 and TA 98 assays (Voogd and van der Stel, 1979).

Two major intercurrent diseases interfered with this study: intestinal obstruction by trichobezoares and respiratory infections. Trichobezoars could be relieved by a change of diet, resulting in a higher crude fiber intake. The prevalence for this condition in females has not been clearified yet. Infections of the respiratory tract occurred, predominantly in treated males, after 60 weeks of exposure; this predominance can be explained partly to the separate housing regimen. However, it cannot be excluded that impairment of immune functions, as described by Munson et al., (1982) by some of these compounds, has played a role. Since these two intercurrent diseases also occurred in other simultaneous experiments in the laboratory, they are not considered to be caused directly by exposure to the compounds under investigation. Several parameters such as bodyweight gain, mortality and neutrophil counts were affected and coincided with these conditions and are considered to be related to them.

Effects on red blood cell parameters and a dose related decrease in water consumption (predominantly in females) remain unexplained: an aversive taste of the drinking water seems unlikely, since at the start of the experiment even a higher water intake was noted.

From the non-neoplastic histopathological findings after 25 months of exposure, only multiple metaplastic bone formation in the lungs in two out of ten top dose males is considered a notable finding. Since no malignant histological features and not any primary tumor with similar appearance was found, these lesions are considered non-neoplastic. A similar condition is described in several other species, including man, with unknown etiology (Borst, 1976). In the rat however, and in particular the strain used in the present study, no such lesions are as yet described in the literature (Burek, 1978, Kroes, 1981). Whether this condition is fortuitous or is to be attributed to exposure to the test compound, or the intercurrent respiratory infections, remains questionable. The tumor distribution, when corrected for age, proves to be not significantly different between control and top dose group. In addition time of appearance of tumors was not influenced by treatment and no unusual tumor types were observed. It should be noted, however, that due to poor solubility of the test compound in the drinking water, the top dose was not at the MTD (maximum tolerated dose) level.

In conclusion, it can be stated that under the conditions of this study this mixture of eleven halogenated hydrocarbons, in a concentration being three orders of magnitude higher than as found in several water wells, exhibited no evident toxic or carcinogenic effect in the rat.

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