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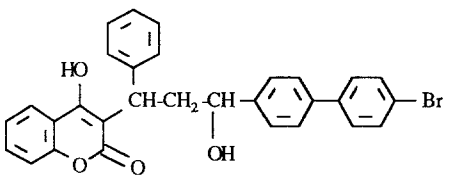
Environmental Contamination and Toxicology

Primary Toxicity of Bromadiolone on the Coypu

M. F. Morin,[1] N. Merlet,[1] G. Naulleau,[2] and M. Dore[1]

[1]Laboratoire de Chimie de l'Eau et des Nuisances, 40, Avenue du Recteur Pineau, 86022 Poitiers Cedex, France and [2]Centre d'Etudes Biologiques des Animaux Sauvages (CEBAS), 79360 Beauvoir, Sur-Niort, France

Bromadiolone, a hydroxycoumarin developped and marketed since 1975, is one of the most effective anticoagulant rodonticides used against a wide variety of rodents (Marsh 1977 ; Redfern and Gill 1980 ; Richards 1981) and it is proving very successful in France in the control of coypus (Myocastor coypus) in swamp areas. The chemical structure of bromadiolone is the following :



This compound is a vitamin k_1 antagonist. It acts on rodents by inhibiting the synthesis of vitamin K-dependent clotting factors. Its efficacy and its acute toxicity to rodents has been widely studied (Grand 1976 ; Gill and Redfern 1980 ; Marsh, Howard and Jackson 1980 ; Malhi 1986 ; Saxena and Singh 1987) as well as its toxicity to some other animals (Lund 1981 ; Grolleau 1983 ; Lorgue, Mally and Nahas 1985). However studies on the quantitative analysis of bromadiolone in tissues of poisoned coypus has yet to be extensively investigated. Only Nahas (1987) has reported results on residues of bromadiolone found in tissues from Rattus norvegicus, but these have given only a fragmentary picture of the poisoning process.

This paper describes the results obtained for a study of acute and chronic toxicity of coypus by bromadiolone and especially the kinetic determination of adsorption and the elimination of this compound by various tissues of the animal (liver, kidney, heart, lungs, muscles).

MATERIALS AND METHODS

Quantitative analyses of bromadiolone were carried out by high pressure liquid chromatography (HPLC) with fluorimetric detection. The analysis of coypus tissues required various stages of extraction and cleaning procedures before

Send reprint requests to M. DORE at the above adress.

analysis. These two analytical procedures has been described elsewhere (Morin, Merlet, Doré and Léchevin 1989) : they allow bromadiolone to be measured at very low levels (detection limit of 0.03 $\mu g\ g^{-1}$) in tissue extracts, with no interference from co-extracted materials.

Feeding tests were carried out on wild, individually caged, coypus caught in the surrounding swamps. The animals were fed with apples and cereal pellets for several days before the start of testing. They were then weighed and offered toxic baits and alternative dry food. In chronic toxicity tests, each coypu received daily 1 kg of carrots poisoned with bromadiolone at a concentration of 100 mg kg^{-1}. Bait that was not consumed was weighed each day to determine bromadiolone intake.

As far as acute toxicity is concerned, the coypus received 1 kg of poisoned carrots only on the first day of the test ; they were then fed with uncontaminated food.

Autopsies were carried out to confirm that deaths were due to anticoagulant poisoning. Animal tissues were frozen (- 20°C) before analysis.

Pure and technical grade bromadiolone was supplied by LIPHA.

RESULTS AND DISCUSSION

The results obtained are presented in Table 1 for chronic toxicity and Table 2 for acute toxicity. The tests for the determination of chronic toxicity were led with high doses of bromadiolone and it would therefore be more accurate to speak about subchronic toxicity. All the animals that died during the tests presented signs of internal bleeding resulting from the action of the anticoagulant rodenticide.

Several conclusions can be drawn from these results. Firstly, the data showed this anticoagulant to be very effective against coypus. In the case of subchronic poisoning, death occurs between 5 to 8 days after the first intake. With acute toxicity, a single consumption of poisoned carrots, containing as little as 7.61 mg of bromadiolone per kg of animal, was sufficient to lead to death after 7 days, that is to say within the same time delay as that obtained during chronic poisoning.

Secondly bromadiolone diffused towards all the tissues of the animals : liver, kidneys, muscles, heart and lungs. Bromadiolone diffused preferentially into livers in which concentrations as high as 16.8 $\mu g\ g^{-1}$ were recorded.

For example, a male coypu of 7.6 kg recovered dead in march, after poisoning with bromadiolone, had bromadiolone concentrations of 1.32 $\mu g\ g^{-1}$ in liver and of 0.5 $\mu g\ g^{-1}$ in kidneys. These concentrations are similar to those registered in dead coypus in experiments with acute toxicity.

Levels of bromadiolone were also high in kidneys, in the order of several $\mu g\ g^{-1}$. All other coypu tissues presented lower but significant concentrations of bromadiolone.

Table 1. Experimental results for chronic toxicity

Coypu n°	1 ♀	2 ♀	3 ♂	4 ♀	5 ♀	6 ♀	7 ♂	8 ♀
Weight (kg)	4.3	3.975	5.025	5.175	3.85	4.2	3.225	3.825
Bromadiolone intake (mg kg^{-1})	60.34	74.59	92.34	57.87	90.39	87.62	11.01	64.84
Day of death	6	6	8	6	6	7	5	8
Bromadiolone concentration in liver (µg g^{-1})	3.4	5.4	3.4	4.4	9.4	5.1	1.3	6.8
Total bromadiolone in liver (µg)	448.8	659.3	596	453.2	772.6	701.2	127.8	698
Bromadiolone concentration in kidneys (µg g^{-1})	2.2	0.6	6	1	0.8	4.3	1.03	1
Total bromadiolone in kidneys (µg)	85.8	16.74	375.6	41.5	28.64	155.23	41.3	27.8
Bromadiolone concentration in muscles (µg g^{-1})	0.1	0.2		0.3	0.7		0.13	
Bromadiolone concentration in heart (µg g^{-1})	0.5				0.33			
Bromadiolone concentration in lungs (µg g^{-1})	0.8				0.89			

Table 2. Experimental results for acute toxicity

Coypu n°	9 ♀	10 ♀	11* ♂	12* ♀	16 ♂	17 ♂	19 ♀
Weight (kg)	4.5	3.4	4.15	2.6	5.175	4.55	3.275
Bromadiolone intake (mg kg^{-1})	7.61	25.45	10.49	28.85	12.37	18.79	21.53
Day of death	7	7			10	8	8
Bromadiolone concentration in liver (µg g^{-1})	1.25	0.85	8.2	16.8	1.05	1.2	1.6
Total bromadiolone in liver (µg)	151.2	107.95	1 363.6	2 054.9	194.2	181.2	158.4
Bromadiolone concentration in kidneys (µg g^{-1})	0.38	0.46	0.95	1.1	0.56	0.26	0.56
Total bromadiolone in kidneys (µg)	13.87		35.62	18.15		14.38	18.94
Bromadiolone concentration in muscles (µg g^{-1})	0	0.16	0.07	0.14			
Bromadiolone concentration in heart (µg g^{-1})	0.05			0.47			
Bromadiolone concentration in lungs (µg g^{-1})	0.26			0.86			

* animals sacrificed 24 hours after the first intake

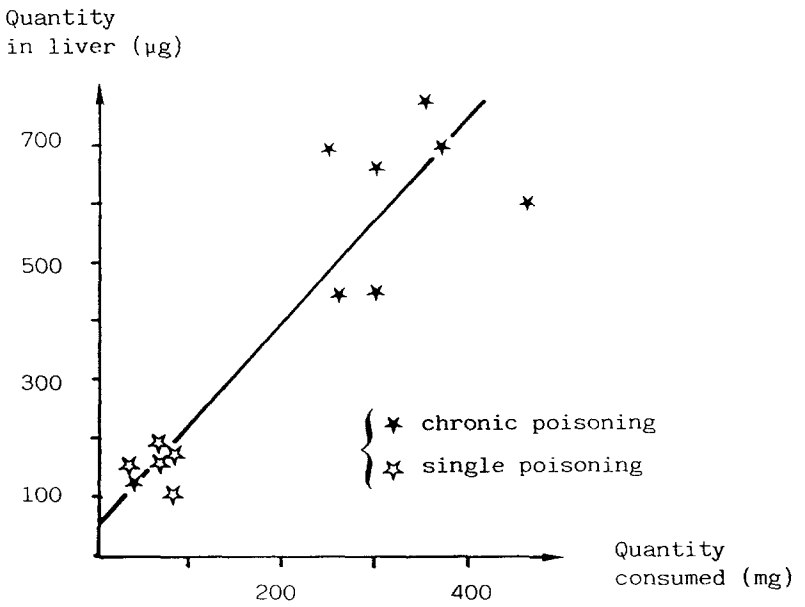


Figure 1 : Bromadiolone fixation in coypu livers

The total amounts of bromadiolone quantified in livers (Q_{liver}) correlated with the total quantities of bromadiolone consumed by each animal ($Q_{consumed}$), both for acute and chronic toxicity. These results, represented in figure 1, led to the following linear relationship :

Q_{liver} (µg) = 1.749 $Q_{consumed}$ (mg) + 50.209

with a correlation coefficient r = 0.932

In addition, Tables 1 and 2 also show that the diffusion of bromadiolone towards all the tissues of coypu was very fast. For example : the quantities of anticoagulant measured in the various tissues of animals 11 and 12 which had been sacrificed showed that these presented high concentrations of the compound.

On the other hand, this fixation was followed by a fast elimination of the poison by the various organs and especially by livers. For identical quantities of intake, the amounts of bromadiolone quantified in livers were lower for the animals poisoned up to death than for the animals sacrificed 24 hours after the first intake. Male coypu number 7 compared with number 11, and female number 10 with number 12. These examples illustrate that elimination from the liver could reach about 90 %.

In the case of the coypus poisoned by a single intake (Table 2), it was possible to calculate the value of the hepatic half-life of bromadiolone ($t_{1/2}$), that is to say, the time which was necessary for the animal's liver to eliminate half of the adsorbed amount. All the values for this calculation are reported in Table 3.

Table 3. Mean values of bromadiolone in livers according to the day of death

Day of death *	Reference numbers of the coypus	Q_t Mean value of bromadiolone in livers ($\mu g\ g^{-1}$)
1	11 and 12	12.5
7	9 and 10	1.05
8	17 and 19	1.40
10	16	1.05

* time limit before death occurs

The hepatic elimination could thus be represented by an exponential function of the type :

$$Q_t = Q_o\ e^{-bt}$$

where :

Q_o is the initial concentration of bromadiolone fixed in the liver
Q_t is the bromadiolone concentration in the liver at the date of the death (t)
b represents the coefficient of hepatic elimination

The results were as follows :

$$\text{Log } Q_t = -0.293\ t + 2.645$$
(with a correlation coefficient r = 0.95)

and the hepatic half-life of bromadiolone was thus :

$$t_{1/2} = \frac{\text{Log } 2}{Q_o} = \frac{\text{Log } 2}{0.293} = 2.4 \text{ days}$$

It would appear thus that 50 % of the bromadiolone initially retained in the liver would be eliminate within a relatively short time, namely 2.4 days.

Moreover, it was significant that bromadiolone did not accumulate in the tissues. This was demonstrated by comparing, for the same time limit of mortality, the total intake of bromadiolone with the hepatic intake, both for acute toxicity and subchronic toxicity (Table 4).

The parameters for this comparison (which should be calculated for the same time limit before death) are the following :

$$A = \frac{\text{Total intake for subchronic toxicity}}{\text{Total intake for acute toxicity}}$$

$$B = \frac{\text{Total hepatic quantity for subchronic toxicity}}{\text{Total hepatic quantity for acute toxicity}}$$

The results obtained (see Table 4) showed good agreement between the coefficients A and B for each date of death (7 or 8 days). It must therefore be concluded that there was no accumulation of bromadiolone during the subchronic poisoning.

Table 4: Comparison between acute and subchronic poisoning

date of death (day)	Technique of poisoning	Reference number of coypus	Mean value of the total intake (mg)	A	Mean value of the total hepatic quantity (µg)	B
7	Subchronic	6	368	6.1	701.2	5.4
	Acute	9 and 10	60.39		129.58	
8	Subchronic	3 and 8	356	4.5	647	3.8
	Acute	17 and 19	78		169.8	

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