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Article *in* Science of The Total Environment · December 2008

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Kinetics of bromadiolone in rodent populations and implications for predators after field control of the water vole, *Arvicola terrestris*

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ARTICLE DATA

Article history:
Received 26 May 2008
Received in revised form
28 August 2008
Accepted 3 September 2008
Available online 26 October 2008

Keywords:
Anticoagulant rodenticide
Secondary poisoning
Pest control
Vertebrate pesticide

ABSTRACT

We document the kinetics of bromadiolone in two rodent populations after a field control of water voles, and their implications for predator exposure. Water voles and common voles were trapped aboveground and underground from 1 to 135 days after bromadiolone treatment in the field. Livers, digestive tracts, and rests of the body were analyzed separately.
Our results indicate that 99.6% of the water voles trapped underground and 41% of the common voles trapped aboveground contain bromadiolone residues. Concentrations were maximal between 3.3 and 6.5 days after treatment, according to the tissues examined and the model applied for water voles, and after 1.3 to 3.7 days for common voles. Water voles appeared available almost exclusively for foraging predators. Common voles, found less likely to be poisoned and exhibiting weaker concentrations, were mainly sampled aboveground. The liver, primarily eaten by some predators and scavengers, contains a larger bromadiolone quantity (59% of the total amount found in water voles). The rejection of the digestive tract by those species may lead to a subsequent consumption of voles with higher bromadiolone concentrations (from +3.8 to +5.8% of concentration) and provide a moderate risk increase. After 135 days, eight of the ten water voles and one of the two common voles exhibited detectable residues. Additionally, one specimen presented higher concentrations than the others, and similar to those measured in Voles trapped between the first 15–20 days. This may have consequences on predator intoxications several months after treatment.
These results integrate individual differences for the two main rodent species present in treated areas. Implications for predator exposure were investigated at the end of the study and suggest that, if the risk of secondary poisoning is maximal during the first 15–20 days when the rodent densities remain high, exposure conditions are maintained for at least 135 days.

1. Introduction

Anticoagulant pesticides are used worldwide to control rodent populations (Erickson and Urban, 2002). Compared to first generation or indandione derivative anticoagulants, Second Generation Anticoagulant Rodenticides (SGARs) are more acutely toxic to mammals and have a longer persistence in vertebrate tissues (WHO — World Health Organization, 1995; Erickson and Urban, 2002). These characteristics provide SGARs with a high risk for non-target mammals and birds,

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doi:10.1016/j.scitotenv.2008.09.003

and the secondary poisoning of predators by anticoagulants via contaminated rodents has been widely reported (McDonald et al., 1998; Murphy et al., 1998; Alterio et al., 1999; Stone et al., 1999; Shore et al., 2003; Fournier-Chambrillon et al., 2004; Walker et al., 2008).

Among SGARs, bromadiolone is extensively used for the field control of rodents, especially in Europe. In accordance with the Ministère de l'Agriculture et de la Pêche (2002), bromadiolone is the only pesticide authorized to control the population outbreaks of the fossorial water vole *Arvicola terrestris Sherman* in France. Laboratory experiments on stoat, *Mustela erminea*, and common buzzard, *Buteo buteo*, fed with voles intoxicated with bromadiolone led to the conclusion that "secondary poisoning was possible, although very unlikely in field conditions" (Grolleau et al., 1989). Moreover, although bromadiolone risk to non-target species is considered to be moderate (birds) to high (mammals), there is a lack of both field and laboratory data to support these assumptions (USEPA, 1998; Erickson and Urban, 2002). However, predictions based on laboratory experiments carried out on captive specimens are inconsistent with secondary poisoning recorded in the field. As for other SGARs, the negative impact of bromadiolone on non-target vertebrates (e.g. common buzzard, red fox and red kite) were reported in eastern France following intensive control operations of vole populations over a large spatial scale (e.g. 60,000 ha in Doubs department during 1998–1999) (SAGIR, 1990–2006; Berny et al., 1997; Raoul et al., 2003; Berny, 2007; Berny and Gaillet, 2008). Therefore, national and international authorities stressed the need for environmental risk assessment of the use of those rodenticides in the field, especially on their transfer in food webs (USEPA, 1998). In addition, survey programs showed that anticoagulant residues in wildlife (e.g. (Eason et al., 1996; Shore et al., 1999; Barnett et al., 2006) have increased over the last decade, heightening worldwide concern regarding the non-target effects of rodenticide use.

Ecotoxicological risk is a function of both exposure and toxicity. According to Erickson and Urban (2002), the assessment of secondary poisoning of non-target fauna by rodenticides must consider the residue burdens available in rodent tissues, which are in part related to the concentration of active ingredient (a.i.) in baits and the duration of exposure (Merson et al., 1984; Grolleau et al., 1989; Erickson and Urban, 2002). During field control operations, the persistence of bromadiolone (characterized by a 50% dissipation time DT_{50}) range from 2.9 to 6.0 days in wheat baits buried in treatment galleries (Sage et al., 2007). However, the storage of baits by voles increases the persistence of bromadiolone up to 10 times, making bromadiolone persistent at high concentrations in baits, i.e., 12 mg/kg 80 days after treatment. This could lead to a delayed exposure of rodent predators during a possible re-colonization of treated parcels by voles (Sage et al., 2007). In laboratory conditions, it was demonstrated that SGARs are persistent compounds in rodents, with a DT_{50} ranging from 170 to 300 days for bromadiolone in rat tissues, the highest concentrations being detected in the liver (Erickson and Urban, 2002). To our knowledge, only Delley and Joseph (1985) and Giraudoux et al. (2006) have studied bromadiolone residues in rodent populations in field conditions. Delley and Joseph (1985) reported residues in water voles exposed to 140 mg of bromadiolone/kg baits and trapped for three days after treatment. Giraudoux et al. (2006) showed that bromadiolone residues in the water vole population were stable for 10 days after treatment, and concluded that such a level of residues in rodents could lead to a daily ingested dose by predators higher than the known-LD_{50}s' (lethal dose of 50% of the population) for different vertebrate species. However no data are available for longer periods of time. It therefore seems essential to complete these data on rodent species present in treated parcels, and to consider the bromadiolone persistence in baits, as well as the vole storage behavior.

The objectives of this study were to assess the kinetics of bromadiolone residues in rodent populations present in treated areas after a field control; in our case in the water vole, i.e. the target species, and the common vole (*Microtus arvalis*), a non-target species. These two grassland rodent species, capable of reaching large densities, are both eaten by predators. For the purposes of our study, the rodents were trapped for 135 days after treatment, and bromadiolone concentrations were determined in the liver, the digestive tract, and the rest of the body. The answers to three vital questions are discussed based on the results obtained: (1) At what time are bromadiolone residues maximal, and how long after the original treatment does bromadiolone persist in rodent populations? (2) Does the distribution of bromadiolone in tissues influence predator exposure? (3) How do the density and accessibility of preys change over time? Then, implications of those results on the secondary poisoning of non-target species over time are discussed.

2. Materials and methods

2.1. Study site

The study site was a parcel of 9.7 ha localized at Pissenavache (N 46°56′34,1″; E 006°16′48,6″; 890–910 m of altitude) on the Jura plateau in Eastern France. In this area, water vole outbreaks occur on six-year cycles and bromadiolone baits have been used for control since the early 1980s. An index line transect (parcel diagonal) was performed to estimate the water vole density according to Giraudoux et al. (1995). Vole indices (burrows, earth tumuli, etc.) were recorded in 50% of 10 m intervals along the transect, approximately corresponding to 200 individuals per ha. Wheat baits were prepared by the process used by farmers: dried wheat grains were mixed with a commercial formulation of bromadiolone Super Caid® A659 (5 g a.i./L, ref. R227002, Liphatech Merck, France) to achieve a nominal concentration of 50 mg of bromadiolone per kg of bait. Then, they were stored at ambient temperature and protected from light and humidity during two weeks until treatments.

The experimental parcel had not been previously treated with bromadiolone since at least four years. In agreement with farmer practices, the baits were distributed into artificial galleries. Sixteen artificial galleries were placed 5 m apart and were 400 m long. This corresponds to a treated zone of 80 m wide. The burrow plough was set to deliver 1 kg of bait per 100 m, at a depth of 15 cm. The quantity of baits used was 20 kg/ha, which corresponds to the higher limit authorized by the French legislation. The field treatment was performed on the 17th of November 2004. To allow for the re-colonization of

the treated zone by rodents living in surrounding areas, only the central part of the parcel was treated.

2.2. Rodent sampling

Rodents were sampled only on the treated part of the parcel each day during the ten days following treatment, and every three or four days after that, by spacing out trapping sessions gradually during 135 days (D1 to D10 and D13; D16; D19; D24; D27; D49; D135). No sampling was possible between D49 and D135 (40 cm of snow on the parcel).

2.2.1. Underground trapping

Depending on the trapping efficiency, an increasing number (from 6 to 50) of underground kill-traps 'Topcat' (topcat GmbH, Wintersingen, Switzerland) were placed, providing burrows with fresh vole indices, in order to randomly catch at least six water voles for each sampling day. 'Topcat' traps were un-baited and were set the morning of the sampling day and controlled every half hour. The variation in the relative density of water voles on the treated parcel over time was estimated by measuring the trapping efficiency expressed as the number of captures per trap per day.

2.2.2. Aboveground trapping

A 400 m long and 40 cm high wood barrier was buried at a depth of 5 cm at the center of the treated area in order to intercept rodents circulating on the ground. Six additional barriers (4 m long) were randomly placed perpendicular to artificial galleries on the treated zone. One hundred and twenty ground traps 'Deadend 2' (topcat GmbH, Wintersingen, Switzerland) were placed every 6 m on both sides of the central barrier. Four ground traps were placed on both sides of each additional barrier, and fifty ground traps were placed over the entire treated parcel, preferentially on the rodent trail. 'Deadend 2' traps were placed in the parcel during the 135 days of the experiment. These were un-baited and were set each evening before the sampling day and controlled in the morning and evening of the sampling days.

2.2.3. Collection of carcasses

As intoxicated rodents could die aboveground of the treated parcel; carcasses were carefully searched on every sampling day.

Each specimen (both trapped and carcasses) was labeled individually and stored frozen (−20 °C) until the tissue processing was performed. The eyes from each specimen were taken and stored in 10% formalin for further determination of the relative age of the specimen, which was performed by weighing the dried crystalline lens (Martinet, 1966; Janova et al., 2003).

2.3. Tissue processing and bromadiolone titration

The specimens were thawed at room temperature, measured, and weighed. The liver, digestive tract, and the rest of the body (referred to as the carcass below) were separated. The liver is usually considered the main storage organ for anticoagulants (USEPA, 1998), and many predators (red fox, stone marten, stoat) feed primarily on this and discard the digestive tract (Delattre, 1987; Artois, 1989). Each tissue category was weighed and prepared for bromadiolone titration. The whole liver was stored separately. The digestive tract was homogenized for 1 min using an Ultra Turrax tissue disperser (T25 Basic S.25. N-18 G, IKA-WERK, Staufen, Germany). Carcasses were immersed in liquid nitrogen (Air liquide, Audincourt, France) for three s, and then crushed in a Blixer 3 Homogenizer (Robocoupe, Vincenne, France) with 150 g of solid carbon dioxide (Cryo Express, Bobigny, France), according to the method of Atterby et al. (2005). The carcass was mixed for three min to obtain a homogeneous powder. Additional solid carbon dioxide was added, if necessary, to keep the sample frozen. Each tissue sample was stored separately at −20 °C until extraction.

Tissues were thawed and an aliquot was weighed (0.2 g for liver samples, 1.0 g for digestive tract and carcass samples) and placed in a 100 mL tube containing difenacoum (lot 0395167, Lipha-Tech, France) as an internal standard (200 μL of a 2.5 μg/mL solution) and 10 mL of acetone (Carlo Erba analytical grade, Italia). This solution was homogenized for 1 min using an Ultra Turrax tissue disperser. The homogenate was centrifuged at 3000 rpm for 5 min. Two mL of supernatant was taken and carefully evaporated to dryness under a stream of nitrogen at 50 °C. The residue was resuspended with 200 μL of the elution solution (Giraudoux et al., 2006), shaken for 20 s with a Vortex (MS2 Minishaker IKA-WERK, Staufen, Germany), ultrasonicated (Kunshan Ultrasonic, Jiangsu, China) for two min to facilitate dissolution, and then centrifuged at 3000 rpm for five min before injection.

Bromadiolone concentrations in tissues were determined by High Performance Liquid Chromatography (HPLC) using methods derived from Hunter (Hunter, 1983a,b), according to Giraudoux et al. (2006). The method was linear at the concentrations tested (r^2>0.99). The limits of detection for bromadiolone were 0.05 mg/kg in the body, 0.1 mg/kg in the digestive tract, and 0.3 mg/kg in the liver (based on the mean noise level +3 standard deviations). Spiked liver, digestive tract, and body samples were used for quality assurance purposes (Bromadiolone, lot Bromo27 Analyse 37261M, Lipha-Tech, France). The percentage of recovery varied between 77.9% and 89.5% for bromadiolone and its internal standard difenacoum in all matrices. The repeatability was excellent (CV<10% over six days). The ratio of bromadiolone to internal standard was used to determine bromadiolone concentrations. From the concentrations in the three entities, we calculated the concentration in the whole body.

2.4. Statistical analysis

The normality of the data distribution was tested using the Kolmogorov–Smirnov test. When the normality could be accepted, or when our data could be transformed to Gaussian, ANOVA was used (Sokal and Rohlf, 1997). Otherwise, non-parametric Kruskal–Wallis or Wilcoxon tests were used (Siegel and Castellan, 1988).

The intoxication kinetics of rodent populations were modeled from the data obtained using non-linear regression. Although Newman (2001) defined the population ecotoxicology, to our knowledge, the pesticide kinetics at the population level are poorly documented in the literature. Two first-order compartment models normally used at the individual level

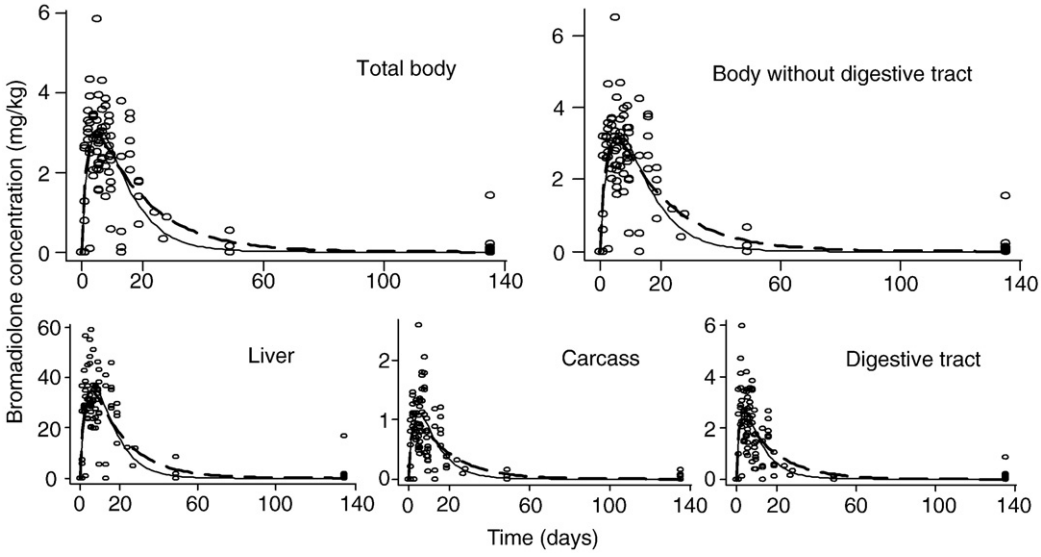

Fig. 1 – Bromadiolone concentrations in the 96 water voles trapped underground. Dotted line: model fit under the hypothesis of voles feeding exclusively in storage, dark line: model fit under the hypothesis of voles feeding exclusively in galleries.

were compared. The first (Pineiro and Bates, 2000) is derived from a compartment model in pharmacokinetics, and describes the concentration of a drug in the serum following a single oral dose. The application of the second model, derived from Widianarko and Van Straalen (1996), is illustrated by curve fitting using observations on the toxicity of an insecticide to a terrestrial isopod and correspond to the modelling of internal body concentration kinetic at individual scale after a single contamination of the medium. These two non-linear models were fitted using generalized least squares and variance functions. Then, they were compared using the information theoretic approach as outlined by Burnham and Anderson (2004). The second model with variance modeling was selected using the Akaike Information Criterion Corrected (Sakamoto et al., 1996). This model was:

$$C(t) = \frac{a}{k_2 - k_0}\left(e^{-k_0 t} - e^{-k_2 t}\right) \quad (1)$$

where $C(t)$ is the internal concentration at time t (mg/kg); a the assimilation rate (mg of bromadiolone/kg_{tissue}/d); k_2 the rate constant for elimination and other loss processes from the body such as metabolism (d^{-1}); and k_0 the rate constant for degradation of the chemical in baits (d^{-1}). This last variable was estimated from the data of Sage et al. (2007) using Eq. (2):

$$C(t) = C(0)e^{-k_0 t} \quad (2)$$

where $C(t)$ is the concentration at time t and $C(0)$ is the initial concentration of bromadiolone in the baits. Since we did not know if rodents fed on baits in galleries (where the persistence of the molecule is smaller) and/or in storage cavities (where the DT_{50} is ten times higher than in artificial galleries), both possibilities were investigated ($k_{0\ \text{galleries}} = 0.106$, $k_{0\ \text{storages}} = 0.057$).

The accumulation and elimination parameters (Eq. (1)) were estimated for the three tissues, the whole body, and the whole body without the digestive tract of each species by fitting the model with an exponential variance function. The variance model retained is a constant plus a power of the absolute value of the variance covariate. The differences in the parameter estimates between tissues and species were judged from the overlap of 95% confidence intervals. The differences between the bromadiolone quantity and concentration in different tissues according to the sex of the specimen were checked by a comparison of the 95% confidence intervals calculated from bootstrap bias-correct adjusted (BCa) limits (1000 permutations) on the standard deviation of the predicts (Wehrens et al., 2000). The relationship between the bromadiolone concentrations or quantities in vole tissues and the age of the individuals was verified by using the General Linear Model. Analyses were performed using R 2.4.1 (R Development Core Team, 2004).

3. Results

3.1. *Rodent sampling and population density estimation*

One hundred and eighty eight animals were trapped in the study area.

3.1.1. *Underground trapping*

Ninety four water voles were trapped, and one water vole was accidentally found dead in its burrow (although these were not normally searched). After six days, the number of water voles caught per trap decreased over time, so the sampling effort was gradually increased 2-fold until D9 and then 10-fold on D20 of the experiment. After D28, the efficiency seemed to increase from 0.04 to 0.2 rodents caught per trap.

3.1.2. *Aboveground trapping*

Sixty eight common voles and five water voles were trapped. The carcasses of fourteen common voles and one water vole were collected. Two common voles were collected in a moribund state. The sampling effort was identical throughout

Table 1 – Estimates of kinetic parameters for bromadiolone persistence in the water vole population trapped underground after a field treatment

		a (mg/kg$_{tissue}$/d)	min/max	p-value	k_2 (d^{-1})	min/max	p-value	r^2	Time of maximum concentrations calculated (d) and value (mg/kg$_{tissue}$)
$k_{0\ galleries}$	Whole b.	1.62[a]	1.23/2.01	<0.001	0.29[a,b]	0.20/0.38	<0.001	0.54	5.5 (3.1)
	Liver	15.05[b]	11.84/18.27	<0.001	0.21[a]	0.14/0.27	<0.001	0.51	6.5 (36.3)
	Carcass	0.62[c]	0.44/0.79	<0.001	0.33[a,b]	0.23/0.43	<0.001	0.46	5.1 (1.1)
	D.t.	2.04[a]	1.28/2.80	<0.001	0.50[b]	0.30/0.69	<0.001	0.48	4.0 (2.7)
	Body without D.t.	1.52[a]	1.17/1.86	<0.001	0.24[a,b]	0.17/0.31	<0.001	0.56	6.1 (3.3)
$k_{0\ storages}$	Whole b.	2.13[a]	1.42/2.85	<0.001	0.55[a]	0.35/0.75	<0.001	0.57	4.6 (3.0)
	Liver	19.48[b]	13.69/25.27	<0.001	0.41[a]	0.26/0.55	<0.001	0.54	5.6 (34.9)
	Carcass	0.76[c]	0.44/1.08	<0.001	0.61[a]	0.34/0.88	<0.001	0.45	4.3 (1.0)
	D.t.	2.53[a,c]	1.06/4.01	0.001	0.88[a]	0.35/1.41	0.002	0.47	3.3 (0.5)
	Body without D.t.	1.99[a]	1.36/2.62	<0.001	0.47[a]	0.30/0.64	<0.001	0.50	5.1 (3.2)

Minimum and maximum values are likelihood-based 95% confidence intervals. For one parameter, within the same k_0, values that share similar letters are not significantly different.
Parameters are presented for the whole body (Whole b.) and each tissue: liver, carcass, and digestive tract (D.t.), with the rate constant k_0 being estimated in treatment galleries and in storage cavities.

the experiment; however, the sampling efficiency was higher at the beginning, as 91% of the common voles and 80% of the water voles were captured during the first 16 days.

3.2. *Kinetics of bromadiolone residues in rodent populations*

No bromadiolone residues were found in the eight rodents trapped before the treatment.

3.2.1. *Underground trapping*

Eighty eight of the 94 water voles trapped underground (94%) contained detectable bromadiolone residues. On the first day after treatment, four of the six water voles trapped underground (67%) exhibited residues in all three tissues analyzed. Following the second day, all of the trapped water voles (n=78) accumulated residues in their tissues for 50 days, except for one specimen which was trapped at D13. Although the maximal concentration of bromadiolone in tissues was registered on D5, even at D135, eight of the ten water voles (80%) had residues in at least one tissue, with three specimens having residues in three tissues, four having residues only in the liver and digestive tract, and one having residues only in the liver. Among the water voles trapped 135 days after treatment, one presented a high bromadiolone concentration (1.43, 16.63, 0.15 and 0.86 mg/kg in the whole body, liver, carcass, and digestive tract, respectively) close to that observed on D1 and D18–D20.

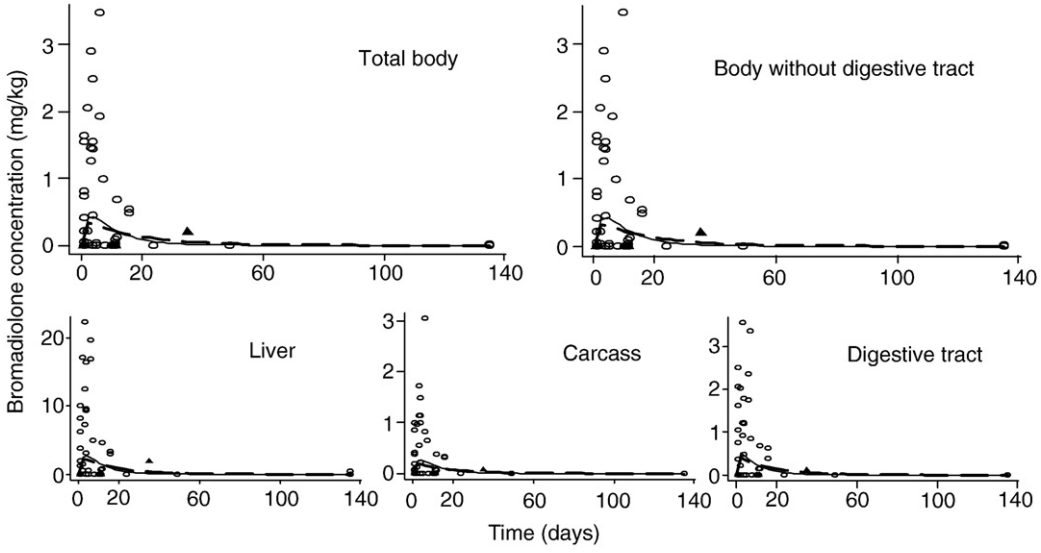

Fig. 2 – Bromadiolone concentration in the 69 common voles (O) and in the five water voles (▲) trapped aboveground. Dotted line: model fit under the hypothesis of voles feeding exclusively in storage, dark line: model fit under the hypothesis of voles feeding exclusively in galleries.

Table 2 – Estimates of kinetic parameters for bromadiolone persistence in common vole and water vole populations trapped aboveground after a field treatment

		a (mg/kg$_{tissue}$/d)	min/max	p-value	k_2 (d^{-1})	min/max	p-value	r^2	Time of maximum concentrations calculated (d) (and value) (mg/kg$_{tissue}$)
$k_{0\ galleries}$	Whole b.	0.43[a]	−0.01/0.86	0.058	0.69[a]	−0.07/1.44	0.078	0.07	3.2 (0.4)
	Liver	2.48[a]	0.14/4.81	0.042	0.62[a]	−0.02/1.26	0.063	0.06	3.5 (2.8)
	Carcass	0.21[a]	0.03/0.39	0.026	0.54[a]	0.01/1.06	0.049	0.06	3.7 (0.3)
	D.t.	0.82[a]	−0.59/2.23	0.262	1.27[a]	−0.99/3.53	0.275	0.08	2.1 (0.5)
	Body without D.t.	0.39[a]	0.03/0.74	0.039	0.61[a]	−0.01/1.23	0.057	0.05	3.5 (0.4)
$k_{0\ storages}$	Whole b.	0.53[a]	−0.75/1.81	0.420	1.38[a]	−2.06/4.83	0.433	0.01	2.4 (0.3)
	Liver	2.91[a]	−2.16/7.97	0.265	1.13[a]	−1.01/3.28	0.305	0.01	2.8 (2.2)
	Carcass	0.26[a]	−0.36/0.88	0.406	1.23[a]	−1.72/4.17	0.419	−0.01	2.6 (0.2)
	D.t.	1.40[a]	−12.76/15.57	0.847	3.20[a]	−28.97/35.38	0.846	0.03	1.3 (0.4)
	Body without D.t.	0.48[a]	−0.63/1.59	0.398	1.29[a]	−1.78/4.35	0.414	0.02	2.5 (0.3)

Minimum and maximum values are likelihood-based 95% confidence intervals. For one parameter, within the same k_0, values that share similar letters are not significantly different. Negative values should be biologically considered as zero values.
Parameters are presented for the whole body (Whole b.) and each tissue: liver, carcass, and digestive tract (D.t.) with the rate constant k_0 being estimated in treatment galleries and in storage cavities.

At the population level, the kinetic model adapted from Widianarko and Van Straalen (1996) describes a dramatic increase of bromadiolone residues in different tissues during the first days (Fig. 1). Bromadiolone concentrations reached the maximum level between day 4.0 and day 6.5 according to tissues for the models fitted with the $k_{0\ galleries}$, and between day 3.3 and day 5.6 for the models fitted with the $k_{0\ storages}$. Whatever the modality, the digestive tract is the organ in which the maximal predicted concentrations are reached the earliest, whereas the liver is the organ in which it happens the latest. The kinetic data show a gradual decrease until D135 (Fig. 1). The parameters estimates and maximum values modeled are listed in Table 1. According to these estimates, no matter the k_0, the assimilation rate (a) is higher in the liver than in the digestive tract, and lower in the carcass (Table 1). Therefore, at the population level, the liver assimilates bromadiolone more quickly (e.g. 25.8 and 7.7 times more quickly than in carcass and the digestive tract, respectively). The liver eliminates the bromadiolone slower than the digestive tract (k_2 is 2.4 times lower) when the data are fitted with the $k_{0\ galleries}$. In spite of an elimination rate of up to 2.2 fold smaller in the liver than in the digestive tract, when the data are fitted with the $k_{0\ storages}$, no statistical differences were found.

The comparison between the two modalities (galleries and storages) did not exhibit statistical differences for parameters of a given tissue (Table 1). The kinetic parameters (a and k_2) were higher (in average 26±4 and 88±8% respectively) when $k_{0\ storages}$ were taken into account, rather than $k_{0\ galleries}$. This explains why predicted values using the $k_{0\ storages}$ parameter are lower than those estimated by

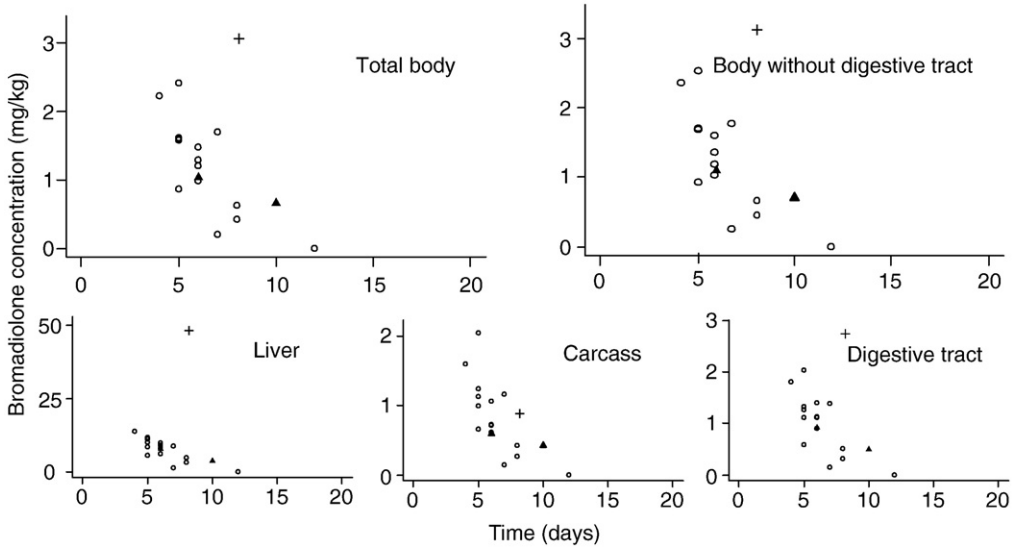

Fig. 3 – Bromadiolone concentration in the 14 common voles collected dead (O) or two moribund (▲) and the water vole (+) collected dead aboveground.

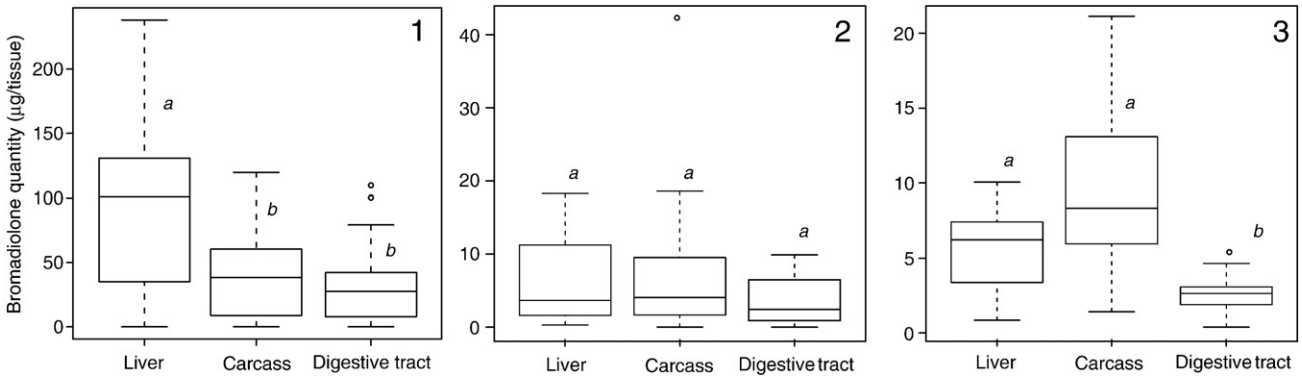

Fig. 4 – Bromadiolone quantity in the liver, carcass, and digestive tract of intoxicated rodents: water vole trapped underground (1); common voles trapped aboveground (2) and collected dead (3). For each boxplot, the median and inter-quartile range are indicated by the horizontal line and box height, respectively. Letters *a* and *b* indicate difference between tissues for a same category (Kruskal–Wallis, $p<0.05$).

using the $k_{0\ \text{galleries}}$ at the time of maximal concentration, but are higher during the phase of concentration decrease (Fig. 1). This also served to explain why bromadiolone persists longer in all tissues when the situation of bait consumption in storages is retained.

Age does not influence bromadiolone concentration in whole body, liver, carcass, or digestive tract (ANOVA, $p>0.05$). The overall sex ratio was 1.14 for water voles trapped underground (50 males/44 females). No differences in concentration in the whole body, liver, and digestive tract were found between sexes (bootstrap BCa limit comparison, $p>0.05$), but bromadiolone concentrations in female carcasses were found to be higher than in males (bootstrap BCa limit comparison, $p=0.036$).

3.2.2. *Aboveground trapping*

Twenty eight of the 69 common voles trapped aboveground (41%) and one of the five water vole (20%) contained detectable bromadiolone residues (Fig. 2). On the first day after treatment, six common voles of the 21 trapped (29%) had residues in the three tissues analyzed. From D2 to D7, 12 of the 22 common voles trapped (55%) had residues in three tissues and two had residues only in the liver. The measured concentrations are on average 1.9 fold higher than those measured at D1. From D8 to D35, only four of the 26 common voles trapped (15%) have residues (two trapped at D11 and D135 only in the liver, one trapped at D11 only in the digestive tract and carcass). The measured concentrations are on average 2.8 fold lower than those measured at D1. Of the five water voles trapped aboveground (Fig. 2) (one at D1, one at D10, two at D11 and one at D35), only the individual trapped on D35 (20%) had residues in its tissues (Fig. 2). The few individual data and the numerous values (59%) with no detectable residues may explain the fact that several parameters of the models are not significantly different from zero, and that the model explains a low fraction (r^2 ranged from 0.01 to 0.08) of the total variation (Table 2). The bromadiolone concentrations in water voles and common voles trapped aboveground showed a dramatic increase during the first 1.3 or 3.7 days according to tissues and k_0 parameters, and then, decreased gradually. The maximum calculated concentrations are presented in Table 2. The overall sex ratio was 1.3 for common voles trapped aboveground (39 males/30 females), and 2.5 for those collected dead (ten males/four females). The two moribund specimens were females. We failed to detect sex and age effects on bromadiolone concentration in any tissues (bootstrap BCa limit comparison, $p>0.05$ for sex and ANOVA $p>0.05$ for age). Whatever the tissue, bromadiolone concentrations were lower in common voles trapped aboveground than in water voles trapped underground, and in most of the cases the assimilation rate of the common vole was lower than for the water vole, while the elimination rate was not significantly different.

3.2.3. *Carcass collection*

All moribund animals and carcasses were found aboveground between D4 and D12 (Fig. 3). All of them had residues in the three tissues analyzed. The water vole found dead in a burrow also had residues in the three tissues analyzed (Fig. 3). The maximum concentration of bromadiolone in different tissues in common voles was registered five days after treatment, after which there was a gradual decrease. (Fig. 3) For the period of time in which dead specimens were collected aboveground, the bromadiolone concentrations in the liver were higher in intoxicated trapped common voles than in common voles collected dead aboveground (ANOVA $p=0.01$). However no

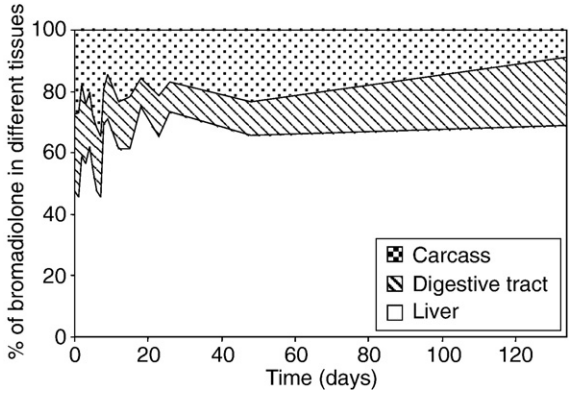

Fig. 5 – Evolution of the bromadiolone distribution in tissues over time in the water vole population.

difference between the two groups was found for other tissues as well as for whole body concentrations.

3.3. *Bromadiolone distribution in rodent tissues*

The results of the bromadiolone quantity measured in intoxicated rodents (those containing bromadiolone residues in at least one tissue) are presented in Fig. 4. For water voles trapped underground (Fig. 4.1), liver is the organ that contains the highest quantity of bromadiolone (58.7±14.2%) (Kruskal–Wallis, $p<0.001$), with the liver mass representing 5.0±0.8% of the total fresh body mass. No difference was observed between the quantity of bromadiolone in the carcass and digestive tract, which contained 22.1±10.5% and 19.3±13.1% respectively (Kruskal–Wallis, $p>0.05$), even though the carcass mass was larger (71.3±3.5% and 22.8±3.1% of the total body mass respectively) (Kruskal–Wallis, $p<0.001$). At the population level, the bromadiolone distribution changes over time (Fig. 5). The percentage of bromadiolone contained in the liver increased during the first two weeks (ANOVA, $p<0.001$) from 47.6±18.5 at D1 to 71.5±6.5% at D20 and then, tended to stabilize (ANOVA, $p<0.05$) to 69.0±15.4% at D135. Different trends were observed for the carcass (27.5±17.5 at D1 to 15.6±5.5 at D20 and 9.0±14.7% at D135) and digestive tract (24.9±30.4 at D1 to 9.3±4.5 at D20 and 22.9±14.3% at D135). Concerning common voles trapped aboveground (Fig. 4.2), no difference was observed in the percentage of bromadiolone accumulated in each tissue (Kruskal–Wallis, $p>0.05$). The liver, carcass, and digestive tract contained 42.9±22.9%, 35.8±16.3% and 21.4±10.14% of bromadiolone, respectively. In common voles collected once dead (Fig. 4.3), the liver and carcass were the two tissues containing the highest quantity of bromadiolone (32.4±5.8% and 52.2±6.2%, respectively) (Kruskal–Wallis, $p<0.025$). The percentage of mass of each tissue in common voles was of the same order as that in water voles. The liver, digestive tract, and carcass mass were 5.5±0.7; 20.9±2.7 and 72.5±8.9% of the total body mass, respectively. Water voles trapped underground and common voles collected dead or moribund exhibited a higher bromadiolone concentration (Wilcoxon, $p<0.001$) without a digestive tract compared to total concentrations for the whole body (on average +5.8% and +3.8%, respectively). However, no statistical differences were found between bromadiolone concentrations measured with or without the digestive tract in common voles trapped alive aboveground.

4. Discussion

Some data are available on the residues of rodenticides in target species organisms in the laboratory (Record and Marsh, 1988). However, little information is available on the total residues of bromadiolone or other SGARs in rodents under field conditions (Giraudoux et al., 2006). Given this lack of data, Lodal and Hansen (2002) calculated the expected contents of target rodents based on a scenario of bait consumption and anticoagulant elimination. The present study describes, for the first time, the evolution over time (135 days) of the kinetics of bromadiolone residues in populations of target and non-target rodents present in the treated area and eaten by predators.

4.1. *Rodent intoxication kinetic pattern*

In our study, a large proportion of water voles trapped on D1 (67%) and 99.9% of those trapped from D2 to D50 had bromadiolone residues in their tissues. These results are in agreement with those reported by Giraudoux et al. (2006) (60% on D1, 100% during the following nine days) and demonstrate that water voles had rapid access to baits. However, during the first ten days, the mean quantity of residues in water voles was twice as high in the present study (196.5 compared to 93.5 μg/ind). Our results for the residue levels in the carcass and digestive tract were similar to those reported by Giraudoux et al. (2006), although liver residues exceeded those reported by this previous study and were also considerably higher than those reported for other species and SGARs in the field (Delley and Joseph, 1985) and in the laboratory (Brown, 1994; Fisher et al., 2004; Atterby et al., 2005). Only the results of Poché (1988) are in accordance with ours: rats fed for one day with bromadiolone baits at 50 mg/kg contained 2.08 mg/kg of bromadiolone the next day, while, in our study, intoxicated water vole concentrations were on average 1.83±0.96 mg/kg. Common voles were found to have rapid access to baits as well, since 29% showed residues in tissues at D1. However, they were exposed to bromadiolone in a fewer number and measured concentrations were lower than for water voles. These vole species do not frequent the same burrow at the same time (Le Louarn and Quéré, 2003) and it is possible that differences in their dietary behavior or metabolism affect the residues.

Despite these differences, the two species have presented the same pattern of intoxication kinetics. While Giraudoux et al. (2006) have observed a relative stability of concentrations during the first 10 days, our data have exhibited non-linear kinetics characterized by a very fast concentration increase lasting until days D1.3 to D6.5, according to the tissue and species analyzed, and then followed by a gradual decrease. While the carcass (muscle, bone, skin…) had a lower assimilation rate, the high affinity for liver reported in the literature at the individual level (Parmar et al., 1987) may explain similar phenomena in our study. SGARs are especially known to persist for prolonged periods in the liver (USEPA, 1998). In our study, the digestive tract was the tissue with the highest elimination rate, while liver has eliminated bromadiolone slower. We observed the same exponential decline of concentrations described by Nahas (1987) and Hawkins et al. (1991) in laboratory rats. The type of toxicokinetic model used to fit our data usually describes the fate of chemicals at the individual level, i.e. the processes of uptake, distribution, metabolism, and elimination in organisms after a single dose (Bernillon and Bois, 2000). Soon after an anticoagulant is ingested, it will begin to be metabolized and excreted (Record and Marsh, 1988). In our study, four processes may explain this decline. The first two express themselves at the individual level, while the last two can be related to population processes: (1) the excretion and elimination of the molecule from the body; (2) its biotransformation into metabolites; (3) the new re-colonization of rodents poisoning themselves by feeding on less concentrated baits (Sage et al., 2007) and (4) after a few days, the most poisoned rodents die and, therefore, will not be trapped. The latter two processes are probably one reason why the second

phase of the exponential concentration decline was so short in our study in comparison with individual level studies (e.g. Hawkins et al., 1991).

Life-history traits weakly influenced rodent intoxication kinetics. Only sex had an influence on water vole carcass residues. Behavior might affect bait consumption and therefore the persistence at the population level but, alternatively, differences in the residue concentration might reflect toxicokinetic differences between sexes. For example, warfarin metabolism and rates of elimination were markedly different between male and female rats (Eason et al., 2002).

4.2. *Ecological reality of modeling*

According to Widianarko and Van Straalen (1996), because the model used only three parameters, the underlying assumptions were necessarily a simplified representation of reality. One of the difficulties in our study concerned the choice of the degradation rate constant of the chemical in baits (k_0). The prediction of a model for a rodent which eats baits alternatively in galleries and in storages should be between our two extreme scenarios, while the ideal model would take into consideration an overall k_0 including two rate constants for uptake $k_{1\ \text{galleries}}$ and $k_{1\ \text{storages}}$ (d^{-1}), each of them weighting the $k_{0\ \text{galleries}}$ and $k_{0\ \text{storages}}$. However, rodent behavior remains unknown and these two k_1 may change with time. For example, rodents may eat bait more in galleries at the beginning of the treatment (bait have still not been stored), and it appears impossible to know the real values of each k_1. Moreover, the assimilation rate (a) ideally invariant across doses and time may change with exposure, e.g., due to behavioral changes during accumulation. Rodents may feed on baits continually but they may also transport baits into storage cavities and/or eat them only when they germinate (Defaut and Sage; personal observations). Furthermore, re-colonizing rodents have access to baits later after treatment. This may explain the high variability of the data obtained. The presence of one non-intoxicated water vole trapped underground at D13, and four of the five trapped aboveground between D1 and D35, may be the consequence of a re-colonization from surrounding parcels and capture before they encountered poisoned baits. Saucy and Schneiter (1998) indicated that young water voles disperse *en masse* aboveground and these individuals were among the younger trapped (results not shown). However, to combine storage behavior and re-colonization in a model is out of reach at this time for lack of field data.

4.3. *Long time persistence of bromadiolone residues*

Spurr et al. (2005) have already observed that residues were still present in the liver of six rats at least 24 months after brodifacoum application. In our study, in addition to a recent re-colonization, another hypothesis may explain how caught individuals still have residues in tissues at least 135 days after treatment. These individuals may have either ingested a non lethal dose or be less sensitive to a given dosage. However, determining the relationship between an exposure and the resulting dose in target tissue is a critical issue encountered in quantitative risk assessment (Bernillon and Bois, 2000). Accumulation of carcass concentrations could be the result of intermittent or repeated exposure to sub-lethal doses and subsequent tissue accumulation, and this does not necessarily infer anything about toxicity.

Giraudoux et al. (2006) have suggest that the persistence of high residues in the digestive tract for a long time reflects that active baits may be consumed for long periods after treatment. In our study, and particularly for one specimen, high concentrations in the total body, including the digestive tract were observed up to 135 days. However, our results and those of Giraudoux et al. (2006) concerning the digestive tract include intestinal contents and tissues. Therefore, the bromadiolone concentration in digested food cannot be separated from its persistence in tissues, preventing us from estimating the real time to intoxication. Moreover, the mechanism of anticoagulant metabolism and excretion are poorly known (Atterby et al., 1999) and to our knowledge, no data are available concerning the duration of the excretion period.

4.4. *Bromadiolone distribution in tissues*

Assimilation rates that were higher, and elimination rates that were lower, in the liver than in other tissues have led to higher concentrations of bromadiolone being measured in this organ. Giraudoux et al. (2006) have observed that the liver contributed to only 25% of the total bromadiolone quantity in a water vole body, while, in our study, the proportion was 2.4 times higher, accounting for 59% of the total bromadiolone. For common voles collected dead, the digestive tract contains the smallest quantity of bromadiolone. This may indicate that just before dying, common voles decrease their activity and they do not ingest baits (Brakes and Smith, 2005). No differences were observed between the concentrations measured in the population trapped alive and those of specimens collected dead, except in the liver where the concentration of trapped common voles was found to be 1.8 times higher than that measured in dead specimens. As suggested by Grolleau et al. (1989), one can hypothesize that the bromadiolone degradation by micro-organisms after death had already begun.

4.5. *Implications for predator exposure*

In addition to the kinetics of residues in rodent populations, the bromadiolone available for predators over time may be determined by several factors, such as:

4.5.1. *Evolution of rodent density*

Underground, the trapping efficiency may be related to the evolution of the water voles density. The frequent checking of traps limited the non-availability of traps and the 'trap engaged effect' (Weihong et al., 1999), making the number of voles trapped each days negligible in comparison with the population density estimation before treatment. The dramatic increase of the trapping effort after the first 6–7 days reflects the diminution in rodent population density and corresponds to the maximum rodent mortality observed by Grolleau et al. (1989) between five and ten days after the first bromadiolone ingestion. Between D20 and D50, the trapping efficiency was very low, suggesting that the treatment was effective and that live preys are lower for predators. After D50, the presence of

40 cm of snow in the study site prevented the capture of specimens by surface predators and allowed vole population movements from surrounding parcels. Re-colonization might explain the relative increase in trapping efficiency at the end of the experiment. Common vole specimens were released when a sufficient number was trapped for residue analysis. As the only information we kept was the number of specimens analyzed, we could not estimate the density variation over time for this species. However the intoxication and the death of common voles showed that the water vole control reduced the local population of non-target rodent species and may limit the food availability of some specialist predators (e.g. Least Weasel, *Mustela nivalis*) or lead to the exposure of predators that do not eat water voles.

4.5.2. *Prey accessibility*

The likelihood of a rodent being fed upon by predators or scavengers is a critical determinant in the matter of secondary hazard (Brakes and Smith, 2005). For predators which do not consume carcass but prefer living prey, we showed that exposure is maximal during 6–7 days but is continuous all throughout the experiment. For surface scavengers, the exposure appears limited between D4 and D12, when mortality in the two species was observed aboveground. SGARs such as bromadiolone are generally defined as single-feeding rodenticides (Kolf-Clauw et al., 1995). However, because of the slow onset of their action and their high persistence in baits (Sage et al., 2007), rodents may continue feeding and will eat more than a LD before dying, thus potentially increasing residues. The time to death after ingestion may permit the rodents to seek cover when they fall ill (Record and Marsh, 1988), and, as previously discussed, to be less available to surface predators. On the other hand, Saucy et al. (2001) demonstrated that 38% of poisoned water voles died aboveground instead of in their underground tunnels. In our study, we saw that this behavior differed among rodent species. Birds of prey such as buzzards have limited access to water vole, i.e., the most poisoned species, while predators such as mustelids and foxes can have direct access to them underground. Furthermore, even if concentrations are not more important in moribund specimens, their reduced escape response could increase the proportion of contaminated individuals in the diet and the likelihood of ingesting a harmful dose (Cox and Smith, 1992).

4.5.3. *Bromadiolone distribution in the rodent body*

The rejection of the digestive tract, where bromadiolone was at low concentrations, by some scavenging species (Delattre, 1987; Artois, 1989) may provide a moderate degree of risk increase for secondary poisoning while they feed primarily on the liver, the most concentrated tissue and the one where bromadiolone accumulation rate was the most important over time. Our results contrast with those reported by Giraudoux et al. (2006) who described a concentration decrease of 12.79% when the digestive tract is discarded. Our study demonstrates a concentration increase from 3.8 to 5.8%.

4.5.4. *Exposure simulation and risk assessment for predators: the case of the Red fox*

The high bromadiolone concentration in 96% of water voles and 51% of common voles is clearly a hazard for their predators. By considering those previous factors influencing bromadiolone availability, the residues in rodent tissues were maximal between three to six days after bait distribution, but the risk appeared maximal during the first 15–20 days. In order to assess the risk for Red foxes that could feed on a treated parcel after a control operation, a "worst case" scenario was developed as follows. We assumed that adult Red foxes ate between 0.3 and 0.6 kg of food per day (Artois, 1989). According to (Weber and Aubry, 1993), they may feed on water vole exclusively during high-density peaks. If those foxes feed on water vole trapped underground exclusively, they may ingest between 0.13–0.25 or 0.14–0.27 mg of bromadiolone per kg of body mass per day on average, depending on if they feed on all the rodent body or if they discard the digestive tract, respectively. Foxes feeding only on live rodents trapped aboveground will ingest on average 0.020–0.041 or 0.021–0.042 mg of bromadiolone per kg of body mass per day. Foxes feeding only on moribund or dead rodents will ingest on average 0.067–0.127 or 0.067–0.133 mg of bromadiolone per kg of body mass per day. As no toxicity parameters for bromadiolone were available for foxes (USEPA, 1998; Erickson and Urban, 2002; AGRITOX INRA, 2004), we compared the ingested doses of bromadiolone calculated above to the LD_{50} of 10 mg/kg established for dogs (USEPA, 1998). However, SGARs are more toxic when ingested for several consecutive days because repeated ingestion did not allows the animals to recover from the effects of the precedent doses (Kolf-Clauw et al., 1995). If the concentrations observed aboveground are less important, the values for water voles trapped underground are near the acute LD_{50} after five days of exposure for dogs (0.50 mg/kg/day, Petterino and Paolo, 2001), and are generally higher than the lowest lethal dose, 0.15 mg/kg/day for five days (Kolf-Clauw et al., 1995). Moreover, the risk is magnified by the persistence of the compound in the rodent population for at least 4.5 months, which could lead to accumulation following repeated exposure. In this regard, it is important to remember that a sub-lethal dose well below the LD will produce significant clotting abnormalities and some hemorrhaging (Eason et al., 1996). Furthermore, while toxicity such as LD_{50} on dogs is mostly obtained under laboratory conditions, with animals housed individually and reduced movements, field conditions may increase the risk of severe bleeding, thereby increasing the toxicity of rodenticides (Petterino and Paolo, 2001; European Commission, 2002; Berny et al., 2006). Moreover, the risk for non-target species may considerably increase with time since high-rodent-density peaks are maintained for months (Giraudoux et al., 1997). This study was carried out in a parcel of 4 ha, but field control operations are undertaken on larger areas (i.e. 60,000 ha treated in 1998/1999 (SAGIR 2000)). On the level of a predator hunting area, all bromadiolone treatments are not performed at the same time by farmers (e.g. there are several weeks between control operations of water vole in the same village). This results in a prolonged exposure period for non-target species.

5. Conclusion

Previous studies have focused only on intoxication of individuals. However, for a full understanding of the consequences of using rodenticides, processes at the population level have to

be investigated complementarily. For the first time, the kinetics of bromadiolone residues on the two rodent populations present on treated areas was monitored extensively after field treatment. A mathematical model of the rodent-body residue was built as required for risk-assessment studies (Erickson and Urban, 2002), and without considering prey/predator interactions, allowed a simulation of the bromadiolone available for secondary poisoning. Processes other than the metabolism of the specimens, such as the re-colonization and intoxication of rodents living in surrounding areas, the death of the most poisoned specimens, and/or the fate of bromadiolone in the environment (soil, baits…), may influence the residue kinetics at the population level. To date, laboratory experiments (i.e. Grolleau et al., 1989) were in contradiction with massive secondary poisoning hazards regularly reported during the past 20 years (SAGIR, 1990–2006; Berny, 2007). Our study clearly and realistically indicates that an important risk of non-target species poisoning does exist during the first 15–20 days, and can be prolonged for longer times (until at least 135 days) after field control operations.

Acknowledgements

This study received support from the *Conseils généraux* of the Doubs and the Jura departments and from the *Région* of *Franche-Comté*. We are grateful to B. Barbier and B. Videmann of the ENVL, D. Truchetet of the *Direction Régionale de l'Agriculture et de la Forêt*, S. Bagnoud of the *Fédération Régionale de Défense contre les Organismes Nuisibles* and to G. Cordier, farmer of the *Zone Expérimentale de Lutte Anti Campagnols* for their participation and technical support.

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