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ORIGINAL ARTICLE

Ethyl-iophenoxic acid as a quantitative bait marker for small mammals

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Abstract

Bait markers are indispensable for ecological research but in small mammals, most markers are invasive, expensive and do not enable quantitative analyses of consumption. Ethyl-iophenoxic acid (Et-IPA) is a non-toxic, quantitative bait marker, which has been used for studying bait uptake in several carnivores and ungulates. We developed a bait with Et-IPA, assessed its palatability to common voles (*Microtus arvalis*), and determined the dose-residue-relation for this important agricultural pest rodent species. Et-IPA concentrations of 40 to 1280 μg Et-IPA per g bait were applied to wheat using sunflower oil or polyethylene glycol 300 as potential carriers. In a laboratory study, common voles were offered the bait and blood samples were collected 1, 7, and 14 days after consumption. The samples were analyzed with LC-ESI-MS/MS for blood residues of Et-IPA. Sunflower-oil was the most suitable bait carrier. Et-IPA seemed to be palatable to common voles at all test concentrations. Dose-dependent residues could be detected in blood samples in a dose-dependent manner and up to 14 days after uptake enabling generation of a calibration curve of the dose-residue relationship. Et-IPA was present in common vole blood for at least 14 days, but there was dissipation by 33–37% depending on dose. Et-IPA meets many criteria for an "ideal" quantitative bait marker for use in future field studies on common voles and possibly other small mammal species.

Key words: baiting, biomarker, *Microtus arvalis*, quantitative bait marker, small mammals

INTRODUCTION

Bait markers are an invaluable tool in ecological research because they allow the study of movement, population dynamics, dispersal, and food choice (Fry & Dunbar 2007) in several carnivores, ungulates, and small mammals (Linhart *et al*. 1993). They are usually applied to mark animals internally via uptake of food or water spiked with a bait marker that is present in the animal's body for long enough to be detected in the course of the study. There are many bait markers available that can be delivered through bait. Some of them such as Rhodamine B or antibiotics (e.g. tetracycline) are systemic and accumulate in several tissues (Jacob *et al*. 2002; Fry & Dunbar 2007; Fry *et al*. 2010). Others such as iophenoxic acid are detectable even in small quantities of blood.

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Bait markers have been used in the past to study the use of resources in small mammals (review in Fry & Dunbar 2007; Ballesteros *et al*. 2013). This is relevant for both basic research and applied science. The management of vertebrate pest populations and pathogens in vertebrates relies mostly on the use of bait-based compounds that kill animals considered pest species (Jacob & Buckle 2018) and/or associated parasites/pathogens (Leirs *et al*. 2001) or reduce target population fertility (review in Jacob *et al*. 2008). The latter is especially important for animals such as wild boar (*Sus scrofa* Linnaeus, 1758) and white-tailed deer [*Odocoileus virginianus* (Zimmermann, 1780)], which can cause vehicle accidents, loss of biodiversity, damage to crops, infrastructure, private gardens, and public parks. In densely populated human habitations, national parks etc., these species cannot be controlled via traditional methods (Raiho *et al*. 2015; Croft *et al*. 2020). Furthermore, bait markers were used to evaluate the (potential) uptake of oral rabies vaccines in Ethiopian wolves (*Canis simensis* Rüppell, 1840) (Sillero-Zubiri *et al*. 2016), raccoons [*Procyon lotor* (Linnaeus, 1758)] (Hadidian *et al*. 1989) and mongooses (*Herpestes auropunctatus* É. Geoffroy Saint-Hilaire, 1818) (Berentsen *et al*. 2020).

Most bait markers used in ecological studies only allow qualitative assessment of whether an animal has consumed bait or semi-quantitation of assessing how often bait was consumed such as the Rhodamine B (Fisher 1998; Spurr 2002a; Fry *et al*. 2010). Rhodamine B is a non-toxic systemic biomarker that stains keratinous tissues (nails, hair, vibrissae), excretions and organs (Jacob *et al*. 2002; Smyser *et al*. 2010; Fry *et al*. 2010). Insufficient persistence is one of the main limitations for physical markers such as beads and glitter (Fry & Dunbar 2007). The oil soluble bait marker Solvent Blue 36 also rapidly declines between day 2 and 5 (Tobin *et al*. 1996). Compounds such as Mirex that are highly persistent may have negative side effects on the environment and cannot be used as a bait marker (Fry & Dunbar 2007). If bait markers degrade slowly, there is the opportunity to detect bait uptake based on the presence of bait marker residue over time. However, for some bait markers (tetracycline and other antibiotics) lethal sampling may be required to collect teeth or bone material for analysis (Crier 1970; Fry & Dunbar 2007).

Ethyl-iophenoxic acid (Et-IPA) has been used as a systemic bait marker in several species due to its lack of negative side-effects, low toxicity (Shapiro 1953; Larson *et al*. 1981) and long persistence in blood (Astwood 1957; Hall & VanderLaan 1961). This includes stoats (*Mustela erminea* Linnaeus, 1758) (Spurr 2002b), wild boar (Massei *et al*. 2009), mongooses (Berentsen *et al*. 2020) and dogs (*Canis lupus* Linnaeus, 1758) (Baer *et al*. 1985; Wiles & Campbell 2006). However, information about Et-IPA in rodents is scarce and restricted to a concept study on the retention of Et-IPA in laboratory rats (*Purdey et al*. 2003) and an unsuccessful attempt to use Et-IPA as a bait marker in rock squirrels [*Otospermophilus variegatus* (Erxleben, 1777)] (Larson *et al*. 1981) and black tailed prairie dogs [*Cynomys ludovicianus* (Ord, 1815)] (Creekmore *et al*. 2002).

Et-IPA contains organic iodine and was originally used as a cholecystographic medium in humans in the 1950s to visualize the gall bladder (Margolin *et al*. 1953; Shapiro 1953). Et-IPA is highly persistent in human blood plasma (Hall & VanderLaan 1961; Mudge *et al*. 1978). After uptake, Et-IPA can be indirectly detected as the elevated blood iodine concentration decreases slowly, with the concentration measured in plasma or serum samples (Larson *et al*. 1981) reflecting the amount consumed for at least 6–8 weeks (Baer *et al*. 1985). Et-IPA can be also detected directly, by liquid chromatography (Jones 1994; Wiles & Campbell 2006; Ballesteros *et al*. 2010), which can be costly. In addition, there is high variability in longevity of Et-IPA residues in blood among species and depending on the ingested concentration (Baer *et al*. 1985; Ballesteros *et al*. 2013; Berentsen *et al*. 2020), which needs to be considered in laboratory and field trials.

Using a serum sample instead of tooth or bone samples offers an excellent advantage (Crier 1970; Ballesteros *et al*. 2013) for welfare reasons and because animals can be sampled repeatedly. Only small quantities of Et-IPA are excreted to the environment, so the potential for environmental contamination seems low but there are no published studies about the risks of Et-IPA metabolites for the environment (Ballesteros *et al*. 2013). Et-IPA can be detected in blood serum samples (10 μl) from laboratory rats (Purdey *et al*. 2003) but there is no published empirical information about the suitability of Et-IPA as a bait marker in rodents.

The common vole [*Microtus arvalis* (Pallas, 1779)] is the major agricultural vertebrate pest species in Europe (Jacob *et al*. 2014, 2020). During outbreaks, it causes massive pre-harvest losses (Jacob *et al*. 2014) and it hosts zoonotic pathogens with high disease risk to humans and livestock (Luque-Larena *et al*. 2015; Mrochen *et al*. 2018; Jeske *et al*. 2018). Common vole populations are usually managed with rodenticidal bait but fertility control methods using bait delivered compounds may be available in the future. In either case, an optimal strategy for bait placement is necessary. The use of Et-IPA could help to develop such a strategy in field studies but palatability

of Et-IPA to common voles and the dose-residue relationship need to be known.

It is crucial to evaluate and optimize bait application in a variety of settings to maximize the efficacy of a baiting system, to improve benefit-cost ratios and to minimize the risk for non-target species. A suitable systemic bait marker could help with the conduct of related laboratory, enclosure and field experiments. In this laboratory study, a bait coated with Et-IPA was assessed and the Et-IPA concentration on the bait was validated. A range of Et-IPA concentrations was delivered orally to common voles to assess palatability. Blood residues were measured 1, 7, and 14 days after consumption to evaluate the systemic persistence of Et-IPA and to develop a dose-residue calibration curve. This new knowledge could be used to conduct future field trials, which compare baiting strategies to define the efficacy of bait delivery—be it a lethal bait or a fertility control bait—to common voles and possibly other rodents and to assess the exposure of non-target species.

MATERIAL AND METHODS

All trials were conducted 2019–2020 in the animal holding facilities of JKI in Münster (51°58′29.6″N 7°34′02.2″E), Germany.

Animals

All animals were captured on agricultural fields near Münster, Warendorf, and Coesfeld (North-Rhine-Westphalia, Germany). Ugglan live-traps were equipped with wood wool for nesting and an apple piece, rolled oats, peanut flips, and pellets as bait. Traps were checked every 12 h and captured animals were transported to the animal holding facility at JKI. They were housed individually in standard rodent cages (410 × 250 × 150 mm: Dieter Wenzel, Detmold, Germany), with softwood chips and hay as bedding, a polycarbonate house, rodent pellets (Altromin 1324; Altromin Spezialfutter GmbH & Co. KG, Lage, Germany), and tap water ad libitum at all times at a natural light–dark cycle. The experiments were conducted with wild-caught common voles and their offspring (F_1 and F_2) with a minimum body weight of 14 g (minimize potential health risk of repeated blood sampling to small individuals with low blood volume).

Et-IPA bait preparation and delivery

Et-IPA (CAS Number: 96-84-4, 97%, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was dissolved in polyethylene glycol (PEG 300) and sunflower oil, respectively, at 6.4% (w/w) of the compound. Both mixtures were prepared by thoroughly stirring at 65°C. Broken wheat was sieved to remove small particles and 1 kg-portions were carefully mixed with the Et-IPA solution to achieve 6 concentrations: 40, 80, 160, 320, 640, 1280 μg/g. The carrier without Et-IPA was used to produce experimental control bait (0 μg/g).

Individual voles were chosen randomly and allocated to seven groups of ten voles each (five males and five females). At day 0, animals were placed in fresh cages with paper (40 × 60 cm, No: 220040601500, Diagonal, Münster, Germany) as bedding. Food pellets were replaced with 1 g of Et-IPA bait (based on results broken wheat + sunflower oil + Et-IPA was used; see below) at 1600 h. Uptake was checked visually the following morning at 0800 h and the animals were returned to their cages with standard bedding, food, and water.

Blood samples were collected directly after the complete consumption on day 1 and after 7 and 14 days to investigate how long the quantitative marker remains in the blood and to what degree Et-IPA blood concentration changes. For each blood sampling, the voles were placed in an anesthesia chamber, pre-flooded with isoflurane (3%) that was reduced to 1.5–2% when the animal was unconscious (for details see Imholt *et al*. 2018). While fully anesthetized, a blood sample (50 μL) was collected by puncture of the retro-bulbar sinus with a capillary pipette (Hirschmann ringcaps 50 μL #9600150). Whole blood samples were stored at −80°C until further analysis.

Body weight was measured with a spring scale (Pesola Medio-Line; Pesola-Werke, Switzerland, supplier: Gottl. Kern & Sohn GmbH, Balingen, Germany) to the nearest gram shortly before Et-IPA-uptake and before each sampling of blood. The Et-IPA dose provided was calculated by dividing the concentrations of 40, 80, 160, 320, 640, 1280 μg/g bait by body weight.

Analysis for Et-IPA residues

Reagents

Et-IPA, propyl-IPA (surrogate) and butyl-IPA (internal standard) were purchased from PR EuroChem Ltd., acetonitrile (Chemsolute) from Th. Geyer, formic acid and trifluoroacetic acid (TFA) from Sigma-Aldrich, the Quechers extraction salt mixture of sodium chloride and magnesium sulfate (1:4) from Agilent Technologies and sodium chloride p.a. from Roth. Deionised water was produced in-house with Arium 611UV (Sartorius). The stock

solutions (1 mg/mL), working and calibration standards were in acetonitrile and stored at −20°C. Samples were stored at −80°C until analysis.

Extraction of samples

0.5 g of a sunflower oil sample or 0.5 g of a PEG 300 sample was placed in a 50 mL pp-tube and spiked with the surrogate propyl-IPA (100 μg/g). After 1 min, the sample was vortexed (VF 2, IKA). With the addition of 10 mL acetonitrile and 5 g of Quechers salt mixture and the subsequent ultrasonic treatment (10 min, Sonorex Super 10P, Bandelin) Et-IPA was extracted. After centrifugation (5000 rpm for 10 min, Heraeus Megafuge 16 R), an aliquot of the supernatant was taken and evaporated to dryness under a nitrogen stream. The residue was redissolved in 1 mL internal standard solution (butyl-IPA in acetonitrile) and filtered through a ROTILABO syringe filter (PTFE, 0.2 μm) in an autosampler vial.

Samples of broken wheat coated with Et-IPA in sunflower oil or with PEG 300 were ground with a ball mill (Retsch 301). 0.5 g of a sample was placed in a 50 mL pp-tube and spiked with the surrogate propyl-IPA (100 μg/g). After 1 min, the sample was vortexed and given 15 min to swell. Further processing was identical to the procedures for sunflower oil and PEG 300.

The preparation of the blood samples was based on Berentsen *et al*. (2019). Mean whole blood sample weight was 44 ± 14 mg (CV 31.5%). The samples were spiked with the surrogate propyl-IPA (5 ng, about 0.1 μg/g related to the average blood sample weight) and, after 1 min, vortexed. Et-IPA was extracted with 1 mL acetonitrile +0.5% TFA with support of a steel ball (Ø 2 mm) and addition of 250 mg sodium chloride by vortex (1 min) and subsequent ultrasonic treatment (10 min). After centrifugation, an aliquot of the supernatant was taken and the processing was carried out as described above.

LC-ESI-MS/MS analysis

The analyses were performed by liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS/MS; 1290 Infinity II, Agilent and QTRAP 6500+, SCIEX) based on Berentsen *et al*. (2019). The chromatographic column Zorbax Eclipse C18 (50 mm × 2.1 mm i.d. × 1.8 μm, Agilent) was loaded with 5 μL of the sample solutions. The mobile phase consisted of a gradient elution of 2 solvents (A: water + 0.1% formic acid; B: acetonitrile + 0.1% formic acid). Eluent A was started with 97% and reached 3% in 3 min and remained for 2 min. For column equilibration, eluent A was switched to 97% and stayed for 1 min. The measurement of Et-IPA was performed in the negative electrospray ionisation mode. Identification, quantification, and confirmation took place with precursor—product ion—transition for Et-IPA (570.6 → 126.8/570.6 → 442.8 *m*/*z*), propyl-IPA (584.6 → 126.8 *m*/*z*), and butyl-IPA (598.6 → 126.8 *m*/*z*). The calibration curves were linear with $R^2 > 0.99$ over the whole range. All samples were measured twice. The concentration of Et-IPA was calculated with peak areas by Analyst 1.7.1 and the reported data were not surrogate-corrected.

The analytical methods were validated with a recovery test for oil, bait and blood matrix (Table 1). Recovery rates were determined with corresponding untreated samples. The reporting limit for the measured concentration for Et-IPA was 0.006 μg/g whole blood.

Statistical analyses

To estimate the impact of dose and sex of voles on Et-IPA blood residues, we devised a generalized linear mixed model with a gamma error distribution and logarithmic link function. Individual Et-IPA residues were used as the dependent variable, while independent factors were "sex"

Table 1 Recovery rates for ethyl-iophenoxic acid (Et-IPA) and the surrogate propyl-IPA based on five samples per matrix: sunflower oil (SF), polyethylene glycol 300 (PEG 300), broken wheat based bait and common vole (*Microtus arvalis*) blood

	SF	PEG 300	Bait		Blood			
μg/g	50	50	100	1000	0.01	0.1	1	100
					%			
Et-IPA	82 (12)	106 (8)	102 (1)	108 (18)	111 (11)	113 (12)	119 (12)	92 (19)
Propyl-IPA	76 (10)	95 (5)	101 (7)	106 (24)	74 (15)	99 (13)	112 (10)	98 (17)

Values are means (standard deviation).

(categorical variable, male/ female), "dose" (continuous) as well as the continuous variable "day" defined as the time after Et-IPA consumption (here day 1, 7, 14). Individual identification numbers ("ID") and "day" were introduced as random factors to account for the repeated measures design. In the global model, all two-way interactions were included and backward model simplification was performed using likelihood ratio tests. Within subject contrasts for day were compared using pairwise "Tukey" comparisons with the *as.glht* function from the *emmeans* package. Analyses were performed using R (R CoreTeam 2020).

RESULTS

The correlation of expected Et-IPA concentration and achieved (recovered) Et-IPA concentration in bait was close and positive for sunflower oil (linear regression $R^2 = 0.95$) and PEG 300 (linear regression $R^2 = 0.95$) (Fig. 1). The slope of the linear regression line was almost double for sunflower oil (77.9) versus PEG 300 (48.6) indicating that more Et-IPA was retained on broken wheat when sunflower oil was the carrier. Therefore, sunflower oil was used to produce Et-IPA bait for the following trial.

Almost all common voles consumed the offered 1 g Et-IPA wheat bait within 12 h independent of Et-IPA concentration. One vole in the 0, 80, and 160 μg/g groups and two voles in the 40 and 640 μg/g groups did not eat the bait. As there was no obvious relationship to the ET-IPA concentration, these animals were excluded from analyses. Bait consumption resulted in Et-IPA doses of 1–71 mg Et-IPA per kg body weight. There was no statistically significant difference in residues between males and females and this factor was excluded during model selection. There was a highly significant effect of dose on Et-IPA concentration in blood ($P < 0.001$) (Tables 2 and 3; Figure 2). Residues generally decreased over time ($P < 0.001$) and the more so the higher the Et-IPA dose was ($P < 0.001$) (Table 3; Fig. 2). The slope of the decline was greater for higher doses compared to lower doses (Interaction "day:dose" $P < 0.025$). Residues were higher at day 1 than at days 7 ($P < 0.001$) and 14 ($P < 0.001$) and there was a significant difference in residues between day 7 and 14 ($P < 0.001$). The decrease in Et-IPA concentration over time ranged from 33% (dose of 1 μg/g day 1 versus day 14) to 37% (dose of 70 μg/g day 1 versus day 14) (Fig. 2). The mean dose-residue relationship (Fig. 3) can be used to calculate the Et-IPA dose consumed based on Et-IPA blood residues using the hyperbolic equation: Et-IPA dose = 99.9456 × residue/(87208.6607 + residue).

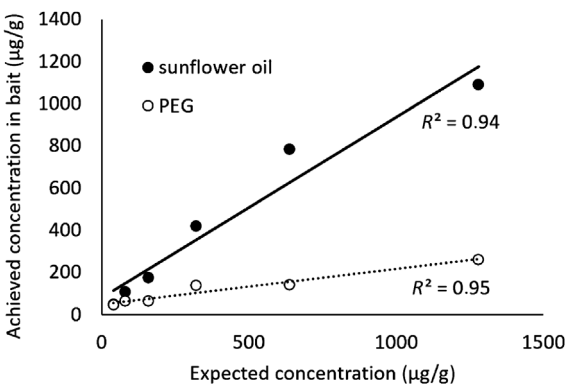

Figure 1 Correlation of expected concentration of ethyliophenoxic acid (Et-IPA) (μg/g) for broken wheat bait and achieved Et-IPA concentration (μg/g) measured in bait. Sunflower oil or polyethylene glycol (PEG) were used as carrier for Et-IPA.

DISCUSSION

With bait developed in this study, Et-IPA was delivered to common voles where dose-dependent residues could be detected in whole blood samples across several doses and for 2 weeks after uptake. This makes ET-IPA a suitable bait marker for small mammals similar to carnivores (Baer *et al*. 1985; Spurr 2002b; Cagnacci *et al*. 2006) and ungulates (Sweetapple & Nugent 1998; Massei *et al*. 2009; Phillips *et al*. 2014).

For both carriers tested, there was a close positive correlation of added Et-IPA concentration and achieved bait concentration. Sunflower oil was the most suitable carrier because more Et-IPA was retained on broken wheat when sunflower oil was the carrier compared to PEG 300 despite identical bait preparation for both carriers. PEG 300 may have a lower binding capacity for IPA or abrasion might have been higher for PEG 300 than sunflower oil. The suitability of sunflower oil confirms other studies, in which oils were successfully used as a carrier for Et-IPA (Fisher & Marks 1997; Larson *et al*. 1981).

Oily substances are usually palatable to small rodents (Jokić *et al*. 2011, 2013) and can increase bait uptake (Schlötelburg *et al*. 2018). The same is true for wheat (Jokić *et al*. 2012; Buckle & Smith 2015) that was used as a bait base. The combination of sunflower oil and wheat may have contributed to the palatability of the bait for common voles resulting in complete consumption of 1 g of the bait within 12 h independent of the Et-IPA concentration (for 93% of common voles). This is similar to mustelids where Et-IPA concentration has no effect on palatability (Spurr 2002b). However, this may be

Table 2 Ethyl-iophenoxic acid (Et-IPA) concentration in bait and corresponding mean and standard error (SE) of the Et-IPA blood concentration in common voles (*Microtus arvalis*) at day 1, 7, and 14 after uptake

	Et-IPA blood concentration μg/g					
Et-IPA concentration in bait μg/g	Day 1 Mean (SE)	*n*	Day 7 Mean (SE)	*n*	Day 14 Mean (SE)	*n*
0	0 (0)	10	0 (0)	10	0 (0)	10
40	9 (0)	8	3 (0)	8	3 (0)	8
80	16 (3)	9	8 (1)	9	7 (1)	8
160	15 (1)	9	12 (1)	9	8 (1)	7
320	33 (3)	10	28 (3)	8	23 (3)	7
640	61 (5)	8	57 (7)	8	37 (3)	7
1280	119 (13)	10	85 (6)	10	61 (8)	8

Table 3 Results of the generalized linear mixed model to test the effect individual Et-IPA doses and day of sampling and their interaction after consumption of Et-IPA on Et-IPA blood concentration in common voles (*Microtus arvalis*)

Source of variation	Coefficient β	SE (β)	*t*-Value	*P*-value	R^2c
Intercept	9.44007	0.09795	96.38	<0.001	0.94
Day	−0.05657	0.00785	−7.208	<0.001	
Dose	0.04285	0.00305	14.05	<0.001	
Day:Dose	0.00047	0.00021	2.236	0.025	
Random	σ^2	StDev			
ID	0.13709	0.37026			
Day	0.00238	0.04873			

SE, standard error; R^2c, conditional R^2; σ^2, variance; StDev, standard deviation; ID, individual identification.

different when foraging constraints vary (Weerakoon & Banks 2011). The willingness of black rats, which were offered a Rhodamine B bait at different concentrations, to consume the bait, was highly affected by the accessibility of other food sources (Weerakoon & Banks 2011).

We detected a strong dose-dependent concentration of Et-IPA residue in common vole blood ranging from 1–71 mg Et-IPA per kg body weight. The residue pattern was independent of the sex of the voles and blood concentration of Et-IPA decreased during the two weeks after uptake. The maximum decrease of 37% between day 1–14 was detected for a dose of 70 µg/g and is acceptable for application in a field study to assess bait uptake.

Persistence of bait markers in small mammals for 5–8 days is deemed sufficient to allow flexibility in sample collection (Cowan *et al*. 1984; Tobin *et al*. 1996). Our results show that Et-IPA was stable long enough for uptake studies and then disappears reasonably rapidly from the blood stream of voles. In addition, Et-IPA is excreted to the environment via urine and feces in very small quantities suggesting limited potential for long-term environmental risks (Mudge *et al*. 1978).

The use of Et-IPA as a bait marker provides several advantages compared to bait markers currently in use. Sampling for Et-IPA uptake is less intrusive than for tetracycline as only small quantities of blood are needed (50 μL) (Purdey *et al*. 2003) but there are still some limitations for its use in small mammals (see below). Although exposure to Rhodamine B can be easily identified, it gives no information about the quantity of bait consumed because there is no reliable dose-residue relationship (Fry & Dunbar 2007; Fry *et al*. 2010). In contrast to Rhodamine B, Et-IPA can be used (with some limitations) as a quantitative bait marker in large vertebrates (Saunders *et al*. 1993), laboratory rats (Purdey *et al*. 2003; Ballesteros *et al*. 2013) and common voles.

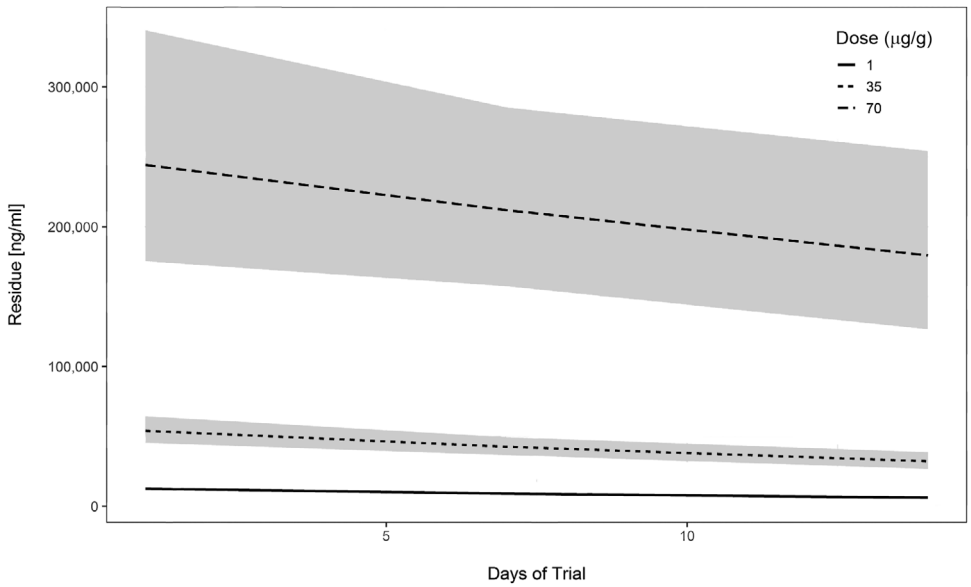

Figure 2 Predicted values of ethyl-iophenoxic acid (Et-IPA) concentration in blood of common voles (*Microtus arvalis*) depending on day after the consumption of various doses of Et-IPA (95% confidence intervals in grey). Based on the generated generalized linear mixed effect model, results were projected for doses representative of the study range.

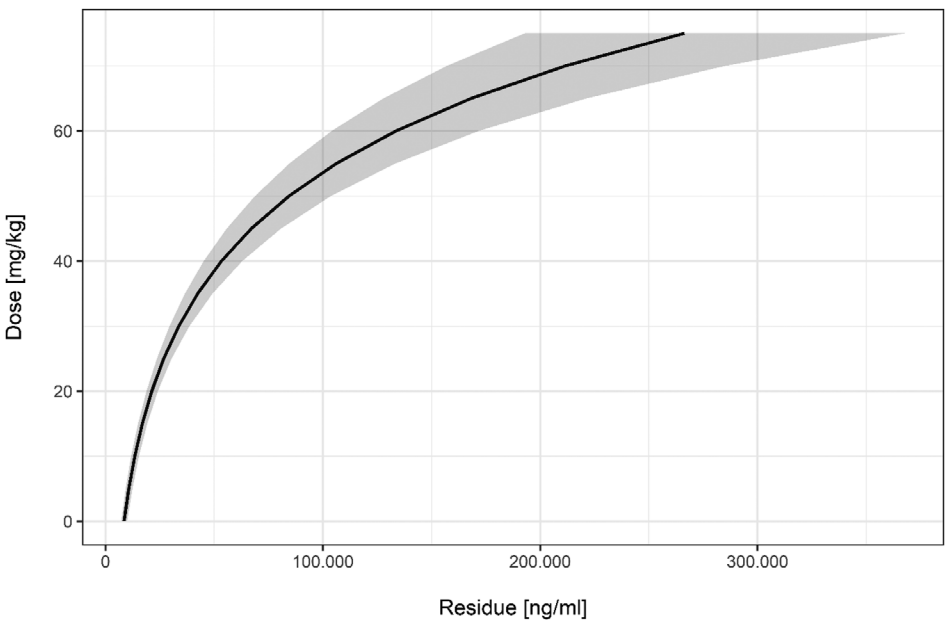

Figure 3 Dose-residue relation—predicted values (95% confidence intervals in grey) of the ethyl-iophenoxic acid (Et-IPA) dose consumed by common voles (*Microtus arvalis*) based on blood residues of Et-IPA.

Et-IPA residues in whole blood of voles were clearly detectable from day 1 to 14 after uptake of Et-IPA bait. This was similar to laboratory rats that received 5 mg Et-IPA per kg body weight, and in which 174, 168, and 58 mg/mL Et-IPA residues were detected at 7, 14, and 21 days, respectively (Purdey *et al*. 2003). Et-IPA may be present for even longer in common voles assuming similar declines to rats (Purdey *et al*. 2003) and other species. In red foxes [*Vulpes vulpes* (Linnaeus, 1758)] Et-IPA persists in blood and elevates serum iodine

levels (indirect detection of ET-IPA) for approximately 13 weeks, and in dogs at least for 52 weeks (Baer *et al*. 1985). In these species, iodine levels continuously decline over time (Baer *et al*. 1985), which is comparable to our short-term results. In common voles, there were differences in residue concentrations between samples collected 1 day after bait uptake and those collected 1 or 2 weeks later. After 1–2 weeks, residue concentrations were lower, especially when low Et-IPA doses were consumed. Therefore, it is recommended to apply the highest dose used in this trial to a bait to be used in field studies of bait uptake.

In a field situation, where bait is free-fed, animals consume bait voluntarily and may eat various amounts of bait at various times, so it is unclear when and how much bait was consumed. A low dose consumed recently and a high dose consumed long ago will yield similar Et-IPA residue concentrations in voles. However, the equation for the dose-residue relation gives a reasonable quantitative indication of minimum uptake when animals are sampled within 1–2 weeks. The quantitative performance of the bait marker seemed better for lower doses consumed (95% CI ≈ 13–15% of mean) than for higher doses (95% CI ≈ 25–34% of mean). This could suggest that using low Et-IPA doses in field studies could be advantageous. However, it is impossible to limit individual uptake to low doses and this may be in conflict with the aim of the uptake studies. Despite this shortcoming, Et-IPA provides a tool to quantitatively estimate uptake of food resources, bait, plant protection actives, biocides, antifertility agents etc. in small mammals as long as these compounds are consumed together with Et-IPA and the Et-IPA metabolism of animals in a field situation is similar to laboratory conditions.

A practical and efficient bait marker should meet several criteria. It needs to be palatable to the target species, easily incorporated into bait, and non-toxic at the concentration used for target-species and non-target species. In addition, the compound should be inexpensive, readily available, and there needs to be a simple and a minimally invasive method for its detection. Furthermore, its presence in the target species should be long lasting with a stable, consistent and strong positive relationship to bait uptake across individuals and times of sampling.

Et-IPA meets most of these requirements for common voles. It can be easily incorporated into food and bait, has no effect on palatability, has low systemic toxicity (Ballesteros *et al*. 2013), and is present in blood for at least 2 weeks. Sampling is not as invasive as for other bait markers but the collection of small volumes of blood can be intrusive, especially for wild animals (Purdey *et al*. 2003). Et-IPA does not occur naturally, so there is no risk for false positives. In conclusion, ET-IPA is a suitable bait marker for small mammals, which allows the quantitative measurement of food uptake for a multitude of potential applications in basic and applied research.

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