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Toxicology, 31 (1984) 237—250
Elsevier Scientific Publishers Ireland Ltd.

EFFECTS OF TRICHLOROETHYLENE EXPOSURE ON MALE REPRODUCTIVE FUNCTION IN RATS*

H. ZENICK, K. BLACKBURN, E. HOPE, N. RICHDALE and M.K. SMITH*

Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH 45267 and [a]Toxicology and Microbiology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268 (U.S.A.)

(Received September 19th, 1983)
(Accepted March 5th, 1984)

SUMMARY

The present study was designed to evaluate the influences of trichloroethylene (TCE) on the reproductive system of male rats. In addition, information was obtained on the distribution and metabolism of TCE. At 100 days of age, male rats were allowed to copulate with ovariectomized, hormonally primed females and copulatory behaviors scored. Fifteen minutes post-ejaculation, females were sacrificed and ejaculate and semen plug recovered from the uterus and vagina for evaluation. These data served as a pre-exposure baseline for each animal. TCE exposure was then initiated with animals intubated with either 0, 10, 100, or 1000 mg/kg of TCE (10 males/group) for 5 days/week for 6 weeks. Copulatory behaviors and semen evaluations were conducted at Weeks 1 and 5 as well as 4 weeks post-exposure. Three males/group were sacrificed at the end of the sixth week of exposure and levels of TCE and its metabolites measured in various organs and blood. The remaining animals were sacrificed at the end of Week 10. TCE-related effects were seen primarily in the 1000 mg/kg group as reduced body weight gain, elevated liver/body weight ratios, and impaired copulatory behavior. However, the copulatory performance of the "affected" males had

*Although the information described in this article has been funded wholly or in part by the USEPA under Co-operative agreement No. CR808880 to University of Cincinnati it has not been subjected to the Agency's required peer and administrative review and therefore does not necessarily reflect the views of the agency and no official endorsement should be inferred.

Address all correspondence to: Dr. Harold Zenick, Department of Environmental Health, University of Cincinnati Medical Center, 3223 Eden Avenue, Cincinnati, OH 45267, U.S.A.

Abbreviations: MAC, maximum allowable concentration; TCA, trichloroacetic acid; TCE, trichloroethylene; TCOH, trichloroethanol.

0300-483X/84/$03.00
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Printed and Published in Ireland

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returned to normal by the fifth week of exposure. Although TCE and its metabolites concentrated to a significant extent in the male reproductive organs, semen evaluations failed to reveal any indices of spermatotoxicity. The initial alterations in copulatory behavior may be attributed to the narcotic properties of TCE. Tolerance to this pharmacological effect may explain the absence of these effects by the fifth week of exposure.

Key words: Trichloroethylene; Male reproductive toxicity; Semen evaluations; Copulatory behaviors

INTRODUCTION

Trichloroethylene (TCE) has been the subject of extensive study for over 100 years [1]. Much of this effort has been directed at delineating its effects on the CNS, hepatic, renal, and cardiovascular systems. Recent interest has also centered around its carcinogenic potential, substantiated in part by the increase in hepatocellular carcinomas in mice chronically exposed to TCE [2]. Yet, in spite of the multitude of research papers, little data are available regarding its reproductive effects.

Most studies of TCE toxicity have been guided by reports that TCE can bind to macromolecules. Thus, studies examining male reproductive effects have employed assays to test the mutagenic properties of TCE. Slacik-Erben et al. [3] found no evidence of an increase in dominant lethality in mice exposed to 50, 202, or 450 ppm for 24 h. In another study examining the mutagenic potential of inhaled anesthetics, Lande et al. [4] exposed mice to 0.1—1 MAC (maximum allowable concentration, — probably 150 ppm) of TCE for 4 h/day for 5 days. Sperm morphology (cauda epididymis) was assessed 28 days after the first exposure. TCE exposure produced 1.68—2.43% abnormal sperm at the highest exposure, a level which was significantly higher than control values (1.42%) but of uncertain biological significance.

The present study was designed to further evaluate the influences of TCE on the reproductive system of male rats. A 6-week exposure and 4-week recovery phase were incorporated into the design. Endpoints measured included the evaluation of sperm count, motility, and morphology in the ejaculate as well as assessment of copulatory behavior. In addition, information was obtained on the distribution and metabolism of TCE.

METHODS

Treatment protocol

Long-Evans hooded male rats (Charles River), 70 days of age at the initiation of this study, were used. All animals were caged individually and maintained on a reverse 14:10 h light/dark cycle with the lights turned on at 2200 h. Purina lab chow (No. 5001) and water were available ad libitum.

Trichlorethylene (electronic grade, >99.9% pure) and corn oil (laboratory grade) were obtained from Fisher Scientific Company. Animals were intubated with TCE in an approximate volume of 0.5 ml corn oil at 30–45 min before the start of the dark cycle. Weekly samples of food, water, and dosing solutions were analyzed by gas chromatography for TCE levels. Values were found to average 1.27 ± 2.07 μg/g and 0.63 ± 0.16 μg/ml for food and water respectively, and 17.5 ± 7.0 μg/ml for the vehicle.

Male reproductive toxicity evaluation

The same experimental strategy was utilized both in a pilot study and in the expanded investigation. Male Long-Evans hooded rats were received in the laboratory at 70 days of age. Over the next 3–4-week period, these males were allowed to mate on several occasions with ovariectomized, hormonally-primed females in order to obtain mating experience. Females received 0.1 mg of estradiol benzoate (s.c.) 48 h prior to mating followed by 0.1 mg of progesterone (s.c.) 4 h prior to mating. Subsequently all the males were mated on a weekly basis for the duration of the experimental period. When 100 days of age, all males were intubated with corn oil for 5 days. This procedure was employed in order to acclimate the animals to the intubation procedure. Two days after cessation of the corn oil intubation, the males were mated with ovariectomized, hormonally-primed females. Mating behaviors were scored and at 15 min postejaculation, the females were sacrificed, semen recovered from the uterine horns and processed for determinations of sperm count, percent motile sperm and sperm morphology (see below). These data were used to determine baseline (pre-exposure) values for each animal. Moreover, the sperm count data were used to match males for assignment to treatment groups, such that the mean sperm count of each group was essentially equivalent.

In the pilot study, males were subsequently intubated with 0, 500, 1000 or 2000 mg/kg of TCE for 5 days a week for 3 weeks (3 males/group). Semen samples were collected weekly with mating trials conducted approximately 4–5 h after intubation. The major finding in this phase was the failure of all animals in the highest dose group (2000 mg/kg) to initiate mating during any of the weeks of treatment. This obviated any attempt to conduct semen evaluations in this group. Similar behavior was displayed by 1 animal in the middle dose (1000 mg/kg) and 1 male in the low dose (500 mg/kg) groups beginning at 1 and 2 weeks of exposure respectively. No treatment-related effects were observed in semen evaluations.

Based upon the pilot data, 4 doses were employed in the present study such that males were intubated with 0, 10, 100, or 1000 mg/kg of TCE (10 males/group). The same time-table was followed as used in the pilot study with one exception. Forty-eight hours after the collection of the baseline sample, males were tail-bled (300 μl), the sample centrifuged and the plasma removed and frozen (−70°C) for subsequent testosterone analysis. Animals were then treated over the next 6 weeks. At the end of Week 1 and Week 5 of exposure, copulatory behaviors and semen samples were again

evaluated. During intervening weeks, females were placed with males in their home cages (end of treatment week) in order to maintain a constant interval between matings. However, no attempts were made to evaluate copulatory behavior at those times.

Males were continued on treatment for a sixth week, at which time a 300 μl sample of blood was obtained from the tail of each animal. The sample was then divided with approximately 200 μl frozen at −70°C for subsequent GC analysis of TCE and its metabolites. The remainder was centrifuged and the plasma removed and frozen (−70°C) for subsequent testosterone analyses. These assays were conducted using Immuchem Covalent-Coat RIA kits (Carson, CA) and procedures detailed in their literature. At this time, 3 animals were randomly selected from each group and sacrificed by CO_2 asphyxiation. Approximately 3 ml of blood were obtained (via cardiac puncture) and the following organs removed and frozen (−70°C): brain, heart, lung, kidney, liver, fat, muscle, testis, adrenals, seminal vesicle, prostate, vas deferens, and epididymis. The caudal portion of the other epididymis was processed to determine a sperm count and sperm morphology.

Tissue and blood levels of TCE and the major metabolites trichloroethanol (TCOH) and trichloroacetic acid (TCA) were analyzed in triplicate by gas chromatography utilizing a modification of the procedure of Humbert and Fernandez [5] described in the accompanying paper [6]. The lower limit of detection was calculated to be 0.5 ng/ml, or 10 ppb based upon a tissue sample size of 100 mg. Standard curves for TCE, TCA, and TCOH yielded correlation coefficients of 0.99, 0.99, and 0.98, respectively.

Exposure for the remaining animals was discontinued at this time. Males were allowed weekly mating experiences in their home cages. Copulatory behavior and semen samples were again evaluated at Week 10 (4 weeks post-exposure). These animals were then sacrificed in a manner identical to that described above.

Copulatory behavior

The male was placed into a Plexiglas® observation cage and allowed to habituate for approximately 15 min. Subsequently, an ovariectomized, hormonally-primed female was introduced into the cage and copulatory behavior monitored. Four behaviors were recorded: (1) mount latency — defined as the elapsed time between introduction of the female and the first mount; (2) number of mounts; (3) number of intromissions — distinguished from mounts by increased vigorous thrusting, back-pedalling following disengagement, and immediate genital grooming; and (4) ejaculation latency — defined as the elapsed time between the initial mount and ejaculation. The presence of a copulatory plug upon sacrifice in 100% of the females confirmed our accuracy in determining time of ejaculation.

Sperm evaluation

Approximately 15 min after copulation, the female was sacrificed under

CO_2. A midline incision was made, the uterine horns exposed, and the contents aspirated into a syringe. Subsequently the uterus and vagina were excised, cut open and flushed to obtain residual sperm and the copulatory plug was removed and weighed. At the end of Week 5 these plugs were frozen for subsequent GC analyses for TCE and metabolite content.

A drop of the uterine sample was placed on a slide in order to subjectively estimate the sperm density (low, moderate, high). An aliquot of the sample was diluted with warmed culture medium (37°C) to provide a sample dilute enough to be scored for motility. This dilution was usually 1:5 or 1:10. Fifteen microliters of this diluent was pipetted onto a slide, cover-slipped and placed on the microscope stage maintained at 37°C with an air curtain incubator. Four areas of the slide were then videotaped on a Panasonic NV1850 videotape recorder. The tape was subsequently played back in slow motion and the percent motility determined for 100 sperm.

Approximately 50 μl (1 drop) of this diluted sample was mixed with an equal volume of Eosin Y stain, smeared onto a slide and allowed to air dry for subsequent scoring of sperm morphology (i.e. percent abnormal shapes seen in 100 sperm according to the categories previously used in this laboratory [7]).

The remaining undiluted sample was transferred to a graduated cylinder. All slides and syringes were rinsed into a cylinder and the final volume diluted to 50 ml. Both chambers of a hemocytometer were charged with 10 μl of sample and sperm counts determined. If counts differed by more than 10%, the chamber was recharged and sperm count redetermined.

Data analyses

A two-way repeated measures analysis of variance (ANOVA) with one between factor (TCE dose) and one within factor (Weeks — baseline, Week 1, Week 5 of exposure) was applied to the analyses of body weights, sperm counts, sperm motility, plug weights, mount latency, ejaculation latency, number of mounts and number of intromissions. Since 3 animals were sacrificed from each group at Week 6, a separate one-way analysis of variance was applied to the Week 10 data. The latency measures were subjected to a logarithmic transformation prior to analysis to reduce heterogeneity of variance. A square root transformation was applied to intromissions and mount data for the same reason, while an arcsine transformation was applied to the percent motility data to normalize the distribution. These transformations are conventional with variables of this nature [8]. Moreover, for each animal a percent of its baseline was calculated for each of these variables on each of the weeks sampled. These data were also analyzed employing a repeated measures ANOVA. All significant trends were further tested using Duncans New Multiple Range Test to contrast the various treatment groups.

Body wt.
Sperm ct.
sperm motility
plug wts.
mt. latency
ejac. latency
mts.
intromissions

RESULTS AND DISCUSSION

In most instances, analyses of raw data and percent of baseline data

yielded identical results. The means and standard deviations for the semen parameters and copulatory behavior for Baseline, Week 1 and Week 5 are presented in Table I. Data for Week 10 are presented in Table II. One animal in the high dose group died following the fifth week of exposure. Necropsy revealed hemorrhagic lungs most likely produced by a misdirected intubation. Comparable conditions were not observed in other animals at time of necropsy.

TABLE I

GROUP MEANS AND STANDARD DEVIATIONS AND PERCENT OF BASELINE ESTIMATES FOR BASELINE AND WEEKS 1 AND 5 OF EXPOSURE[a]

Treatment (mg/kg/day)	Baseline	Week 1		Week 5	
A. Body wt (g)					
0	402 ± 20	413 ± 26	(103%)	472 ± 31	(117%)
10	392 ± 30	398 ± 22	(102%)	449 ± 27	(115%)
100	395 ± 20	405 ± 30	(102%)	465 ± 44	(117%)
1000	397 ± 24	396 ± 28	(99%)	424 ± 45	(107%)[b]
B. Plug wt (mg)					
0	99 ± 23	110 ± 14	(118%)	105 ± 26	(115%)
10	98 ± 22	100 ± 15	(104%)	105 ± 22	(106%)
100	113 ± 28	108 ± 23	(99%)	103 ± 35	(94%)
1000	108 ± 15	110 ± 30	(103%)	107 ± 19	(100%)
C. Sperm count ($\times 10^6$)					
0	47 ± 13	42 ± 17	(91%)	46 ± 21	(95%)
10	43 ± 11	46 ± 23	(106%)	48 ± 19	(113%)
100	44 ± 12	47 ± 18	(109%)	48 ± 29	(112%)
1000	47 ± 18	41 ± 20	(93%)	36 ± 26	(75%)
D. Sperm motility (%)					
0	50 ± 12	33 ± 21	(69%)	32 ± 24	(70%)
10	34 ± 15	29 ± 10	(97%)	38 ± 19	(114%)
100	37 ± 13	35 ± 12	(97%)	49 ± 13	(125%)
1000	30 ± 14	30 ± 14	(99%)	35 ± 22	(119%)
E. Sperm morphology (percent normal)					
0	99.2 ± 0.80	97.8 ± 2.14	(99%)	98.3 ± 1.40	(99%)
10	99.1 ± 0.94	98.3 ± 2.10	(99%)	99.3 ± 0.67	(100%)
100	98.1 ± 2.87	98.8 ± 2.06	(99%)	98.7 ± 1.55	(100%)
1000	98.6 ± 2.07	98.1 ± 1.54	(99%)	99.1 ± 0.92	(101%)
F. Mount latency (s)					
0	8.2 ± 2.4	8.2 ± 2.6	(117%)	7.4 ± 2.3	(116%)
10	6.0 ± 1.6	4.6 ± 1.6	(97%)	8.8 ± 2.6	(122%)
100	12.2 ± 2.7	8.3 ± 1.9	(76%)	6.5 ± 2.6	(101%)
1000	10.2 ± 2.9	—[c]		12.1 ± 1.7	(149%)

TABLE I (*Continued*)

Treatment (mg/kg/day)	Baseline	Week 1	Week 5
G. Ejaculation latency (s)			
0	330 ± 17	299 ± 18 (109%)	244 ± 15 (95%)
10	358 ± 23	428 ± 20 (104%)	327 ± 19 (99%)
100	369 ± 21	347 ± 22 (100%)	311 ± 22 (98%)
1000	428 ± 18	—[c]	395 ± 19 (101%)
H. Mounts ($\sqrt{}$)			
0	1.36 ± 0.40	1.68 ± 0.87 (123%)	1.25 ± 0.37 (103%)
10	1.62 ± 0.45	1.97 ± 0.50 (132%)	2.06 ± 0.72 (133%)
100	1.27 ± 0.46	2.04 ± 0.70 (137%)	1.90 ± 0.64 (127%)
1000	1.78 ± 0.55	—[c]	1.78 ± 0.65 (105%)
I. Intromissions ($\sqrt{}$)			
0	4.06 ± 0.80	4.32 ± 1.22 (106%)	3.65 ± 0.64 (90%)
10	4.35 ± 0.95	4.24 ± 0.85 (101%)	4.02 ± 0.68 (94%)
100	4.15 ± 0.72	4.61 ± 0.68 (115%)	4.16 ± 0.63 (103%)
1000	4.29 ± 1.40	—[c]	4.32 ± 1.27 (109%)

[a] $N = 10$/group.
[b] Significantly different from controls, $P < 0.001$.
[c] The majority of males in this group exhibited abnormally protracted mating behaviors obviating inclusion of the data in the analyses (see text for details).

TABLE II

GROUP MEANS AND STANDARD DEVIATIONS FOR WEEK 10: 4 WEEK POST EXPOSURE[a]

	Treatment (mg/kg/day)			
	0	10	100	1000
Body wt (g)	541 ± 18	538 ± 20	522 ± 44	476 ± 30
Plug wt (mg)	108 ± 20	115 ± 20	124 ± 19	105 ± 20
Sperm count ($\times 10^6$)	59 ± 30	66 ± 9	58 ± 20	44 ± 26
Sperm motility (%)	34 ± 13	36 ± 12	58 ± 20[b]	44 ± 26
Sperm morphology (%)	99.2 ± 1.91	97.1 ± 1.47	99.4 ± 1.34	97.1 ± 1.76
Mount latency (s)	5.1 ± 2.0	7.5 ± 1.3	6.4 ± 2.1	8.6 ± 1.8
Ejaculation latency (s)	213 ± 14	317 ± 17	337 ± 17	344 ± 20
Mounts ($\sqrt{}$)	0.69 ± 0.07	0.57 ± 0.53	1.52 ± 0.91	0.62 ± 0.97
Intromissions ($\sqrt{}$)	3.73 ± 0.31	3.94 ± 0.94	4.54 ± 1.30	3.90 ± 0.72

[a] $N = 7$/group for Groups 0, 10, 100 and 6/group for Group 1000.
[b] $P \leq = 0.05$ significantly different from controls.

TABLE III

GROUP MEANS AND STANDARD DEVIATIONS FOR SPERM COUNT AND MORPHOLOGY, BODY WEIGHT, ORGAN WEIGHTS AND ORGAN/BODY WEIGHT RATIOS (IN PARENTHESES) AT 4 WEEKS POST EXPOSURE

	Treatment (mg/kg/day)			
	0	10	100	1000
	(N = 7)	(N = 7)	(N = 7)	(N = 6)
Body weight	541 ± 180	538 ± 203	522 ± 44	476 ± 30
Testis	1.78 ± 0.22	1.74 ± 0.13	1.82 ± 0.19	1.82 ± 0.16
	(3.29 × 10^3)	(3.25 × 10^3)	(3.51 × 10^3)	(3.69 × 10^3)
Seminal vesicle	2.49 ± 0.43	2.31 ± 0.36	2.57 ± 0.45	2.59 ± 0.38
	(4.66 × 10^3)	(4.31 × 10^3)	(4.91 × 10^3)	(5.35 × 10^3)
Cauda epididymis	0.21 ± 0.08	0.19 ± 0.03	0.23 ± 0.08	0.21 ± 0.05
	(3.93 × 10^4)	(3.55 × 10^4)	(4.46 × 10^4)	(4.30 × 10^4)
Epididymis	0.61 ± 0.11	0.63 ± 0.07	0.65 ± 0.05	0.64 ± 0.06
	(1.12 × 10^3)	(1.17 × 10^3)	(1.26 × 10^3)	(1.35 × 10^3)
Vas deferens	0.10 ± 0.02	0.09 ± 0.02	0.016 ± 0.14	0.10 ± 0.02
	(1.84 × 10^4)	(1.76 × 10^4)	(3.18 × 10^4)	(2.18 × 10^4)
Prostate	0.65 ± 0.20	0.63 ± 0.07	0.58 ± 0.22	0.57 ± 0.12
	(1.20 × 10^4)	(1.14 × 10^4)	(1.12 × 10^4)	(1.17 × 10^4)
Adrenal	0.029 ± 0.1	0.057 ± 0.06	0.044 ± 0.02	0.043 ± 0.02
	(5.26 × 10^2)	(11.38 × 10^2)	(8.40 × 10^2)	(8.50 × 10^2)
Brain	1.64 ± 0.12	1.59 ± 0.16	1.56 ± 0.18	1.71 ± 0.21
	(3.1 × 10^3)	(3.0 × 10^3)	(3.0 × 10^3)	(3.7 × 10^3)
Kidney	2.00 ± 0.19	1.78 ± 0.23	1.96 ± 0.20	1.83 ± 0.23
	(3.7 × 10^3)	(3.7 × 10^3)	(3.6 × 10^3)	(3.7 × 10^3)
Liver	17.76 ± 2.24	17.59 ± 2.61	18.72 ± 1.81	17.76 ± 1.50
	(3.3 × 10^2)	(3.3 × 10^2)	(3.6 × 10^2)[a]	(3.6 × 10^2)[a]
Sperm count (× 10^6)	711 ± 180	771 ± 204	783 ± 243	867 ± 214
Sperm morphology (%)	98.2 ± 1.43	99.3 ± 1.19	97.9 ± 1.67	96.6 ± 2.74

[a] $P < 0.05$ significantly different from controls.

Body weight and organ weight data

All groups exhibited significant increases in body weight over the course of the experiment ($P \leqslant 0.001$). However, this increase was significantly less ($P \leqslant 0.001$) in the 1000 group (seven percent) relative to the other 3 groups (15—17%) by the end of the fifth week of exposure (Table I). These differences persisted but were no longer statistically significant at Week 10, 4 weeks post-exposure (Table II).

Organ and organ/body weight ratios were evaluated at the end of treatment (Week 6) and 4 weeks post-exposure. The only significant effect ($P \leqslant 0.05$) was that the 100 and 1000 mg/kg treatment groups had higher liver/body weight ratios than the controls at week 10 (Table III). Other authors have also reported increases in liver weights accompanying TCE exposure [e.g. 9—11]. Stott et al. [11] have proposed that the increase is a hyperplasia as reflected in increased hepatic DNA synthesis.

Semen and testosterone evaluations

No significant TCE-related effects were seen in semen plug weights or sperm count, sperm motility or sperm morphology during any phase of the study (Tables I and II). There was a non-significant decline in sperm count at Week 5 and Week 10 in the high dose group relative to the other groups. However, analyses of sperm concentration in the cauda epididymis at Weeks 6 and 10 in this group showed no evidence of deficiencies in sperm production.

TCE treatment produced no effects on plasma testosterone levels when evaluated at the end of the sixth week of exposure or 4 weeks post-exposure. These values are presented in Table IV.

TABLE IV

MEANS AND STANDARD DEVIATIONS FOR TESTOSTERONE CONCENTRATIONS (ng/ml) FOLLOWING 6 WEEKS OF TCE EXPOSURE AND 4 WEEKS POST-EXPOSURE (WEEK 10)

Treatment (mg/kg/day)	Baseline	Week 6	Week 10
0	2.61 ± 1.39 (10)[a]	2.17 ± 1.01 (10)	1.95 ± 0.37 (6)
10	3.02 ± 1.30 (10)	2.02 ± 0.87 (9)	1.43 ± 0.95 (7)
100	3.51 ± 1.90 (10)	2.37 ± 2.75 (10)	1.65 ± 1.01 (7)
1000	2.88 ± 1.86 (9)	2.29 ± 1.68 (9)	1.55 ± 0.44 (6)

[a] No. in parenthesis = sample size.

Copulatory behavior

In general, the more temporally-protracted behavioral endpoints (number of intromissions, ejaculation latency) showed the greatest stability across groups over the weeks of testing. Increased variability was seen in number of mounts and mount latency.

TCE-related effects on copulatory behavior were evident only in the high dose group. The animals in this group exhibited protracted mating behavior. Beginning at Week 1, these males exhibited extreme disruption in the conduct of mating as reflected in 2 observations: (1) neglect of the female for extended periods of time; and (2) numerous instances of incomplete genital contact. At these times, the male would approach the female and clasp her flanks but then quickly release. There was a total lack of the vigorous thrusting normally observed in male rats in the presence of a receptive female. (Female receptivity was checked by placing the female with other males who immediately initiated the copulatory sequence). The introduction of a new female made slight difference with 40% of the high dose males still requiring 2—3 h to complete a mating trial. Even in the interim week (Weeks 2—4), when the female was placed in the male's home cage, it was observed that these males required an hour or more before any evidence of copulation was evident (copulatory plug, sperm in the vaginal wash).

Similar behaviors were not present in the controls or the other 2 TCE groups. Moreover, by week 5, the behavior of these high dose males had returned to within normal range. No behavioral differences were seen in any of the groups at 4 weeks post-exposure (Table II).

The disruption in copulatory behavior did not appear to be a result of a TCE-produced anesthesia per se. The high dose males actively explored the mating cages during the adaptation period and similarly, actively investigated the female upon her introduction. Rather the disrupted behaviors may reflect other narcotic properties of TCE. Decreased potency has been reported to be a complaint in workers occupationally exposed to TCE [12]. The interference with copulatory behavior would also be similar to that reported for narcotics and alcohol in both animals [13] and man [14]. Moreover, the return to "normal" copulatory performance by Week 5 in the presence of continued TCE administration may be an expression of tolerance to this pharmacological effect.

Distribution metabolism data

Three animals were sacrificed from each group at the end of Week 6, with tissues and blood analyzed for TCE and metabolites. Median values for each group are presented in Tables V and VI. In most instances mean and median values were quite similar, but, because of the small sample size, the median was used to reduce the influence of an occasionally deviant value. TCE was detected in control tissues with fat showing relatively high levels. The reason for this is not clear and most likely a result of sample contamination as for this is not clear and most likely a result of sample contamination as opposed to TCE accumulation in the fat. The presence of TCE in other tissues may reflect TCE concentrations in the tap water, food, and corn oil

TABLE V

MEDIAN BLOOD (μg/mg) AND TISSUE LEVELS (μg/g) OF TRICHLOROETHYLENE (TCE) TRICHLOROACETIC ACID (TCA) AND TRICHLOROETHANOL (TCOH) AT THE SIXTH WEEK OF EXPOSURE (N = 3/GROUP)

Organ	Treatment (mg/kg/day)											
	0			10			100			1000		
	TCE	TCA	TCOH	TCE	TCA	TCOH	TCE	TCA	TCOH	TCE	TCA	TCOH
Blood	1.21	0.17	*	1.39	2.5	0.13	1.20	14.32	1.66	17.90	28.13	8.00
Heart	1.04	0.007	*	2.67	5.5	0.03	2.76	5.42	1.61	7.70	7.92	4.46
Testis	1.89	0.06	*	2.16	1.06	0.20	3.42	10.56	1.90	9.40	13.57	7.57
Vas deferen	8.2	0.22	0.05	8.36	2.47	0.02	5.36	8.24	5.35	13.65	21.09	6.25
Seminal vesicle	2.40	0.023	0.04	2.95	0.89	0.23	3.86	8.97	4.28	8.05	21.86	28.80
Prostrate	1.69	0.04	0.03	3.09	0.96	0.39	4.32	6.49	5.89	8.85	15.79	17.58
Epididymis	3.17	0.06	0.02	7.77	0.58	0.03	6.78	7.90	5.45	9.17	9.84	5.49
Adrenals	8.45	*	*	6.63	1.81	1.72	8.62	5.94	0.86	6.52	9.06	1.82
Fat	53.95	0.06	0.06	55.24	0.110	0.24	52.76	3.78	3.76	265.90	4.19	11.04
Liver	1.70	0.001	0.012	3.10	1.16	1.81	4.67	13.82	11.76	13.64	9.44	41.57
Kidney	0.145	0.01	0.04	5.0	1.96	2.56	4.12	10.28	14.83	18.47	28.25	81.17
Muscle	0.83	0.01	0.01	1.94	0.86	0.02	3.96	9.21	4.06	3.57	6.57	4.80
Lung	2.48	0.07	*	3.99	2.71	0.13	2.65	6.82	1.68	15.48	10.65	4.99
Brain	1.12	0.006	*	1.91	0.53	*	2.33	5.20	3.11	10.42	3.37	3.97

* Non-detectable levels.

[a] Samples collected 4—5 h after dosing.

TABLE VI

MEDIAN SEMEN PLUG CONCENTRATIONS (μg/mg) OF TRICHLOROETHYLENE (TCE), TRICHLOROACETIC ACID (TCA) AND TRICHLOROETHANOL (TCOH) AT WEEK 5 OF EXPOSURE

Treatment (mg/kg/day)	TCE	TCA	TCOH
0 ($N = 8$)	2.13	0.06	0.29
10 ($N = 10$)	2.68	0.69	0.46
100 ($N = 10$)	2.38	5.03	0.49
1000 ($N = 9$)	3.99	8.24	2.21

vehicle. Moreover, since controls and treatment were housed together, TCE exhaled shortly after intubation may have contributed, inadvertently to control exposure.

A marginal increase was seen in TCE concentrations at the lower doses with no consistent distinctions between the control and 10 mg/kg groups for the various tissues analyzed. At 100 mg/kg of TCE, only slight additional increases in parent compound were seen. Only at 1000 mg/kg of TCE was there a marked increase in TCE levels. Only muscle and adrenals had concentrations comparable to those seen at 100 mg/kg of TCE. The marked increase in blood TCE reflects in part the dosing 4—5 h earlier.

TCA was either non-detectable or present in extremely low concentrations in the control animals. Thus, levels of TCA in the 10 ppm group, although small, were several orders of magnitudes greater than controls. Compared to the 10 mg/kg group, the 100 mg/kg group had TCA levels that were 4—10 times higher, except for essentially equivalent levels in the heart. The 1000 mg/kg group showed the highest levels of TCA for most organs. Directly relevant to this study was the fact that the reproductive organs had 30—150% more TCA than the 100 mg/kg group. The only decrements relative to 100 mg/kg of TCE were seen in the liver, muscle, and brain.

Dose-related trends were also seen in TCOH concentrations. Again, negligible levels were apparent in control tissues and for the most part at the low dose (10 mg/kg). Organs with the highest levels in that group were the kidney, liver, and adrenal. As was true for TCE levels, adrenal concentrations of TCOH did not differ appreciably between groups. Comparison of TCOH concentrations in the reproductive organs in the 100 and 1000 mg/kg groups showed little change in the epididymis and vas deferens but a 3—7-fold increase in the testis, prostate, and seminal vesicle. Increases of similar magnitude were also seen in fat, liver, kidney, lung and blood. This GC data reflects the ability of the reproductive organs to accumulate TCE and metabolites with increasing dose, thus creating the potential for interference with reproductive function.

This latter observation is further supported by examining the levels of TCE and metabolites in the seminal plugs recovered following copulation

at Week 5 (Table VI). Again, the parent compound increased only at the 1000 mg/kg where there was an approximate 2-fold increase. Metabolites showed more apparent dose-response trends, especially TCA. The increased accumulation of the parent compound seen only at 1000 mg/kg, suggests possible saturation of pathways for TCE at this dose.

Finally, correlations were performed examining the correspondence between TCE and metabolite levels in blood and semen plug as well as the correlation between blood and semen plug levels. For both blood and semen plugs, TCE was significantly correlated with each metabolite which were in turn highly correlated with one another. Moreover, blood and semen plug concentrations were also significantly correlated, with correlations ranging between $r = 0.42$ and 0.75.

The data from this study suggest that TCE exerts minimal direct effects on the male reproductive system in terms of spermatotoxicity in spite of elevated concentrations of TCE and metabolites in the reproductive organs. Rather, the central nervous system and liver appear to have lower thresholds for toxicity, as seen in altered copulatory behaviors and elevated liver/body weight ratios. Although the dose required to impair copulatory performance (1000 mg/kg) was substantially elevated relative to environmental contamination, the possibility exists that this threshold may be substantially lowered in the presence of concurrent exposure to other neurotoxicants or drug intake, specifically alcohol. Other investigators have demonstrated ethanol potentiation of TCE toxicity, accompanied by marked elevations of TCE and decreased metabolite production [15]. Similar interactions could have deleterious effects on sexual behavior and indirectly impact on fertility. The potential interaction between other environmental neurotoxins, ethanol, or other drugs (e.g., narcotics) and TCE on copulatory behaviors remains to be studied.

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