Postnatal Development and Resistance to *Plasmodium yoelii* Infection of Mice Prenatally Exposed to Triphenyltin Hydroxide

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ABSTRACT: In this study, we evaluated the effects of prenatal exposure to triphenyltin hydroxide (TPTH) on the postnatal development of Swiss Webster mice. Females were treated by gavage (0, 7.5 15 and 30 mg TPTH/kg/day) on days 6–17 of gestation. After birth, the progeny was examined for deaths, body weight gain and appearance of developmental landmarks. On postnatal day 50, one male and one female of each litter were inoculated with *Plasmodium yoelii* and the time-course of infection was monitored. TPTH was embryolethal at doses $\geq$15 mg/kg/day. Body weight at birth was decreased, but no alteration of pup body weight was observed after postnatal day 5. Except for an advancement of incisor eruption in the group treated with 15 mg/kg/day, no alteration of somatic development was noted. A shorter latency to peak parasitemia and a reduced malaria-induced spleen enlargement were observed in mice prenatally exposed to TPTH. In conclusion, prenatal exposure to TPTH at doses $\geq$15 mg/kg enhanced neonatal lethality, reduced pup birth weight and interfered with the response to infection with *P. yoelii* in adulthood.
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Keywords: triphenyltin; TPTH; *Plasmodium yoelii*; host resistance to infection; prenatal exposure

INTRODUCTION

Organotin compounds (OTC), including triphenyltin (TPT) and tributyltin (TBT) derivatives, have been used as active ingredients of marine antifouling paints for boats, ships, fish nets and cages, as wood preservatives, as disinfectants, as molluscicides and agricultural pesticides, and as industrial catalysts and polyvinylchloride (PVC) stabilizers (Piver 1973; WHO, 1980, 1999).

TBT has been reported to cause imposex in mollusks by blocking aromatase-catalyzed conversion of testosterone into 17-β-estradiol (Morcillo and Porte, 1999). Owing to the marked toxicity of TBT to aquatic organisms its use in antifouling paints has been restricted by several countries (Fent, 1996).

In mammals, toxicity of OTCs has been revealed by a variety of experimental studies and the main targets for TPT and TBT derivatives induced toxicity seem to be the immune system and the reproductive function (Boyer, 1989; WHO, 1999). TBTO, for instance, was shown to reduce thymus weight and to impair resistance to *Trichinella spiralis* infection in rats (Vos et al., 1990). Moreover, it was reported that, in the rat, TBT adversely affects male and female reproductive organs (WHO, 1999; Ogata et al., 2001; Omura et al., 2001; Grote et al., 2004) while, in the mouse, TBTO was found to be teratogenic (Davis et al., 1987). Various studies have demonstrated that TPT derivatives are immunotoxic as well. Nishida et al. (1990), for instance, found that subacute exposure of mice to triphenyltin chloride (TPTC) reduced the weight of thymus and spleen and inhibited the T-cell-dependent humoral and cellular responses. Vos et al. (1984) also reported that, in the

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rat, exposure to TPTH for 3 or 4 weeks induced lymphopenia and lymphocyte depletion in thymus-dependent areas of spleen and lymph nodes. Toxicity of triphenyltin to reproductive organs has been shown by several studies as well. TPT derivatives were reported to cause Leydig cell hyperplasia and morphological changes of the endometrium (WHO, 1999). Recently, it was reported that peripubertal exposure to TPT (6 mg/kg) affected sexual development of male and female rats (Grote et al. 2004, 2006). A few studies in rats have evaluated the prenatal toxicity of TPT derivatives by examining near term fetuses removed by caesarean section (Chernoff et al., 1990; Noda et al., 1991; Ema et al., 1999). As far as the authors are aware, however, a few studies have investigated the effects of prenatal exposures to organotin compounds on postnatal development (Makita et al., 2003, 2004; Grote et al., 2007).

The present study was undertaken to evaluate the effects of prenatal exposure to TPTH on the postnatal development of mice. In addition, we also investigated the hypothesis that *in utero* exposure to TPTH might cause a postnatally persistent impairment of host defense mechanisms against malaria parasites (i.e., *Plasmodium yoelii*).

MATERIALS AND METHODS

Animal Maintenance

Male and nulliparous female Swiss Webster mice, from the Oswaldo Cruz Central Animal House breeding stock, were used. The animals were housed in standard plastic cages with stainless steel covers and wood shaving as bedding. All animals were kept under controlled temperature (21 ± 2°C), relative humidity (approx. 70%) and at a constant light/dark cycle (lights on from 9 a.m. to 9 p.m.). A commercially available rodent diet (Nuvilab, Nuvital, Curitiba, PR, Brazil) and tap water were provided *ad libitum*. The research protocol was approved by the Ethics Committee on the Use of Laboratory Animals of Oswaldo Cruz Foundation (CEUA-FIOCRUZ).

Mating Procedure

Mating was performed as described by Chahoud and Kawasigroch (1977). Briefly, two females were placed into the cage of one male at 7 a.m. At 9 a.m. females were removed, pooled and checked for vaginal plugs. The first 24 h after detection of a vaginal plug were considered as day 0 of pregnancy.

Treatment with TPTH

Triphenyltin hydroxide (TPTH, purity: 97.3%, lot number AARA 00192, AGREVO, Brazil) was dissolved in corn oil and solutions were prepared once a week. After detection of a vaginal plug, females were randomly assigned to one of four experimental groups. Female mice were then treated by gavage either with TPTH (7.5, 15 or 30 mg/kg body weight/day) or with the vehicle alone (corn oil, Mazola, Brazil, 10 mL/kg body weight/day) on gestation days (GD) 6–17. Treatment was always in the morning between 9 and 11 a.m. and, starting immediately after dosing, mice were examined for behavioral changes or any other sign of toxicity for 7 h. Maternal body weight was determined on gestation day 1 (GD1) and thereafter on a daily basis during the treatment period.

Delivery and Evaluation of Postnatal Development

From pregnancy day 18 onwards dam's cages were inspected twice a day (8 a.m. and 5 p.m.) for births, and the day on which they delivered their pups was designated as postnatal day 1. As soon as possible after birth the number of viable and dead newborns per litter was recorded, and all pups were sexed and weighed. The death of a newborn on postnatal day 1 was considered a stillbirth. Females that did not give birth within 22 gestation days and those that cannibalized the whole litter after delivery were killed by cervical dislocation and postmorten examined. Maternal liver, spleen, thymus and uterus weights were recorded and the number of implantation sites was determined by the method of Salewiski (1964). Offspring weight gain was recorded on postnatal days 1, 5, 10, 15, and 20. Each and every pup was examined daily and days on which developmental milestones appeared were recorded as follows:

- *Ear unfolding*: the first sign of detachment of the external pinnae of the ear from the side of the head.
- *Incisor eruption*: the first sign of eruption through the gums of both lower incisors.
- *Development of the fur*: the first detection of downy hair.
- *Eye opening*: separation of upper and lower eyelids and complete opening of both eyes.
- *Vaginal opening*: the first sign of detachment of the vagina.
- *Testes descent*: the first sign of testis descent, determined by scrotum palpation.

At weaning (postnatal day 21) all mothers were killed by cervical dislocation and postmortem examined. Pups were then separated according to sex and housed (five per cage) in mouse standard plastic cages with stainless-steel lids and wood shavings as bedding.

Evaluation of Resistance to Infection with *Plasmodium yoelii*

When mice were ~50-day-old, a challenge test with *P. yoelii* took place. Animals (01 male and 01 female/litter) were inoculated—by the ip route—with 10^3 *P. yoelii*

TABLE I. Effects of TPTH (0, 7.5, 15, 30 mg/kg body wt/day) orally administered to mice from days 6 to 17 of pregnancy on maternal and offspring parameters evaluated at birth and on the number of viable litters on post-natal day 21 (PND 21)

	TPTH (mg/kg body wt/day)			
Treatment	0	7.5	15	30
Treated mice, *N*	20	21	19	19
Pregnant mice, *N* (%)	17 (85%)	18 (86%)	19 (100%)	18 (95%)
Maternal deaths, *N* (%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)
Maternal weight gain (Λg)				
GD, 6–18	18.2 ± 9.2	20.9 ± 8.1	13.3 ± 8.8[a,b]	4.5 ± 8.7[a,b,c]
GD, 0–18	21.2 ± 9.6	23.9 ± 8.0	15.8 ± 9.0[a,b]	7.2 ± 8.9[a,b,c]
Pre-term births[d]	0 (0%)	0 (0%)	1 (5%)	2 (11%)
Prenatal whole litter loss[e]	3 (18%)	2 (11%)	4 (21%)	9 (50%)[a,b]
Peri- and post-natal (whole litter) deaths[f]	0 (0%)	2 (11%)	8 (42%)[a,b]	6 (33%)[a]
Viable litters on PND 1	14 (82%)	15 (83%)	8 (42%)[a,b]	2 (11%)[a,b,c]
Viable litters on PND 21	14 (82%)	14 (78%)	7 (37%)[a,b]	2 (11%)[a,b]

Maternal weight gain (mean ± SD) was analyzed by the Kruskal-Wallis test followed by the Mann–Whitney *U* test. All other parameters were analyzed by the χ^2 test. Differences ($P \leq 0.05$) are indicated as indicated below.

[a] ≠ from control group.

[b] ≠ from 7.5 mg TPTH/kg group.

[c] ≠ from 15mg TPTH/kg group.

[d] Females that delivered before GD18.

[e] Females (with implantation sites) that did not give birth within 22 days.

[f] Mortality of whole litter, all deaths occurred on PND 1 with two exceptions, i.e., one litter (15 mg TPTH/kg) on PND 4 and one litter (7.5 mg TPTH/kg) on PND 20.

(Py17XNL)-infected erythrocytes, obtained from Swiss Webster mice. Erythrocytes were counted by using a Neubauer chamber. Mice infected with *P. yoelii* were checked daily for clinical signs and symptoms and blood smears were prepared every other day by taking a drop of blood from the tip tail. Blood smears were stained with Giemsa's dye and at least 1000 erythrocytes were examined to determine the rate of parasitemia (percentage of erythrocytes bearing parasites). One month after infection all infected mice were killed by cervical dislocation and thymus, liver, and spleen were removed and weighed.

Statistical Analysis

Data were evaluated by one-way analysis of variance (ANOVA) or, alternatively, by the Kruskal-Wallis test whenever the data did not fit a normal distribution. Differences between groups were tested by using a two-sided Student's *t* test or Mann–Whitney *U* test. Proportions were analyzed by the chi-square test. In any case, differences were considered significant at $P \leq 0.05$.

RESULTS

Pre- and Postnatal Mortality

The death (on GD17) of one out of nineteen dams treated with the highest dose (30m kg body wt/day) suggested that this dose level was maternally toxic (Table I). No sign of maternal toxicity was noted at lower doses of TPTH. Among mice treated with the highest dose, however, the overall pregnancy weight gain (weight on GD18 minus weight on GD0) was lower, and the proportion of dams that did not deliver until day 22 was higher than that observed in controls and lower dose groups. Implantation sites were detected in the uteri of those dams thereby indicating that exposure to 30 mg TPTH/kg body wt on GD6–17 increased the number of postimplantation whole-litter losses (Table I).

On postnatal day 1 (PND 1), there was also a high mortality of pups prenatally exposed to the two highest doses of TPTH. All pups of six out of eighteen litters (33%) exposed to 30 mg TPTH/kg and all pups of eight out of nineteen litters (42%) exposed *in utero* to 15 mg TPTH/kg died before or within 24 h after birth (Table I). Most pups found dead on PND1 were partially cannibalized by their mothers and thus a necropsy was not feasible. Among those TPTH exposed newborns that were examined, however, cleft palate was a relatively common finding. Owing to this pronounced pre and neonatal mortality, the number of viable litters on PND 1 was drastically reduced among mice prenatally exposed to the intermediate (15 mg/kg body wt) and high (30 mg/kg body wt) doses of TPTH (Table I). Moreover, duration of pregnancy was shortened by TPTH because one (5%) and two (11%) dams treated with 15 and 30 mg TPTH/kg, respectively, gave birth before day 18 of pregnancy while no preterm birth was noted in the control group as well as in the lowest dose group (Table I).

TABLE II. Effects of TPTH (0, 7.5, 15, 30 mg/kg body wt/day) orally administered to mice from days 6 to 17 of gestation on litter size and postnatal weight gain of exposed offspring

	TPTH (mg/kg body wt/day)			
	0	7.5	15	30
Litters (*N*)	14	15	8	2
Postnatal day:				
PND 1				
body wt (g)	1.66 ± 0.12	1.62 ± 0.16	1.41 ± 0.12[a,b]	(*)
litter size (*N*)	10.1 ± 1.3	10.5 ± 2.9	9.1 ± 1.2	(*)
PND 5				
body wt (g)	2.95 ± 0.38	2.99 ± 0.57	2.68 ± 0.34	(*)
litter size (*N*)	9.6 ± 1.5	10.1 ± 2.9	8.3 ± 1.9	(*)
PND 10				
body wt (g)	5.11 ± 0.55	5.19 ± 1.02	5.24 ± 0.58	(*)
litter size (*N*)	9.4 ± 1.8	9.9 ± 2.8	7.9 ± 2.1	(*)
PND 15				
body wt (g)	6.77 ± 0.49	6.71 ± 1.55	7.43 ± 1.15	(*)
litter size (*N*)	9.4 ± 1.8	9.9 ± 2.8	7.7 ± 2.4	(*)
PND 20				
body wt (g)	8.58 ± 1.11	8.48± 2.09	9.03 ± 2.08	(*)
litter size (*N*)	9.2 ± 1.9	9.0 ± 3.4	7.4 ± 2.2	(*)

Values (mean ± SD) were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. Differences ($P < 0.05$) are indicated by superscripts as explained below.

[a] ≠ from control group.

[b] ≠ from 7.5mg TPTH/kg group. Litter was considered as the experimental unit.

(*): two viable litters only.

Postnatal Development

Mortality of mice prenatally exposed to TPTH during postnatal period was much lower after PND 1. The death of all pups of one litter of the intermediate dose (15 mg/kg) group occurred on PND 4 and all pups of one litter of the lowest dose group were found dead on PND 20 (Table I). Litter size was also reduced in the intermediate dose group due to pup deaths, but the litter size at weaning (PND 20) did not differ between the lowest dose and the control group (Table II).

Although body weight at birth was clearly reduced in the group treated *in utero* with the intermediate dose (15 mg/kg), after PND 5 the body weight of pups treated with the lower and the intermediate doses of TPTH did not differ from that of controls (Table II).

Except for 1-day advancement of incisor eruption at the group treated with 15 mg/kg/day and possibly also at the low dose group, prenatal exposure to TPTH did not alter the day of appearance of landmarks of somatic development such as ear unfolding, primary coat, eye opening (Table III). The day of vaginal opening and testes descent, which are developmental landmarks evaluated after weaning, were not altered by prenatal treatment with TPTH either (Table III).

Challenge Test with *Plasmodium yoelii*

To investigate whether exposure to TPTH during gestation would cause long lasting adverse effects on the immune function, control and exposed mice were inoculated with a nonlethal strain of *P. yoelii* when they were ~50 days old. After infection, the percentage of red blood cells bearing parasites (parasitemia) was determined every other day for 30 days. No deaths were noted and no difference was found between controls and TPTH-exposed mice regarding the highest level (peak) of parasitemia attained after infection with *P. yoelii* (Table IV). The time to peak parasitemia, however, was shorter in the group exposed to the intermediate dose as compared to controls and mice exposed to the lowest dose of TPTH during gestation (Table IV). The proportion of infected mice exhibiting a complete cure (i.e., showing no parasites in the blood smear) within 30 days, and the lowest level of parasitemia after the peak, on the other hand, did not differ between controls and TPTH-exposed groups (Table IV). The latency to the lowest level of parasitemia after the peak did not differ between controls and TPTH exposed groups either (Table IV).

As shown in Table V, prenatal exposure to TPTH did not modify the absolute and relative weights of liver, spleen and thymus in adult (55 day old) mice. Absolute and relative weights of thymus of infected mice were not changed by prenatal exposure to TPTH either (Table V). As expected, infection with malaria parasites increased both absolute and relative weights of spleen in controls. Among *P. yoelii*-infected mice, absolute and relative weights of spleen did not differ between controls and the lowest dose group but the intermediate dose of TPTH reduced

TABLE III. Postnatal day (PND) on which some landmarks of somatic development appeared in the offspring of mice exposed to TPTH (0, 7.5, 15, 30 mg/kg body wt/day) on pregnancy days 6–17

	TPTH (mg/kg body weight)			
	0	7.5	15	30
Ear unfolding PND	5 (3–6)	5 (3–6)	5 (4–6)	(2)
Primary coat PND	5 (4–7)	5 (4–7)	5 (5–6)	(2)
Incisor eruption PND	11 (10–12)	11 (8–12)[a]	10 (9–11)[a]	(2)
Eye opening PND	16 (14–19)	16 (13–20)	16 (15–20)	(2)
Vaginal opening PND	25 (20–31)	27 (23–36)	26 (23–33)	(1)
Testes descent PND	24 (20–35)	26 (20–36)	26 (22–32)	(2)

Values are median and range (min–max.). Data were analyzed by the Kruskall-Wallis test followed by the Mann–Whitney *U* test. PND: postnatal day.

[a] Significantly different ($P < 0.05$) from control group. Weaning was on PND 21.

TABLE IV. Effects of prenatal exposure to TPTH (0, 7.5, 15, 30 mg/kg body wt/day) on gestation days 6–17 on the susceptibility of adult mice (PND 50) to *P. yoelii* infection

	TPTH (mg/kg body weight)			
	0	7.5	15	30
Infected mice (*N*)	25	26	13	3
Mice showing complete recovery within 30 days after infection (%)	20 (80)	22 (85)	12 (92)	–
Peak parasitemia (%)	7.1 (1.6–34.7)	6.3 (1.4–21.2)	6.3 (2.8–12.1)	–
Latency to peak parasitemia (PI day)	12 (8–16)	12 (6–14)	8 (6–14)[a]	–
Lowest post-peak parasitemia (%)	0 (0–4.6)	0 (0–4.4)	0 (0–1.6)	–
Latency to lowest post-peak parasitemia (PI day)	18 (12–20)	18 (18–20)	18 (18–20)	–

Values are median (min–max). Complete recovery: number of mice with no parasites in the blood smear. Proportions were analyzed by the chi-square test. All other parameters were analyzed by the Kruskall-Wallis test followed by the Mann–Whitney U-test.

[a] Significantly different ($P < 0.05$) from control group.

infection-caused splenomegaly (Table V). A small reduction of the relative weight of the liver was also detected in mice exposed to the intermediate dose of TPTH during gestation (Table V).

DISCUSSION

Results presented in this paper showed that TPTH, administered to mice by the oral route on gestation days 6–17, was maternally toxic at a dose as high as 30 mg/kg body wt, and embryolethal at doses ≥15 mg/kg body wt. Newborn mortality on PND 1 was also enhanced in the groups treated with doses ≥15 mg/kg body wt, but only a few additional deaths were noted thereafter. Although some individual deaths occurred within litters, TPTH-caused mortality was mainly due to whole-litter deaths. Most pups found dead on PND 1 had been partially cannibalized by their mothers. In those cases in which a postmortem examination was feasible, cleft palate was common finding in pups from TPTH-

TABLE V. Weight (g) of thymus, spleen and liver of mice prenatally exposed to TPTH. Non-infected mice were killed on day 55 and those which were postnatally challenged with *P. yoelii* on day 50 were killed on day 80

	TPTH (mg/kg body weight)			
	0	7.5	15	30
Non-infected mice (PND 55)				
Litters*	13	14	7	2
Thymus	0.086 ± 0.009	0.094 ± 0.015	0.086 ± 0.008	–
(% of body weight)	0.266 ± 0.042	0.293 ± 0.057	0.272 ± 0.039	–
Spleen	0.185 ± 0.045	0.165 ± 0.030	0.147 ± 0.026	–
(% of body weight)	0.556 ± 0.111	0.513 ± 0.104	0.457 ± 0.083	–
Liver	1.93 ± 0.34	1.90 ± 0.24	1.88 ± 0.21	–
(% of body weight)	5.76 ± 0.45	5.79 ± 0.29	5.78 ± 0.54	–
Body weight (g)	33.27 ± 4.75	32.75 ± 3.60	32.45 ± 2.47	–
Infected mice (PND 80)				
Litters**	13	14	7	2
Thymus	0.084 ± 0.016	0.079 ± 0.011	0.071 ± 0.012	–
(% of body weight)	0.227 ± 0.045	0.225 ± 0.036	0.199 ± 0.039	–
Spleen	0.405 ± 0.09	0.363 ± 0.162	0.235 ± 0.063[a,b]	–
(% of body weight)	1.090 ± 0.241	1.010 ± 0.380	0.652 ± 0.182[a,b]	–
Liver	2.19 ± 0.30	2.01 ± 0.35	1.91 ± 0.34	–
(% of body weight)	5.89 ± 0.44	5.58 ± 0.44	5.16 ± 0.54[a]	–
Body weight (g)	37.05 ± 3.84	35.80 ± 4.64	37.06 ± 2.29	

All values (g) are mean ± SD. In any case the litter was considered the experiment unit. Absolute values were analyzed by one-way analysis of variance and Student's *t*-test. All other parameters were analyzed by the Kruskall-Wallis test followed by the Mann–Whitney *U* test. Statistically significant differences between groups ($P \leq 0.05$) are indicated as shown below.

[a] ≠ from control group.

[b] ≠ from 7.5 mg TPTH/kg group.

* ca. 55 days old; ** ca. 80 days old.

treated dams. It is of note that an increased incidence of cleft palate was observed in mice exposed *in utero* to TPTH (Sarpa et al., 2007), and also in rats (Ema et al., 1999) and mice (Davis et al., 1987; Faqi et al., 1997) prenatally exposed to other OTCs.

One dam of the intermediate and two dams of the high dose group delivered their pups before term (GD18). This finding suggests that TPTH shortened the duration of pregnancy. A similar effect of other OTCs on the duration of pregnancy was described in rats (Grote et al., 2007) and mice (Baroncelli et al., 1995). Since OTCs were reported to interfere with maternal blood levels of several hormones, and hormones play a key role in the maintenance of pregnancy in mammals, earlier parturitions may have resulted from a TPTH-induced hormonal imbalance in late gestation. The mechanism by which OTCs shortened the duration of pregnancy in rodents, however, remains to be elucidated.

Prenatal toxicity of TPTH was also apparent as a reduction of pup body weight at birth (PND 1) at the intermediate dose level. Despite the pronounced lethality observed at birth, *in utero* exposure to TPTH at doses $\geq$15 mg/kg body wt had only minor effects on further postnatal somatic development of survivors. Differences of body weight between controls and TPTH-exposed groups were not seen after PND 5 and, except for 1-day advancement of incisor eruption, prenatal exposure to TPTH did not alter the day on which appeared the developmental milestones recorded in this study. The foregoing findings were consistent with the idea that a catch up postnatal growth occurred after *in utero* exposure to TPTH had been discontinued on GD17.

Despite the long time elapsed between exposure to TPTH (GD6-17) and the challenge test with *P. yoelii* (PND 50), the response of mice which had been exposed to 15 mg/kg body wt was found to be altered. Although parasites were cleared from almost all infected mice within 30 days, and the peak parasitemia did not differ between control and TPTH-exposed groups, time to peak parasitemia was shortened among mice from the intermediate dose group. Furthermore, malaria-induced spleen enlargement, clearly seen in control and lower dose groups, was attenuated in the group that had been prenatally exposed to the intermediate dose of TPTH. It should be pointed out that prenatal exposure to TPTH did not affect the weight of lymphoid organs (thymus and spleen) and liver on PND 55 in noninfected mice. Atrophy of the thymus and reduction in spleen weight following repeated exposures to immunotoxic OTCs have been found by different rodent studies (WHO, 1999). Thymus atrophy associated with a lymphocyte depletion in the thymic cortex seems to be the predominant effect of immunotoxic trialkyltin compounds (Snoeij et al., 1985). Data provided by Snoeij et al. (1985) seemed to indicate that thymus atrophy is to some extent reversible after exposure is discontinued. There have been, however, very few studies on the post-treatment persistence of OTC immunotoxic effects. In the present study, in noninfected mice we found no change of thymus and spleen weights 2 months after the exposure to TPTH had been ceased, but spleen enlargement triggered by *P. yoelii* infection was substantially attenuated in mice that had been exposed to 15 mg/kg of TPTH. The relative weight of liver was also decreased in the group of infected mice that was prenatally exposed to the intermediate dose of TPTH. Spleen plays a crucial function in the control of malaria infection and is primarily involved in the clearance of damaged and parasitized erythrocytes. Erythropoiesis and hematopoiesis as well as generation of pathogen-specific T and B cell responses are also among the roles of spleen in malaria infection (Engwerda et al., 2005). Splenectomized mice infected with *P. yoelii*, for instance, were reported to exhibit a higher peak parasitemia and a prolonged blood parasitemia (Sayles et al., 1993). Splenectomy, nevertheless, has also been shown to protect mice from cerebral malaria after challenge with *P. berghei* (Hermsen et al., 1998). Therefore, as pointed out by Engwerda et al. (2005), the spleen seems to be involved in both protective and pathological responses after infection with malaria parasites. Data provided by this study showed that prenatal exposure to TPTH advanced peak parasitemia and reduced malaria-induced splenomegaly in mice. The highest level of parasitemia, however, did not differ between control and treated groups, and almost all infected mice cleared the parasites within 30 days after infection. Based on the foregoing findings, therefore, it is not possible to say that *in utero* exposure to TPTH reduces host resistance to a malaria infection in adulthood. Results, however, strongly suggest that exposure to TPTH during embryo-fetal development may cause a persistent functional alteration of the immune system still detectable in adulthood.

In conclusion, taken together, findings from the present study in the mouse indicated that *in utero* exposure to TPTH markedly enhanced prenatal and neonatal mortality and interfered with host response to malaria parasites (*P. yoelii*) in adulthood. Based on data presented in this paper, a NOAEL (no observed adverse effect level) for developmental toxicity of TPTH was set at 7.5 mg/kg body wt/day by the oral route.

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