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Project Summary

Effects of Selected Organic Drinking Water Contaminants on Male Reproduction

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Because of the recent increase in exposure of individuals to potentially harmful chemicals, it has become increasingly important to test the potential of environmental chemicals to cause adverse reproductive effects. The Division of Toxicology within the Department of Pharmacology, Medical College of Virginia has responded to this need by examining the abilities of Kepone, hexachlorobenzene, 2,4-dinitrotoluene, 1,2,3,4-tetrabromobutane, chloral hydrate, 1,1,2-trichloroethylene, 1,2-dichloroethylene, 1,2-dichloroethane, dibromochloromethane, trichloromethane, and 1,1,1-trichloroethane to elicit harmful reproductive effects. The following tests were used to assess the extent of these effects: analysis of effects upon rat ejaculate volume and sperm morphology, distribution studies in rats, determination of the cellular sites of action in a P388D$_1$ lymphoid neoplasm cell line, performance of a multigeneration murine experiment which included dominant lethal and teratologic studies, and analysis of the inhibition of mouse testicular DNA synthesis.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Results

Inhibition of Mouse Testicular DNA Synthesis

A group of structurally related compounds were tested for their effects upon mouse testicular DNA synthesis. The compounds were administered intratesticularly, in two equal doses, one dose per testis (0.02 ml/testis, 0.04 ml/mouse). One and one-half hours later, tritiated thymidine was administered (10 μCi/testis, 20 μCi/mouse). The mice were sacrificed one-half hour after the injections of tritiated thymidine, and their testes surgically removed. The testicular DNA was then isolated using a modified Shibko method in which successive PCA precipitations were used to isolate DNA free of protein, lipids, and contaminating RNA.

In each experiment, three fractions obtained from the DNA isolation procedure were analyzed. The first fraction was the homogenate which contained both testes of an individual mouse, homogenized in PBS buffer (0.15 M KPO_4, in 0.85% NaCl, pH 7.2). The total counts in the homogenate fraction measured the tritiated thymidine remaining in the testes at the time of sacrifice. The second fraction was the supernatant fluid removed after centrifugation of the homogenate. The counts in this fraction were a measure of non-PCA-precipitable tritiated thymidine, or tritiated thymidine pool size. The third

fraction analyzed contained relatively pure DNA. The counts in this fraction quantitated the amount of DNA synthesized during the one half-hour after the injections of the tritiated thymidine.

All results were analyzed using an ANOVA, Dunnett's t-test, Duncan's multiple range test, and linear regressions of the dose-response curves of the various fractions studied.

Chloral hydrate (CHL)

Doses ranging from 10 to 900 mg/kg were tested. The 600 and 900 mg/kg doses caused a significant increase in the number of counts in the homogenate fraction. These data imply a mechanism of transport of tritiated thymidine out of the testes. This mechanism is inhibited at these doses of CHL, causing a greater amount of total testicular counts, relative to control values, to remain in the testes at the time of sacrifice of the animals.

Doses greater than or equal to 75 mg/kg caused a significant inhibition of testicular DNA synthesis. The amount of testicular DNA synthesis was 30% and 3% of the vehicle control values at 75 and 300 mg/kg, respectively.

1,1,2-Trichloroethylene (TCE 2)

The range of doses tested was 24 to 1000 mg/kg. Doses greater than or equal to 500 mg/kg caused an increase in tritiated thymidine pool size, and this was accompanied by a decrease in testicular DNA synthesis. The amount of DNA synthesis at 500, 700, and 1000 mg/kg was 40%, 18% and 2% of the vehicle control values, respectively

1,1,2-Trichloroethane (TCE 1,2)

Doses ranging from 3 8 to 1200 mg/kg were tested. A dose-related increase in tritiated thymidine pool size and decrease in testicular DNA synthesis was observed These deviations from control values became statistically significant at doses greater than or equal to 300 mg/kg. At 1000 mg/kg, DNA synthesis was inhibited to 2% of the vehicle control value.

1,1,1-Trichloroethane (TCE 1,1)

Two doses of TCE 1,1 were tested, 100 and 1000 mg/kg. Both doses caused an increase in tritiated thymidine pool size accompanied by a decrease in testicular DNA synthesis. The amount of DNA synthesis at these doses was 60% and 27% of the vehicle control values, respectively.

1,2-Dichloroethane (DCE 1,2)

DCE 1,2 was administered over a dose range of 4 9-250 mg/kg. The only significant effect observed was elicited at 250 mg/kg where a decrease in DNA synthesis to 47% of the vehicle control value was effected.

Compound Comparisons (CHL, DCE 1,2; TCE 1,1; TCE 1,2; TCE 2)

In order to contrast the relative capabilities of the above compounds to inhibit testicular DNA synthesis, their dose-response curves were compared The mean percent DNA synthesis versus log (millimolar dose/kg) was plotted. Four of the five compounds, CHL; DCE 1,2; TCE 1,2, and TCE 2 were found to have approximately equal slopes, although the curve for CHL was to the left of the others These observations suggest that these four compounds may be causing their effects through the same mechanism which is most sensitive to CHL CHL differs from the other compounds in that it contains an aldehyde group. This could explain the marked inhibition of testicular DNA synthesis. As only two does of TCE 1,1 were tested, conclusions cannot be drawn concerning the shape of the TCE 1,1 dose-response curve

2,4 Dinitrotoluene (DNT)

No dominant lethal effects were caused by the oral administration of either 60 or 180 mg DNT/kg/day for five days in rats. The 60 mg/kg/day dose produced no adverse changes in male reproductive performance. The moderately adverse effects of 180 mg/kg/day on mating and fertility indices were reversible Severe reproductive and moderate dominant lethal effects were seen at the 240 mg/kg/day level. These effects persisted for at least eight weeks, indicating that this dose was severely debilitating.

1,2,3,4-Tetrabromobutane (TBB)

The oral administration of TBB at 10 and 40 mg/kg/day for five days in rats failed to produce the classical picture of dominant lethal effects elicited by the positive control, triethylenemelamine (TEM). TEM caused a mutagenic response in the first few weeks of mating. TBB induced significant effects in weeks 6 and 7, but these were reversed by week 14 of mating and were not considered significant evidence of dominant lethality. TBB, at both doses, impaired male reproductive performance as evidenced by a decrease in the mating index (mating index = percentage of mated females which became pregnant), but not as severely as TEM.

Kepone

Evaluation of Kepone in a dominant lethal study (oral administration for five days in rats at 3.6 and 11.4 mg/kg/day) demonstrated no significant changes in either male fertility or dominant lethal mutations. The doses administered produced tremors as previously reported in the literature

Following a single oral administration (40 mg/kg, 2.5 μCi/ml), Kepone and/or its metabolites were found to distribute in the ejaculate via the seminal vesicle and appeared to bind to spermatozoa.

Both i.p. (18 and 36 mg/kg, single acute dose) and p.o. (9 mg/kg/day for 14 days) delivery of Kepone produced an increase in the percent of morphologically abnormal sperm. The effect of orally administered Kepone on sperm morphology persisted for up to 7 weeks post dosing.

Kepone interfered with cellular energy production in P388D$_1$ cells in a manner similar to the classical uncoupler of oxidative phosphorylation, dinitrophenol (DNP). Kepone was approximately 80 times more potent than DNP in inhibiting cellular proliferation and stimulating oxygen consumption, suggesting that the mitochondria may be the ultimate site of subcellular toxicity. Kepone also significantly altered the mitochondrial calcium distribution and the phagocytic process in the P388D$_1$ cell.

Hexachlorobenzene (HCB)

HCB at 70 and 221 mg/kg/day p.o. for five days in rats did not induce classical dominant lethal events during weeks 1-5 of mating. Statistically significant effects during weeks 10-14 of mating were inconsistent in their extent and direction, and were attributed to weekly fluctuations in the test groups. Male reproductive performance (mating index) was impaired by HCB in a dose-related manner.

Fourteen daily oral administrations of 35 mg/HCB/kg significantly altered male body weight, ejaculate volume, sperm count and the percent of morphologically abnormal sperm for up to 7 weeks post-dosing.

Dibromochloromethane (DBCM) and Trichloromethane (TCM)

In a two-generation study, impairment of reproduction was observed among mice ingesting 4 mg/ml DBCM or 5 mg/ml TCM (nominal doses of 685 and 855 mg/kg/day, respectively). Significant decreases were observed in fertility, litter size, and adult female survival during gestation for both compounds at these levels. Inconsistent decreases were observed in postnatal survival and body weight gains. No significant adverse effects were observed for either halomethane in dominant lethal and teratology screening studies.

Recommendations

1. A holistic approach to the evaluation of potential toxicity should be considered. Test animals should all receive the same doses and multiple systems should be evaluated simultaneously.
2. The doses should be multiples of maximum anticipated human exposure (10X, 100X, 1000X, etc.).
3. Molecular mechanisms of adverse effects should be determined.
4. Potential genotoxic effects should be determined using the following test systems: DNA damage, DNA repair, sister chromatid exchange, adduct formation, and DNA single strand break analysis.
5. *In vitro* tests should be developed to evaluate toxicity.

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***Kirby I. Campbell** is the EPA Project Officer (see below).*
The complete report, entitled "Effects of Selected Organic Drinking Water Contaminants on Male Reproduction," (Order No. PB 82-259 847; Cost: $13.50, subject to change) will be available only from:
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