APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2001; **15**: 727–732
DOI: 10.1002/aoc.220

Effects of exposure to dibutyltin dichloride on sperm density, viability and morphology in male mice

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Experiments on the effects of 0.025–0.40 μg of dibutyltin dichloride (DBTCl) per kilogram body weight (kg bw), on sperm density, viability and morphology in mature mice were conducted by daily intraperitoneal injection for 7 days at 22 ± 2 °C and 12 h light–dark cycle conditions. The results demonstrated that DBTCl exhibited strong toxicity on sperm quality. Dosed with ≥ 0.05 μg DBTCl/kg bw groups, the testes weight, sperm density and the rate of survival of sperm decreased, whereas the rate of sperm abnormalities increased significantly. In addition, treatment with 0.05 μg DBTCl/kg bw resulted in increasing rate of sperm head abnormalities, whereas administration at 0.20 and 0.40 μg DBTCl/kg bw significantly increased the rate of sperm tail abnormalities. In the group treated at ≥ 0.10 μg DBTCl/kg bw, the mice body weights decreased. It appeared there was a noticeable dose–response relationship between DBTCl and the parameters studied. ED_{50} values (7 days) of DBTCl for survival rate of sperm and density were 0.17 μg/kg bw and 0.19 μg/kg bw respectively. The present study provides a possibility for early diagnostic indicators and methods for sperm quality induced by organotin compounds (DBTCl) in mammals. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: dibutyltin dichloride (DBTCl); toxicity; sperm; mammals; mice

Received 8 December 1999; accepted 23 January 2001

INTRODUCTION

Organotin compounds are used in a variety of applications, such as stabilizers for polyvinylchloride (PVC), industrial and agricultural biocides, wood-preserving and anti-fouling agents, and catalytic agents and catalysts in a variety of industrial processes.[1,2] There is growing concern about the biological effects of organotin compounds widely distributed in the environment. Despite the large amounts of toxicological data, only recently have the teratological and embryolethal effects of organotins on mammals been reported for a limited number of chemical species.[3–5] Scientific interest has focused on tributyltin (TBT) because it is introduced directly into the environment by its use as an anti-fouling agent in paints. The presence of this compound in the aquatic environment has been reported.[6,7] Food chain bioamplification of TBT has been demonstrated in oysters, *Crassostrea gigas*,[8] mud crabs, *Rhithopanopeus harrisii*,[9] marine mussels, *Mytilus edulis*[10] and the chinook salmon, *Oncorthyncus tshawytscha*.[11] Concerning the effects of organotin compounds on mammals, only very few data have been reported. Penninks and Seinen[2] reported that organotin compounds, especially dioctyltin-, triphenyltin- and dibutyltin-chlorides are known to be immunotoxic. Ananie *et al.*[12] previously reported that 0.01–0.10 μg of tributyltin di-chloride (TBTCl) per kilogram of body weight (kg bw) and 0.01–0.13 μg of monobutyltin dichloride (MBTCl)/kg bw enhanced the immune system in mice, but concentrations higher than these suppressed the immune system. At both low and high concentrations, dibutyltin dichloride (DBTCl) caused immune suppression. The adverse effects of organotin compounds on reproduction have been reported, TBT is known to induce imposex (imposition of male sex characters onto the female) in the dog-whelk, *Nucella lapillus*.[13] Short and Thrower[13] found that a low concentration of TBT decreased

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hormone content in the dog-whelk. Li *et al.*[14] reported that triphenyltin oxide (TPTO) affected the ovaries and testis and embryonic development in the oyster. Triphenyltin acetate (TPTOAc) and triphenyltin chloride (TPTCl) produced degenerative changes in ovarian tissues of rats, indicating decreased ovulation potential and fertility.[15] Winek *et al.*[16] reported that triphenyltin hydroxide (TPTOH) caused anti-fertility effects in female rats after oral administration on days 1–7 of pregnancy. Harazono *et al.*[17] reported that TBTCl on days 0–7 of pregnancy or on 0–4 days of pregnancy produced a significant and dose-dependent reduction in fertility in mice. The effects of DBTCl on reproduction have not been studied. The present study was conducted to examine the effects of DBTCl on testis weight, sperm viability, sperm density and morphology.

EXPERIMENTAL

Materials and methods

DBTCl (Alarch. Corp. USA; 98% purity) was dissolved in 50% of ethanol (95% purity). Healthy Kunming male mice of about 7–8 weeks of age and weighing 35 ± 5 g were purchased from the Institute of Genetics, Chinese Academy of Sciences. They were acclimated to our laboratory for 1 week prior to the experiment. All mice were weighted and randomly distributed into five treatment groups plus one control group. Seven or eight animals were assigned to each group and were housed one mouse per plastic cage with pine sawdust as bedding. Mice were provided rodent chow and tap-water *ad libitum* throughout the experiment. A 12 h light–dark cycle, an ambient temperature of 22 ± 2 °C and humidity of $60 \pm 5\%$ were maintained. The experimental groups were administered by intraperitoneal injection (ip) 0.025, 0.05, 0.10, 0.20, and 0.40 μg DBTCl/kg bw per day for 7 days. The volumes given to the treated and control groups were based on body weight, which was monitored every day. At 24 h after the completion of dosing, body weight gains were measured and mice were killed by cervical vertebral dislocation.[18] Blood samples were collected from the retro-ocular venous plexus for subsequent determination of sex hormone concentrations; both right and left testes and epididymes were then carefully trimmed; only testes were weighed. Testes were dissected free, weighed and processed for subsequent ultrastructural analysis and intertesticural spermitid count. Determination of sperm viability, density and morphology was accomplished by dissecting out the epididymis and teasing it in a known volume of cultural solution. Sperm were incubated at 37 °C for 2 h, during which time the viability and motility were assessed. Sperm density and determination were done using a haematocytometer count according to the methods of Egbunke and Elemo,[19] Mattison,[20] Kempinas *et al.*,[21] and Muller.[22] Morphological changes in the head of sperm were accomplished by placing a drop of sperm suspension on a clean slide and spreading gently to make a thin film. The film was air dried, fixed with ethanol for 5 min and then observed under an optical microscope for changes in sperm morphology.[21,23] The criteria chosen for head abnormality were head without hook, with excessive hook, amorphous, and short head. Tail abnormalities recorded were coiled flagelium, bent flagelium, bent flagelium tip and twisted flagelium. The results are presented as percentages. The data were subjected to standard statistical analysis according to Steel and Torrie.[24]

RESULTS AND DISCUSSION

There were no deaths or signs of overt toxicity in any of the treatment groups, although in the mice dosed with 0.40 μg DBTCl/kg bw the experimental animals were less active than mice in the other groups. Compared with the control group exposure to DBTCl slightly decreased body weights at doses of 0.025 and 0.05 μg DBTCl/kg bw, whereas doses of 0.10, 0.20 and 0.40 μg DBTCl/kg bw significantly decreased both body and testes; testes weight also decreased significantly at 0.05 μg DBTCl/kg bw (Table 1). It has been reported that organotin compounds can inhibit metabolism enzyme activities, such as ATPase;[25] this might be the reason that DBTCl decreased mice body weight. In addition, organotins are known to induce tissues and cells necrosis and also to decrease sex hormone production;[9,12,15,26] this could be one of the possibilities for reduction in mice testes weight, or the cause of testes atrophy.

The reason that DBTCl decreased mice body and testis weights may be due to inhibition of this compound on metabolic enzymes, such as ATPase. Although DBTCl exhibited inhibitory effects on the parameters studied, when analyzed for its effects on the sex hormones estradiol and testosterone the

Table 1 The effects of DBTCl on body and testis weights in mice[a]

DBTCl (μg/kg bw)	Number of mice (head/group)	Initial body weight (g)	Final body weight (g)	Testes weight[b] (g)
0.00	7	39.39 ± 1.4	42.82 ± 2.1	0.40 ± 0.08
0.025	7	38.58 ± 1.8	38.10 ± 1.1	0.34 ± 0.09
0.05	7	39.66 ± 1.2	38.22 ± 1.3	$0.31 \pm 0.07^{*}$
0.10	7	39.85 ± 1.6	$35.12 \pm 1.4^{*}$	$0.29 \pm 0.07^{**}$
0.20	7	38.71 ± 1.1	$33.23 \pm 1.7^{**}$	$0.24 \pm 0.05^{**}$
0.40	7	40.21 ± 1.5	$32.22 \pm 2.1^{**}$	$0.22 \pm 0.06^{**}$

[a] Data presented as mean ± standard error.
[b] Testis weights are weights of the right and left testes.
* Significantly different from the control at $P < 0.05$.
** Significantly different from the control at $P < 0.01$.

results were different. The results demonstrated that when DBTCl ≥ 0.05 μg/kg bw, both estradiol and testosterone contents in treated mice increased significantly with increasing dose and presented dose–response relationships. The estradiol content increased more significantly than that of testosterone.[27] The effect of DBTCl on the testis resulting in testis atrophy may be due to necrosis of testis caused by DBTCl, and not due to the elevation in testosterone content.

The data presented in the Table 2 indicate that 0.025 μg DBTCl/kg bw had no effect on sperm viability, density and morphology in mice; whereas doses ranging from 0.05 to 0.40 μg DBTCl/kg bw caused significant dose-related changes in these parameters.

Under the optical microscope, sperm head and tail abnormalities were observed and the percentage of each abnormality in correspondence to the dose used was calculated. The results demonstrated that sperm with amorphous heads were dominant, whereas sperm with twin heads were very few (Table 3). On the other hand, sperm tails appeared to be more sensitive than other parts of sperm (Table 4).

The probability of depressed sperm viability and density being correlated to the log of DBTCl was high (Fig. 1). The ED_{50} values of DBTCl in terms of sperm viability and density were 0.17 μg/kg bw and 0.19 μg/kg bw respectively. The incidence of morphological abnormalities of sperm was significant after exposure to doses of DBTCl ranging from 0.05 to 0.40 μg/kg bw. The correlation between the incidence of sperm abnormalities and dose are presented in Fig. 1. The data mentioned above suggest that all of these parameters were more sensitive indicators of DBTCl exposure than body weight. This finding is in agreement with the effect of DBTCl on the immune system, including body weight and spleen index in mice.[13] 0.10–0.40 and

Table 2 The effects of DBTCl on sperm viability, density and morphological abnormalities in mice[a]

DBTCl (μg/kg bw)	Number of mice (per group)	Incubation time (min)	Sperm viability (%)	Sperm density ($\times 10^6$ /ml)	Sperm abnormality (%)
0.00	7	120	99.72 ± 2.1	3.56 ± 2.4	0.20 ± 0.02
0.025	7	120	98.93 ± 1.9	3.52 ± 1.3	0.18 ± 0.04
0.05	7	120	$89.22 \pm 4.6^{*}$	$3.11 \pm 1.9^{*}$	$6.23 \pm 1.4^{**}$
0.10	7	45	$68.55 \pm 3.8^{**}$	$2.64 \pm 2.6^{**}$	$16.48 \pm 2.1^{**}$
0.20	7	30	$48.22 \pm 7.8^{**}$	$1.75 \pm 3.2^{**}$	$21.74 \pm 3.5^{**}$
0.40	7	15	$18.31 \pm 5.2^{**}$	$0.92 \pm 4.1^{**}$	$29.22 \pm 2.3^{**}$

[a] Data presented as mean ± standard error.
* Significantly different from the control at $P < 0.05$.
** Significantly different from the control at $P < 0.01$.

Table 3 The percentages of sperm head abnormalities in mice[a]

DBTCl (μg/kg bw)	No. sperm	No hook (%)	Excessive hook (%)	Amorphous (%)	Short head (%)	Twin heads (%)
0.00	200	0.02 ± 0.00	0.00	0.00	0.04	0.00
0.025	200	0.32 ± 0.02	0.00	0.03 ± 0.00	0.00	0.00
0.05	200	$2.41 \pm 0.04^{*}$	0.32 ± 0.00	$3.10 \pm 0.01^{*}$	1.27 ± 0.04	0.00
0.10	200	$4.82 \pm 0.11^{**}$	1.44 ± 0.02	$5.01 \pm 0.62^{**}$	1.79 ± 0.32	0.72 ± 0.02
0.20	200	$8.00 \pm 0.63^{**}$	$1.78 \pm 0.03^{*}$	$9.54 \pm 0.22^{**}$	$2.09 \pm 0.51^{*}$	1.03 ± 0.21
0.40	200	$9.11 \pm 1.02^{**}$	$2.49 \pm 0.41^{*}$	$13.01 \pm 0.16^{**}$	$2.22 \pm 0.73^{*}$	1.68 ± 0.01

[a] Data presented as mean ± SE.
* Significantly different from the control at $P < 0.05$.
** Significantly different from the control at $P < 0.01$.

0.10–0.05 μg DBTCl/kg bw slightly decreased mice body weights and spleen indices respectively, whereas 0.10–0.40 μg DBTCl/kg bw significantly decreased mice spleen indices. In addition, 0.10–0.40 μg DBTCl/kg bw demonstrated a very high toxicity with an ED_{50} value of 0.18 μg/kg bw on B-lymphocytes by inhibiting antibody secretion significantly.

The results demonstrated that DBTCl induced a dose-related decrease in sperm viability. After 2 h of incubation, the percentage sperm mortality induced by 0.025 μg/kg bw and 0.05 μg/kg bw was 1.07% and 10.78%, respectively. At a dose of 0.10 μg/kg bw, DBTCl induced 31.15% of sperm mortality during a 45 min incubation period. 0.20 μg DBTCl/kg bw induced 51.78% mortality a after 30 min incubation period. 0.40 μg DBTCl/kg bw induced 81.69% mortality within 15 min, and for these two last groups the survival sperm had a strange motility. All sperm died at doses of 0.20 and 0.40 μg DBTCl/kg bw after 2 h of incubation. At the same time the density of sperm was also decreased. These data suggest that DBTCl can be considered to be spermatocidal, like other organometallic compounds, such as organoarsenic, organomercuric and organovanadium species.[25,27] It is clear that the decrease in sperm density was due to the atrophy of the testes. In mice injected with 0.05 μg DBTCl/kg bw, sperm with immature heads were dominant (73.3%). In mice treated with 0.10 μg DBTCl/kg bw, sperm with teratic heads (no hook, short head, pin head, or amorphous head), and bent tails were more prevalent than other teratic sperm (66.6% and 25% respectively). In mice administered 0.20 μg DBTCl/kg bw or 0.40 μg DBTCl/kg bw, sperm with twin heads and tails, and sperm with only twin tails were equally prevalent. These results were consistent with previous reports about organometallic compounds and heavy metals.[23,28] The present study also showed dose-dependent

Table 4 The percentages of sperm tail abnormalities in mice[a]

DBTCl (μg/kg bw)	No. sperm	Coiled flagelium (%)	Bent flagelium (%)	Bent flagelium tip (%)	Twisted flagelium (%)	Twin flagelium (%)
0.00	200	0.00	0.03	0.00	0.02	0.00
0.025	200	0.04 ± 0.02	0.00	0.00	1.21 ± 0.04	0.00
0.05	200	1.25 ± 0.33	$3.29 \pm 1.24^{*}$	$5.67 \pm 1.22^{**}$	$4.51 \pm 0.23^{*}$	0.02 ± 0.01
0.10	200	$1.67 \pm 0.23^{*}$	$5.02 \pm 1.65^{**}$	$8.95 \pm 2.13^{**}$	$5.21 \pm 1.02^{**}$	0.47 ± 0.02
0.20	200	$4.55 \pm 1.23^{**}$	$6.98 \pm 2.22^{**}$	$12.01 \pm 3.12^{**}$	$7.33 \pm 2.12^{**}$	1.03 ± 0.03
0.40	200	$8.96 \pm 2.56^{**}$	$9.88 \pm 3.04^{**}$	$15.41 \pm 3.78^{**}$	$9.06 \pm 2.56^{**}$	1.56 ± 0.01

[a] Data presented as mean ± SE.
* Significantly different from the control at $P < 0.05$.
** Significantly different from the control at $P < 0.01$.

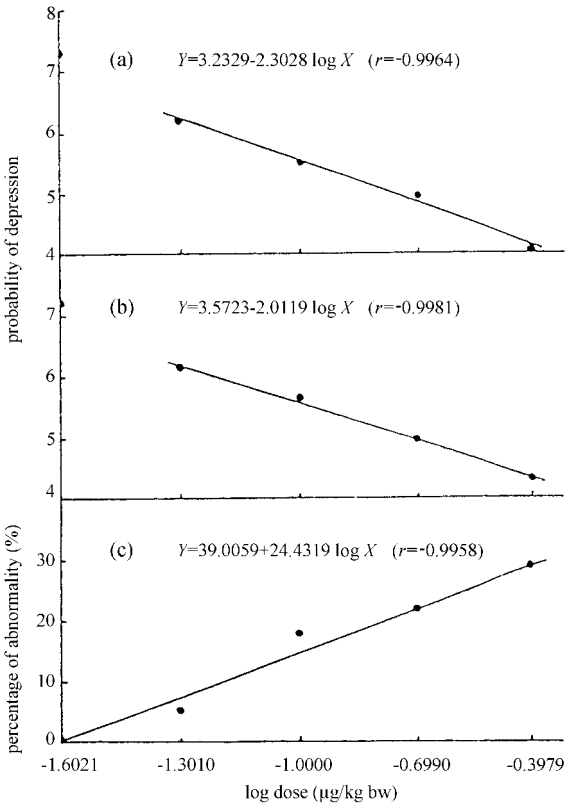

Figure 1 The dose–response relationship between the sperm survival rate (a), density (b), abnormality rate (c) in mice and DBTCl.

decreases in sperm viability, sperm density, and increased sperm morphological abnormalities. This may be due to the effects of DBTCl on various morphological and biochemical events related to spermatogenesis, or it may due to the atrophy of the testes, which led to a reduction of sperm number.[25,29] In addition, the organotin compounds are well known to be potent inhibitors of enzyme activities,[26,28] which can lead to destruction of seminiferous epithelium and losses in germinal elements. This, in turn, could result in a reduction in the number of spermitids associated with the decrease in sperm production in testes. Low sperm production could further lead to decreased sperm release in the epididymes of DBTCl-treated mice.

The current study revealed that DBTCl might interfere with fertility in mammals by causing atrophy of the testes, including different types of abnormality and reductions in sperm viability and mortality. Further research is needed to show the mechanism by which DBTCl reduces fertility in mammals.

Acknowledgements We want to thank the Dean of The Chinese Academy of Sciences (CAS) and the Director of the Institute of Zoology, CAS, who supported this project. We also want to express our deepest gratitude to Professor Dayuan Chen for his advice and for being our consultant from the beginning of the project. We are grateful to Professor Chen Zhu, the director of Key State Laboratory of Reproduction, Institute of Zoology, CAS, for his recommendations to the project. Special thanks go to Professor Qingyuan Sun and Mrs Xiangfen Son for their expertise and technical assistance.

REFERENCES

1. Alam MS, Husain R, Seth PK, Srivastava SP. *Bull. Environ. Contam. Toxicol.* 1993; **50**: 286.
2. Penninks AH, Seinen W. In: Gibson GG, Hubbard R, Parke DV. eds, *Immunotoxicology*. Academy Press: London, 1983; 27.
3. Davis A, Barale R, Brun R, Forster R, Gunther T. *Mutat. Res.* 1987; **188**: 65.
4. Noda T, Morita S, Shimizu M, Yamano T, Yamadaa A. *Health Environ. Sci.* 1988; **50**: 66.
5. Noda T, Nakamura T, Shimizu M, Yamano T, Morita S. *Bull. Environ. Contam. Toxicol.* 1992; **49**: 715.
6. Margui RJ, Tkacs RJ, Chau YK, Bengert GA, Wong PTS. *Chemosphere* 1986; **15**: 253.
7. Margui RJ. *Water Pollut. Res. J. Can.* 1991; **26**: 243.
8. Gibbs PE, Bryan GW. *J. Mar. Biol. Assoc. UK* 1986; **66**: 767.
9. Waldock MJ, Thain JE. *Mar. Pollut. Bull.* 1983; **14**: 411.
10. Evans DW, Laughlin Jr RB. *Chemosphere* 1984; **13**: 213.
11. Laughlin Jr RB, French W, Guard HE. *Environ. Sci. Technol.* 1986; **20**: 884.
12. Ananie D, Chen X, Zhang Y, Dai S. *Chin. Environ. Sci.* 1998; **18**: 378.
13. Short JW, Thrower FP. *Mar. Pollut. Bull.* 1986; **17**: 542.
14. Li Q, Osaka M, Takahashi K, Matsutani T, Mori K. *Bull. Environ. Contam. Toxicol.* 1997; **58**: 489.
15. Newton DW, Hays RL. *J. Econ. Entomol.* 1978; **61**: 1668.
16. Winek CL, Marks Jr MJ, Shanor SP, Davis ER. *Clin. Toxicol.* 1978; **13**: 281.
17. Harazono A, Ema M, Ogawa Y. *Arch. Environ. Contam. Toxicol.* 1998; **34**: 94.
18. Sinha N, Narayan R, Saxena DK. *Bull. Environ. Contam. Toxicol.* 1997; **58**: 79.
19. Egbunke GN, Elemo A. *J. Reprod. Fert.* 1978; **54**: 245.
20. Mattison DR. *Reproductive Toxicology: Progress in Clinical Biological Research* vol. 117. Alan R. Liss: New York, 1983; 1.
21. Kempinas WDG, Suarez JD, Roberts NL, Strader L, Ferrel J. *Biol. Reprod.* 1998; **59**: 890.
22. Muller B, Kirchner C. *J. Reprod. Fert.* 1978; **54**: 167.

23. Ananie D. The effect of some organotin compounds on immune system. PhD thesis, Nankai University, 1998.
24. Steel RGD, Torrie JH. *Principles and Procedures of Statistics*. McGraw-Hill: New York; 1960.
25. Gupta PK, Salunkhe DK. *Modern Toxicology*. Metropolitan Book Co. Pvt. Ltd: India, 1985; 340.
26. Aldrine NW, Cremer JE. *J. Biochem.* 1955; **61**: 406.
27. Ananie D, Huang Y. *Chin. J. Appl. Environ. Biol.* 2000; **6**: 549.
28. Piasek M, Kostial K. *Bull. Environ. Contam. Toxicol.* 1987; **39**: 448.
29. Robb GW, Amann RP, Killiam GJ. *J. Reprod. Fert.* 1978; **54**: 103.

