

Iophenoxic acid as a systemic blood marker for assessment of bait acceptance by stoats (*Mustela erminea*) and weasels (*Mustela nivalis*)

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Abstract Iophenoxic acid (IA) is a compound that causes an increase in the plasma and serum iodine concentration of animals that ingest it, and was evaluated as a marker for assessing bait acceptance by stoats and weasels. The baseline plasma or serum iodine concentration was measured in 12 captive and 10 wild stoats, and one captive weasel. Iophenoxic acid was mixed into broken hen eggs in amounts of 1, 2, 4, or 5 mg per egg (5 mg equals 0.01% in a 50 g egg), and the eggs were then fed to the captive animals. The mean plasma or serum iodine concentration in the dosed stoats was significantly higher than the mean baseline concentration (3.3 µg/100 ml) after 7 days (309.5 µg/100 ml) and after 14 days (24.0 µg/100 ml), but not after 21 days (6.4 µg/100 ml). The duration of plasma or serum marking in stoats (c. 2 weeks) was not as long as reported in most other studies of eutherian mammals (most more than 8 weeks). Not enough data were collected to determine the duration of marking in weasels.

Keywords stoat; weasel; *Mustela erminea*; *Mustela nivalis*; blood markers; bait markers; iophenoxic acid

INTRODUCTION

Stoat (*Mustela erminea*) predation is an important factor in the continued decline of several species of native birds in New Zealand (Dowding & Murphy

1996; Elliott et al. 1996; McLennan et al. 1996; O'Donnell 1996; O'Donnell et al. 1996; Wilson et al. 1998). Weasels (*Mustela nivalis*) also kill native birds, but the impact of their predation is unknown. Traditionally, stoats have been controlled using Fenn traps set in tunnels lured with hen eggs (King et al. 1994). Weasels are also caught in these traps. Recently, a new stoat control technique has been developed using poisoned hen eggs placed in bait stations (Spurr 1996, 1999, 2000; Miller & Elliot 1997; Dilks & Lawrence 2000). An important step in the development of new toxic baits for stoat control is to determine the proportion of stoats that eat the baits. Consequently, a biomarker is required that can be added to baits and identified in stoats that have eaten marked baits.

Iophenoxic acid (α -ethyl-3-hydroxy-2,4,6-triiodobenzene propanoic acid) (IA) in baits has been shown to be a suitable marker for a number of animal species (Appendix 1). It binds to blood proteins, causing an increase in the total plasma and serum iodine concentration. Larson et al. (1981) found no difference in the iodine concentration in the plasma or serum, and subsequent studies have analysed either plasma or serum, presumably depending upon whether heparinised collection tubes were available. The duration over which iodine has been elevated has varied with species, and with the amount of iophenoxic acid ingested. In most eutherian mammals studied, doses of 0.5–5.0 mg of iophenoxic acid per kg of body weight elevated the plasma or serum iodine concentration for at least 8 weeks (Savarie et al. 1992; Appendix 1). In dogs (*Canis familiaris*), the iodine concentration remained elevated for at least 52 weeks (Baer et al. 1985). However, in rock squirrels (*Spermophilus variegatus*), the iodine concentration was not elevated by iophenoxic acid (Larson et al. 1981), and in two marsupials that have been studied, the brushtail possum (*Trichosurus vulpecula*) and swamp wallaby (*Wallabia bicolor*), it was elevated for less than 2 weeks (Eason et al. 1994; Fisher & Marks 1997).

Doses of iophenoxic acid up to about 70 mg/kg have been administered to animals in some studies

in an attempt to prolong the duration of marking (Appendix 1). Generally, it has been found that the higher the dose the greater the intensity and longer the duration of marking (Larson et al. 1981; Baer et al. 1985; Eason & Batcheler 1991; Saunders et al. 1993; White et al. 1995; Hartley & Hamilton 1997; King et al. 1998; Sweetapple & Nugent 1998; Knowlton & Olmstead 2001). However, Knowlton & Olmstead (2001) found that the iodine concentration in the serum of Angora goats (*Capra hircus*) became saturated after iophenoxic acid doses of 25–30 mg/kg. Dosing above this level did not appreciably increase the intensity or duration of marking in this species.

With respect to mustelids, captive ferrets (*Mustela furo*) dosed with 5 mg of iophenoxic acid per kg of body weight had a post-dose serum iodine concentration significantly higher than the pre-dose concentration for at least 4 weeks (Ogilvie et al. 1996; Ogilvie & Eason 1998). All ferrets captured in a field trial 2–12 days after bait containing 0.02% iophenoxic acid had been placed in bait stations, had a significantly elevated serum iodine concentration. One stoat captured at the same time also had a serum iodine concentration significantly higher than the baseline concentration in captive ferrets (Ogilvie et al. 1996). The objective of the present study was to evaluate iophenoxic acid as a systemic blood marker for assessment of bait acceptance in stoats and weasels.

METHODS

Blood samples for determination of the baseline iodine concentration were collected from 12 stoats (all males) held in captivity at the Landcare Research animal facility between November 1997 and March 1998, and from 10 stoats (5 males and 5 females) captured in the field and fitted with radio-transmitters between February and April 1998 (Table 1). A baseline blood sample was also collected from a captive male weasel in December 1997. The animals in captivity were anaesthetised with 4% fluothane gas, and in the field by intra-muscular injection of 0.25 ml/kg ketamine. Blood (1–2 ml) was collected from anaesthetised animals by cardiac puncture, using a 22-gauge needle and 3 ml hypodermic syringe, because sufficient could not be obtained from other sources such as the caudal vein. A 2 ml sample of blood represented 1% of the live weight of a 200 g animal, equivalent to about 15% of the circulating blood volume (AWAC 1996).

Iophenoxic acid (Aldrich Chemical Company, Milwaukee, Wisconsin, USA) was mixed into broken hen eggs in amounts of 1, 2, 4, or 5 mg in 1 ml of methyl cellulose solution per egg, to provide a range of dose rates for the stoats and weasels. A dose of 5 mg of iophenoxic acid in a 50 g egg represented 0.01% iophenoxic acid per egg (by weight). Eggs containing 1, 2, and 4 mg of iophenoxic acid were fed to 3, 3, and 4 randomly selected captive stoats, respectively, on 27 November 1997, and 5 mg of iophenoxic acid to another 6 captive stoats on 12 March 1998 (weights in Table 1). The average dose rates fed to these stoats were 3 mg/kg, 6 mg/kg, 12 mg/kg, and 17 mg/kg, respectively. Eggs containing 1 and 2 mg of iophenoxic acid were also fed to 2 captive male weasels on 27 November 1997. Their dose rates were 9 and 15 mg/kg, respectively.

Blood samples were taken from the captive stoats 7, 14, and 21 days after dosing, and from the captive weasels 21 days after dosing. Because not more than 15% of the estimated circulating blood volume should be removed in any 4-week period (AWAC 1996), the same animals could not be re-sampled each time. Three stoats were re-sampled 23 days after the first blood sampling (14 days after dosing), and then were euthanased with an overdose of pentobarbital while still under anaesthesia, because the interval between the two samples was less than 4 weeks. Six other stoats were re-sampled 30 days after the first blood sampling (21 days after dosing), and it was not necessary to euthanase them.

The blood samples were placed in heparinised tubes, when available, centrifuged, and the plasma was frozen for later analysis. When heparinised tubes were not available, the blood samples were centrifuged and the serum was frozen for later analysis. The iodine concentration in the plasma or serum was measured using the methods described by Hadidian et al. (1989), and modified by Eason & Frampton (1992), as an indirect measure of the iophenoxic acid concentration. An aliquot of each sample was digested with a perchloric acid digest solution at 195°C. On cooling, the ceric and arsenious redox reagents were added and the absorbency was measured on an ultraviolet-visible spectrophotometer at a wavelength of 420 nm after a constant time interval. Results were calculated by comparison with a set of standards run concurrently, and reported in µg iodine/100 ml plasma or serum, with a 95% confidence interval of ± 1.1 µg/100 ml.

Plasma or serum iodine concentrations in relation to dose rate and days after dosing were compared

using a two-factor analysis of variance (Wilkinson 1996). The analysis used data from Days 14 and 21 only, because Day 7 samples were collected only for the 5 mg dose rate. Post-dose plasma or serum iodine concentrations for all dose rates combined were then compared with baseline concentrations using Wald's test, after fitting a linear mixed-effects model to the square root of the data (Pinheiro & Bates 2000). The mixed-effects model was used because some of the post-dose blood samples were from the same animals as in the baseline samples (within stoats) and some were from different animals (between stoats). Significance levels for the analyses were set at $P = 0.05$. The decision on whether or not a post-dose iodine concentration in the plasma or serum of an individual stoat was significantly higher than the population baseline iodine concentration was made using a t -test for comparing a single observation with the mean of a population (Sokal & Rohlf 1995). Significance levels tested were for $P = 0.05$, 0.01, and 0.001.

The research reported here was carried out with the approval of the Landcare Research Animal Ethics Committee (Approval No. 96/5/2).

RESULTS

The mean baseline concentration of iodine in the plasma or serum was 3.48 $\mu\text{g}/100\text{ ml}$ (range 0–8 $\mu\text{g}/100\text{ ml}$) in the 12 captive stoats, and 3.04 $\mu\text{g}/100\text{ ml}$ (range 1–8 $\mu\text{g}/100\text{ ml}$) in the 10 wild stoats. There was no significant difference between the two means ($F_{1,20} = 0.138$, $P = 0.715$), and no reason to believe that the response to iophenoxic acid would be different in captive and wild animals, so the two samples were combined. There was also no significant difference between the means for male and female wild stoats ($F_{1,8} = 0.428$, $P = 0.531$). The baseline plasma iodine concentration in the single captive weasel sampled was 1 $\mu\text{g}/100\text{ ml}$, which was within the range of the larger sample of stoats.

All the stoats and weasels ate the broken hen eggs containing iophenoxic acid at all the dose rates tested (1–5 mg per egg). In the two-factor analysis of variance of Day 14 and Day 21 results, the iodine concentration in the plasma or serum of stoats did not differ between dose rates ($F_{3,6} = 4.133$, $P = 0.066$) (Table 2), but did differ between Day 14 and Day 21 ($F_{1,6} = 24.311$, $P = 0.003$). There was no interaction between dose rate and day ($F_{3,6} = 3.097$,

Table 1 Number of stoats and weasels sampled pre-dose (baseline) and sampled or re-sampled post-iophenoxic acid (IA) dose for determination of iodine concentration in plasma or serum.

Number and sex	Weight range (g)	Number sampled pre-dose	IA dose given (mg)	Number sampled or re-sampled* post-dose		
				Day 7	Day 14	Day 21
Captive stoats						
3 males	280–333	3	0	–	–	–
3 males	326–420	3	1	–	1*	2*
3 males	312–371	3	2	–	1*	2*
3 males	283–367	3	4	–	1*	2*
1 female	220	–	4	–	–	1
2 males	268–324	–	5	2	–	–
2 males	274–310	–	5	–	2	–
2 males	312–362	–	5	–	–	2
Wild stoats						
5 males	270–370	5	0	–	–	–
5 females	150–240	5	0	–	–	–
Captive weasels						
1 male	120	1	0	–	–	–
1 male	111	–	1	–	–	1
1 male	136	–	2	–	–	1

$P = 0.111$). Consequently, the results from the different dose rates were combined.

In the mixed-effects-model analysis (all dose rates combined), the mean plasma or serum iodine concentration in dosed stoats was significantly higher than the baseline after 7 days ($t_7 = 21.57$, $P < 0.001$) and 14 days ($t_7 = 6.38$, $P < 0.001$), but not after 21 days ($t_7 = 1.98$, $P = 0.09$) (Table 3). However, one of the five stoats sampled on Day 14 had an iodine concentration of only 6 $\mu\text{g}/100$ ml, lower than the maximum baseline value (8 $\mu\text{g}/100$ ml). The other four stoats sampled on Day 14 had more than 20 $\mu\text{g}/100$ ml. In contrast, seven of the nine stoats on Day 21 had an iodine concentration below 6 $\mu\text{g}/100$ ml, while the other two had 16 and 18 $\mu\text{g}/100$ ml.

Given the mean iodine concentration in the baseline sample of stoats (3.3 $\mu\text{g}/100$ ml), the standard deviation (2.7 $\mu\text{g}/100$ ml), and sample size (22 stoats), assuming a normal distribution of data, and using the t -test for comparing a single observation with the mean of a population, an individual stoat would need to have an iodine

Table 2 Iodine concentration in the plasma or serum of 14 captive stoats, 14–21 days after dosing, in relation to the dose of iophenoxic acid (IA) fed in hen eggs (mg/kg body weight).

Dose of IA	<i>n</i>	Iodine ($\mu\text{g}/100$ ml)		
		Mean	SE	Range
1 mg (3 mg/kg)	3	15.7	6.8	3–26
2 mg (6 mg/kg)	3	4.0	1.0	3–6
4 mg (12 mg/kg)	4	11.5	8.2	3–36
5 mg (17 mg/kg)	4	18.3	9.2	5–30

Table 3 Iodine concentration in the plasma or serum of 19 captive and 10 wild stoats, before and after being fed hen eggs containing iophenoxic acid (IA), with doses 1–5 mg IA combined. Day 14 includes three stoats earlier used in the baseline sample; Day 21 includes six stoats earlier used in the baseline sample.

Time	<i>n</i>	Iodine ($\mu\text{g}/100$ ml)		
		Mean	SE	Range
Baseline	22	3.3	0.6	0–8
Day 7	2	309.5	1.5	308–311
Day 14	5	24.0	5.1	6–36
Day 21	9	6.4	2.0	3–18

concentration greater than 9.1, 11.2, or 14.0 $\mu\text{g}/100$ ml for it to be significantly greater than 97.5%, 99.5%, or 99.95% of the mean baseline iodine concentration, respectively; i.e., for the individual to be considered to have eaten bait containing iophenoxic acid.

The plasma iodine concentration in two captive weasels, 21 days after being dosed with 1 mg or 2 mg of iophenoxic acid, was 8 and 236 $\mu\text{g}/100$ ml, respectively.

All captive animals survived blood sampling, but three of the 10 wild radio-transmitted stoats died within a few days of blood sampling.

DISCUSSION

Calculation of the natural upper limit of plasma or serum iodine concentration in a population of animals is necessary to determine which animals have eaten bait containing iophenoxic acid. The observed maximum baseline iodine concentration recorded in stoats in this study (8 $\mu\text{g}/100$ ml) is based, as in most studies, on a relatively small sample size (22 stoats). An estimate is needed of the likely maximum baseline iodine concentration in a larger sample of animals. Different researchers have used different ways to calculate the expected boundary between baseline and elevated iodine concentrations. For example, Follmann et al. (1987) and Southey et al. (2001) used a value of twice the mean baseline iodine concentration to separate animals that had eaten bait from those that had not. Using this formula for stoats, then any individual with an iodine concentration of more than 6.6 $\mu\text{g}/100$ ml (twice the mean) would be considered to have eaten bait. However, this is less than the observed maximum baseline iodine concentration. Ogilvie & Eason (1998) and Sweetapple & Nugent (1998) used the formula (mean + 2 \times standard deviation), Fletcher et al. (1990) used (mean + 3 \times standard deviation), and Fleming (1997) used (mean + 3.08 \times standard deviation), to include 97.73%, 99.87%, and 99.95%, respectively, of the likely baseline iodine concentrations. Using these formulae for stoats, then any individual with an iodine concentration greater than 8.7, 11.4, or 11.7 $\mu\text{g}/100$ ml, respectively, would be considered to have eaten bait. However, these formulae all use the sample mean and standard deviation as if they were population values. The t -test for comparing a single observation with the mean of a population (Sokal & Rohlf 1995) allows for the fact that the mean and standard deviation are taken from

a sample of the population. Nevertheless, the values calculated to include 97.5%, 99.5%, and 99.95% of the likely baseline iodine concentrations (9.1, 11.2, and 14.0 µg/100 ml, respectively) are similar to those calculated above. Which level of confidence should be used (97.5%, 99.5%, or 99.95%) depends upon the certainty that an investigator wishes to include or exclude animals that have eaten bait. If the limit is set too low, some animals that have not eaten bait may be included in the bait consumer category, whereas if it is set too high some animals that have eaten bait may be excluded from the bait consumer category. Using 97.5% as a conventional level, neither of the two Day 7 values was below 9.1 µg/100 ml, but one of the five Day 14 values and seven of the nine Day 21 values were below this concentration. The results would be no different for the other significance levels.

The baseline plasma or serum iodine concentration in stoats measured here (3.3 ± 0.6 µg/100 ml) (mean \pm standard error) was lower than recorded in ferrets (8.5 ± 0.7 µg/100 ml) by Ogilvie & Eason (1998). However, it was within the range of 0–23.2 µg/100 ml found in other species (Appendix 1). The post-dose peak iodine concentration, expected after 1 day, was not recorded in this study, but the iodine concentration after 7 days in stoats (309.5 µg/100 ml) was similar to that recorded by Ogilvie & Eason (1998) in ferrets (384 µg/100 ml). However, the duration of elevated iodine in stoats (c. 14 days) was not as long as in ferrets (>28 days, but <56 days). Furthermore, the duration in both stoats and ferrets was less than reported in most other eutherian mammals (see Introduction and Appendix 1). Thus, iophenoxic acid is useful as a systemic marker in stoats for up to about 14 days, but it is not as useful as in other eutherian mammals that are marked for longer. There was not enough data collected to determine accurately the duration of marking in weasels, but the iodine concentration in one of the two weasels sampled after 21 days was below the maximum baseline iodine concentration found in stoats.

The lack of a clear dose-dependent response in stoats in this study may, at first sight, be considered surprising, given that it has been observed in other species, and that the doses given to stoats (3–17 mg/kg) were within the range of doses given to other species. However, the sample sizes for stoats were small. A dose-dependent response may have been detected if a larger sample of stoats had been tested, if more stoats had been sampled nearer to the peak iodine concentration (1–7 days after dosing), and if some stoats had been given higher doses of

iophenoxic acid. More stoats were not available for testing, and more samples were not collected during the first 7 days because marking was expected to last at least 28 days, as in ferrets (Ogilvie & Eason 1998). As it transpired, most samples were collected near to or beyond the limit of detection of marking (14–21 days after dosing) for the dose rates given. Higher doses of iophenoxic acid (e.g., 25–30 mg/kg) may be needed to prolong the duration of marking in stoats.

The death of some stoats blood-sampled in the field indicates a potential further limitation to the usefulness of iophenoxic acid as a systemic marker for stoats. The stoats blood-sampled in captivity were returned to warm, dry housing, but stoats in the field were released into colder and/or damper conditions more likely to induce hypothermia. The effects of the anaesthetic, cardiac puncture, and reduced blood volume would have increased the risk of hypothermia in the field. A systemic marker of hair, such as Rhodamine B (Fisher 1998) or clenbuterol (Gleixner et al. 1998), would require less invasive sampling than a systemic marker of blood, such as iophenoxic acid. Research on the use of Rhodamine B as a systemic hair marker for stoats is currently in progress.

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Appendix 1 Studies on the use of iophenoxic acid (IA) as a biomarker in mammals (* indicates field studies).

Species	Base blood iodine level (µg/100 ml)	Dose of IA administered (c. mg/kg)	Duration of mark (weeks)	Reference	
Coyote (<i>Canis latrans</i>)	0.8–6.4	c. 0.5	>8	Larson et al. 1981	
	2.3–4.6	c. 1.0	>16	Knowlton et al. 1987	
		c. 1.5	>16		
	8.0	?	>16	Knowlton & Olmstead 2001	
Dog (<i>Canis familiaris</i>)	4.6–12.2	c. 0.5	34–52	Baer et al. 1985	
		c. 1.0	>52		
Red fox (<i>Vulpes vulpes</i>)	2.0–5.0	c. 1.0	6–8	Larson et al. 1981	
	8.0–8.2	c. 2.2	6–13	Baer et al. 1985	
		c. 4.4	13–34		
	?	?	>8	Trewhella et al. 1991*	
	5.4	3.6	6–10	Saunders et al. 1993	
		7.3	6–10		
		10.9	>10		
	4.3–6.3	?	>2	Fleming 1997*	
Arctic fox (<i>Alopex lagopus</i>)	9.5	c. 4.0	13	Follmann et al. 1987	
Cat (<i>Felis catus</i>)	?	1.5	>20	Eason et al. 1994	
Badger (<i>Taxidea taxus</i>)	0.8–1.8	<1.0	>8	Larson et al. 1981	
Badger (<i>Meles meles</i>)	6.3–7.8	?	>16	Southey et al. 2001*	
Raccoon (<i>Procyon lotor</i>)	1.4–12.4	c. 1.0	>8	Larson et al. 1981	
	1.6–11.0	?	>3	Hadidian et al. 1989*	
	12.0–21.0	?	>2	Fletcher et al. 1990*	
	5.0–19.0	?	>3	Linhart et al. 1994*	
Striped skunk (<i>Mephitis mephitis</i>)	3.5–6.1	c. 1.0	>8	Larson et al. 1981	
Ferret (<i>Mustela furo</i>)	7.2	5.0	>4	Ogilvie et al. 1996*	
Rabbit (<i>Oryctolagus cuniculus</i>)	–	0.7	3	Ogilvie & Eason 1998*	
		3.6	>6	Hartley & Hamilton 1997	
		7.1	>6		
		7–10	1.5	13	King et al. 1998
			5.0	15	
		10.0	17		
Swamp wallaby (<i>Wallabia bicolor</i>)	2.9–12.3	15.0	c. 0.5	Fisher & Marks 1997	
		30.0	c. 0.5		
Brush-tail possum (<i>Trichosurus vulpecula</i>)	?	1.5	<1	Eason et al. 1994	
		10.0	<2		
Pig (<i>Sus scrofa</i>)	2.6–12.0	?	>2	Fletcher et al. 1990*	
Goat (<i>Capra hircus</i>)	4.0–10.0	1.5	>12	Eason & Batcheler 1991	
		?	>1	Parkes 1991*	
		1.5	>20	Eason & Frampton 1992	
		?	>1	Forsyth & Parkes 1995*	
	5.3	c. 3–67	>17	Knowlton & Olmstead 2001	
White-tailed deer <i>Odocoileus virginianus</i>	10.7–23.2	0.1	<1	White et al. 1995	
		1.2	<1		
		5.6	>1		
		5.0	3		
Red deer <i>Cervus elaphus scoticus</i>	6.6	0.3	>6	Sweetapple & Nugent 1998	
		2.3	>6		
		6.1	>6		