Pesticide Effects on Body Temperature of Torpid/Hibernating Rodents (*Peromyscus leucopus* and *Spermophilus tridecemlineatus*)

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Abstract. Environmental contaminants have been shown in the lab to alter thyroid hormone concentrations. Despite the role these hormones play in the physiological ecology of small mammals, no one has investigated the possible effects of thyroid-disrupting chemicals on mammalian thermal ecology and thermoregulatory ability. Because the energetic impact of such a disruption is likely to be most dramatic during times already energetically stressful, we investigated the effects of two common pesticides (atrazine and lindane) on the use of daily torpor in white-footed mice, and the use of hibernation in 13-lined ground squirrels. Fortunately, we found that these strategies for over-wintering success were not impaired.

Introduction

Recent studies have provided convincing evidence that a number of chemicals have disruptive effects on the endocrine system (Colborn and Clement, 1992). These "endocrine disruptors" include many pesticides and polychlorinated biphenyls (PCBs) and can compromise an animal's survival, or a species' continued existence, without directly killing any individuals. Because winter is already an energetically stressful time of year, any additional stress imposed by an endocrine disruptor may be especially detrimental at this time.

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For mammals, the energetic difficulties associated with winter are two-fold. First, since reduction of heat loss is not usually sufficient, there are the increased thermoregulatory demands of remaining euthermic. These must be met via either shivering or nonshivering thermogenesis (NST). Second, winter is usually associated with decreased quantity and quality of available food. To avoid these energetic problems, many species of small mammals have evolved the ability to enter torpor, either on a limited basis (daily torpor) or a seasonal basis (hibernation).

The relationship between mammalian thermoregulation, torpor, and the thyroid gland has been examined extensively but is still not completely understood. We know that thyroid function in small mammals is altered by cold exposure and by approaching winter (Tomasi and Mitchell, 1996), and that thyroid changes occur prior to and during hibernation (Tomasi and Stribling, 1996; Tomasi et al., 1998; Nicol et al., 2000). Since brown fat function is also dependent upon thyroid hormones (thyroxine – T_4 and triiodothyronine – T_3), changes in the plasma levels could affect thermogenensis and/or the use of daily torpor (Himms-Hagen, 1990). Because of these relationships, improper thyroid function could potentially interfere with the use of torpor.

Many environmental contaminants have been documented to alter thyroid function, mostly in laboratory rodents (Brucker-Davis, 1998; Crisp et al., 1998), either inhibiting the thyroidal system or mimicking it (Cheek et al., 1999). Possible mechanisms for this disruption include direct interaction with thyroid hormone receptors in target cells, either as agonists or antagonists; altering serum transport of thyroid hormones; or interfering with thyroid hormone and cell membrane interactions. In most of these cases, exposure to such compounds is correlated with decreased serum levels of thyroid hormone, particularly thyroxine (Colborn et al., 1996; Curran and DeGroot, 1991), and it is assumed that there may be some negative consequences to this disruption of normal thyroid function. Unfortunately, direct measurements of parameters linked to energetics and winter survival are rare (French et al., 2001; Tomasi, 2001).

The emerging evidence linking synthetic chemicals to thyroid disruption is especially worrisome because of the potential bioaccumulation and biomagnification of these chemical within animals feeding at different levels of the food chain (Guillette et al., 1995). Some are extremely persistent in the environment, taking decades to break down. Lipophilic contaminants are also stored in animals' fat reserves until they are metabolized during energetically expensive events, such as thermogenesis in the cold. Furthermore, since hibernating mammals accumu-

late extra body fat during the prehibernation period, and "live" off this fat during hibernation, any lipophilic toxins will be released from the fat all winter.

Atrazine is a triazine herbicide and is reported to be the most widely used herbicide in the world (Hayes et al., 2002). It is used for weed control in agriculture and is highly persistent in soil and water. Atrazine effects on thyroid function (reviewed by Brucker-Davis, 1998) include increased plasma levels of T_4 and T_3.

Lindane is an organochlorine, and the active component of the insecticide hexachlorocyclohexane (HCH). It is used on agricultural crops, and in the treatment of hardwood logs and stored grain products. It is also highly persistent in soil and water, and its effects on thyroid function (reviewed by Brucker-Davis, 1998) include reduced plasma levels of T_4 and T_3 and elevated thyroid-stimulating hormone (TSH).

White-footed mice (*Peromyscus leucopus*) use daily torpor when the energetic conditions dictate. These torpor bouts in white-footed mice and in deer mice (*P. maniculatus*) usually last from 2–10 hours. Body temperature (T_b) is typically reduced from about 37° C to around 20° C, while metabolic rate is reduced by 75% (Gaertner et al., 1973; Nestler, 1991; Tannenbaum and Pivorun, 1988).

The 13-lined ground squirrel (*Spermophilus tridecemlineatus*) is widely distributed in the northern Great Plains of the USA. It readily uses deep seasonal torpor (hibernation) generally from October to March, depending on latitude, age, and gender (Streubal and Fitzgerald, 1978).

Although previous studies suggest that pesticides can alter thyroid function by interfering with thyroid hormone levels in laboratory rodents, it is also important to determine if such a disruption will ultimately affect over-winter survival of rodents under natural conditions (Van den Berg, et al. 1991; Brucker-Davis, 1998).

The objectives of this study are to determine whether the purported thyroid-disrupting chemicals atrazine and lindane disrupt thyroid function such that they interfere with temperature regulation during euthermia, during bouts of daily torpor in white-footed mice, and during hibernation in 13-lined ground squirrels. Rates of metabolism and concentrations of thyroid hormones were also measured but are not reported herein.

Methods

White-footed mice (n = 18) were trapped (Sherman traps) in southwest Missouri (Greene County), and 13-lined ground squirrels (n = 30) were trapped (tube traps) in northwest Iowa (Dickinson County). Both were housed in an approved

animal room in the Biology Department of Southwest Missouri State University, in individual polypropylene cages (16x20x27 cm) maintained in environmental chambers under controlled temperature and photoperiod. Both protocols were approved by the SMSU IACUC.

Animals were provided *ad lib.* rodent diet (Purina #2010) and water, except when food was reduced for white-footed mice to 75% of *ad lib*. consumption to induce torpor. To simulate seasonal changes for these mice, the photoperiod was gradually reduced from 13L:11D to 9L:15D, and the ambient temperature (T_a) was reduced gradually from 18/13° C (day/night) to 13/8° C. For the ground squirrels, hibernation was induced by a gradual reduction of the photoperiod from 14L:10D to 8L:16D, followed by a reduction of T_a from 26° C to 5° C.

Pesticide (herbicide or insecticide) treatments were prepared by spraying each pesticide solution onto a single layer of rodent chow, and letting this chow then dry before feeding it to the animals. "Control" chow was sprayed with water.

For the white-footed mice, four treatments groups were established (three atrazine doses and a control, with males and females divided evenly among treatments), and an application rate of 25 mL/2.5 kg of chow was used. The atrazine treatments, based on an average food consumption of approximately 4 g/day, were: "low" = 0.376 ug/day (0.235 mg/25mL, 0.094 mg/kg chow); "medium" = 0.075 mg/day (47 mg/25mL, 18.8 mg/kg chow); and "high" = 1.5 mg/day (940 mg/25mL, 376 mg/kg chow). The low dose was chosen to match the recommended crop application rate and the higher doses to simulate possible bioaccumulation in the environment. Each dose was administered chronically, starting in mid-September. The food restriction to induce torpor was started 15 weeks later, three weeks prior to the onset of T_b data collection, and continued for about six more weeks.

For the 13-lined ground squirrels, five treatment groups were established (four atrazine and/or lindane doses, plus a control), each with comparable males/females, and 1.96 kg of food for each group was sprayed with 280 ml of the appropriate solution. Treatments were based on a mean food consumption of 35 g/day, and consisted of: "high lindane" = 17.5 mg/day (0.98 g/280mL, 500 mg/kg chow); "low lindane" = 1.75 mg/day (0.098g lindane/280mL, 50 mg/kg chow); "atrazine" = 205 mg/day (11.508 g/280mL, 5871 mg/kg chow); and "low lindane/atrazine." The latter treatment received both solutions applied to its food, and was designed to determine if any synergistic effect occurred. Treatments were started six weeks before measurements were initiated, and were maintained for the duration of the study (six months).

Body temperatures of white-footed mice (measured eight at a time) were continuously monitored (every three minutes) for six to eight days at 75% of *ad lib*. food intake, using implanted (IP, under sodium pentobarbitol anesthesia) temperature-sensitive transmitters and automated data acquisition system (VM-FH transmitters and Vital View Series 3000: Mini-Mitter Co., Sun River, OR). Torpor was defined as T_b below 30° C. Each group of mice monitored simultaneously included individuals from all treatments. Data for each torpor bout were analyzed for mean euthermic (midnight–4:00 a.m.) and minimum temperatures (° C), time spent in torpor, torpor "area" (a calculated integration of torpor depth and duration: degree-hours), and heat loss and heat gain rates between 25° C and 30° C (° C/hr). Parameters for an animal were calculated for each bout with reliable T_b recordings, and data from multiple torpor bouts were averaged for that animal to avoid pseudoreplication.

Body temperatures of 13-lined ground squirrels (measured 12 at a time) were continuously (every 10 minutes) monitored for approximately four weeks with VM-XF-LT-DISC transmitters and the hardware/software described above. Each group of ground squirrels monitored simultaneously included individuals from all treatments. Data for each hibernation bout were analyzed for euthermic and torpid mean temperatures (° C), and heat loss and heat gain rates (° C/hr) between 10° C and 30° C. Because T_b data obtained while entering and arousing from torpor were used to calculate heat loss and gain rates, we defined "torpor duration" as time spent under 10° C. For animals that maintained a hibernating T_b at or near 10° C, the heat loss rate was calculated 30° C to 15° C, and the bout was considered terminated when the animal initiated a rapid increase in T_b. Data from multiple hibernation bouts were averaged to avoid pseudoreplication. Shallow bouts at the beginning of the hibernation season were excluded.

The data were analyzed via one-way ANOVA (Minitab), using a GLM procedure and $P \leq 0.05$ for significance.

Results

Most of the white-footed mice entered torpor fairly regularly with a 25% reduction in food. Their euthermic temperatures were stable at 36–37° C, and their torpor bouts generally lasted 3–7 h with a minimum temperature about 20° C (Table 1). When entering torpor, they cooled down about 50% as quickly as they heated up when arousing from torpor. Although the mice that received the low dose of atrazine appeared to use torpor less, with a torpor "area" of less than half that seen in the other treatment groups, this was not statistically significant

(P = 0.07). The mice showed no atrazine-treatment effects on any of the torpor parameters measured (Table 1).

The 13-lined ground squirrels showed typical hibernation/arousal patterns, arousing about every four to seven days (longer in the middle of the hibernation season). Including the torpor entry and arousal times in each torpor bout increases the torpor duration by about 8 hours but demonstrates the same statistical results. Animals maintained T_b a few degrees above the environmental chamber (5° C) while torpid (Table 2). Their rate of heat gain during an arousal was similar to that of the white-footed mice, but their rate of heat loss when entering torpor was only about 12%–22% of this rate. However, none of these parameters varied among pesticide treatments (Table 2).

Discussion

Under our simulated laboratory conditions, both species used torpor in predictable manners, based on previous studies on these or related species (Stribling and Tomasi, unpubl data; Barnes and Ritter, 1993; Gaertner et al., 1973; Nestler, 1991; Tannenbaum and Pivorun, 1988). Both species also demonstrated a typi-

Table 1. Body temperature (T_b) parameters in relation to daily torpor bouts, in white-footed mice (n = 18) chronically fed atrazine-treated food (means ± SE).

	Atrazine Treatment				
Parameters	**Control**	**Low**	**Medium**	**High**	**P =**
n =	**3–6**	**4**	**4–5**	**4**	
Torpor frequency (% of days)	87 ± 13	70 ± 10	78 ± 20	89 ± 7	0.79
Euthermic T_b (°C)	36.36 ± 0.23	36.95 ± 0.29	36.70 ± 0.30	36.33 ± 0.25	0.37
Torpor T_b (°C)	20.54 ± 1.39	22.93 ± 1.49	19.15 ± 0.57	19.38 ± 0.13	0.13
Torpor duration (h)	5.42 ± 1.18	3.26 ± 0.62	6.31 ± 0.60	6.05 ± 0.38	0.10
Heat loss rate (°C/h) [30–25° C]	8.20 ± 0.86	7.05 ± 0.53	8.90 ± 0.50	8.80 ± 0.54	0.25
Heat gain rate (°C/h) [25–30° C]	14.30 ± 1.94	17.60 ± 4.40	20.85 ± 1.88	18.93 ± 4.06	0.51
Torpor "area" (degree-hours)	48.32 ± 11.74	21.03 ± 6.48	57.17 ± 9.86	50.24 ± 2.68	0.07

Table 2. Body temperature (T_b) parameters in relation to hibernation bouts in 13-lined ground squirrels (n = 30) chronically fed atrazine-treated or lindane-treated (high or low dose) food (means ± SE).

		Pesticide Treatment				
Parameters	**Control**	**Atrazine**	**Atrazine/ lindane (L)**	**Lindane (L)**	**Lindane (H)**	**P =**
n =	5	5–6	6	6	6–7	
Euthermic T_b (° C)	36.39 ± 0.26	36.23 ± 0.15	36.23 ± 0.26	35.81 ± 0.16	36.14 ± 0.18	0.38
Hibernation T_b (° C)	6.49 ± 0.38	7.93 ± 0.71	8.36 ± 0.75	7.70 ± 0.32	7.60 ± 0.70	0.35
Hibernation bout duration (h)	78.8 ± 19.8	139.9 ± 32.4	157.8 ± 20.7	121.4 ± 18.3	168.6 ± 30.0	0.16
Heat loss rate (° C/h) [30–10° C]	3.08 ± 0.25	3.64 ± 0.76	3.32 ± 0.40	2.42 ± 0.37	2.58 ± 0.14	0.50
Heat gain rate (° C/h) [10–30° C]	20.02 ± 1.60	16.65 ± 2.19	18.30 ± 1.70	16.17 ± 1.47	21.15 ± 1.53	0.20

cal eutherian T_b. The slower cooling rate of the ground squirrels is likely related to their larger body mass (allowing more insulation) and lower surface-to-volume ratio, so it is interesting that they were able to produce heat fast enough to warm up at the same rate as the mice. This might be explained by the same higher insulation and lower surface-to-volume ratio that resists passive cooling, if this species also has more NST capacity, a logical prediction for an obligate hibernator *vs.* a facultative daily-torpor species.

It appears that the pesticides used in this study (at these doses) do not impact the use of torpor in these species, although they have been reported to cause thyroid disruption (Brucker-Davis, 1998; Crisp et al., 1998). This lack of significant physiological effects may be due to small sample sizes; however, the equipment hardware limited the number of transmitters that can be monitored simultaneously. As we rotated different groups of animals onto the receivers, the groups contained individuals from each pesticide treatment, to minimize seasonal effects, but any such effect would still increase the within-treatment variation. In addition, to avoid pseudoreplication, we further reduced our sample size by using in

this analysis the average value for each animal. When statistically separating the within-individual variation from the treatment variation (ANOVA with "animal" nested within "treatment"), and using all torpor/hibernation bouts for which we have reliable data, some of the P-values are reduced below 0.05. Finally, using our operational definitions for the duration of torpor in mice (T_b < 30° C) and ground squirrels (T_b < 10° C), and the T_b ranges for calculating rates of temperature change (25–30° C and 10–30° C, respectively) was somewhat arbitrary. These were chosen to avoid inclusion of nontorpor variations in T_b and to obtain values for maximum rates of T_b change. Recalculations using different temperature ranges generally lead to the same statistical conclusions.

The animals used in this study were also subjected to measurements of resting metabolism (nontorpid), NST capacity, and serum concentrations of thyroid hormones (Elsken-Lacy, unpublished thesis data; Perry, unpublished thesis data). In general, the results from those studies support the conclusions of the T_b data presented here. There were few parameters where significant differences were obtained between treatment groups, and these were not sufficiently consistent to conclude that exposure to these levels of these pesticide would negatively impact the over-wintering success of these species in the wild.

The lack of energetic problems noted in these studies, despite previous reports that these pesticides are thyroid disruptors at similar doses (Brucker-Davis, 1998, Crisp et al., 1998), is good news for the wild rodents and for the people who manufacture and use these pesticides. However, we believe that guarded optimism is appropriate. The potential still exists that man-made thyroid-disrupting chemicals are affecting the delicate energetic balance of nontarget species in subtle ways, compromising their over-wintering ability or reducing the reproductive potential of survivors in the following year. Vigilance is still necessary, and thyroid-disruption possibilities should be investigated where poor over-wintering success is otherwise unexplained.

There are many possible explanations for the different conclusions between previous studies on lab rodents and the studies reported here. Since previous studies only measured thyroid hormone levels, and ours are among the first to include ecologically relevant energetic parameters, it is possible that the laboratory rodents were not really compromised. They may compensate for decreased serum levels by altering some other aspect of thyroid function or metabolism not monitored in those studies. Alternatively, wild rodents (particularly the herbivorous species) may possess a better ability to detoxify pesticides because of

their continuous exposure to the numerous toxic plant secondary compounds in their diets.

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