# WHICH USEFUL TOXICOLOGICAL INFORMATION CAN BE DRAWN FROM STUDIES ON THE HEPATIC FIXATION OF ANTICOAGULANT RODENTICIDES

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ABSTRACT: Anticoagulant rodenticides act at the hepatic level where they are more or less fixed according to their lipophilic nature. The studies on kinetics and metabolism carried out with no toxic doses are useful to know how products act but do not allow to anticipate the toxicity risks for non target species, because of low residual contents. These risks can only be assessed after the administration of toxic doses taking into account the residue levels. The use of half-life to express the results is not sufficiently accurate and may lead to wrong conclusions. The studies involving the residue and secondary toxicity levels are more accurate for assessing the risks. Some examples are given in particular for bromadiolone.

Proc. 15th Vertebrate Pest Conf. (J. E. Borrecco & R. E. Marsh, Editors) Published at University of Calif., Davis. 1992

## INTRODUCTION

Anticoagulant rodenticides exert their antivitamin K effect at the hepatic level by preventing the transformation of vitamin K epoxide into reduced vitamin K. Coagulation factors depending on vitamin K being no longer activated, blood coagulation does not work properly and induces the onset of internal haemorrhages (Figure 1, Meehan 1984).

The hepatic fixation of anticoagulant rodenticides varies in time according to products. It seems closely related to the lipophilic nature of the molecule and that explains the more potent activity of products called second generation products. Therefore, bromadiolone turns out to be more lipophilic than warfarin because of the length and structure of its side chain; presence of 2 phenyl rings and a bromine atom (Figure 2).

The first data on the hepatic behaviour of products are given by the studies on kinetics and metabolism classically carried out on lab rodent strains which are given low but no lethal doses in order to keep animals in life as long as necessary. These studies interesting for the knowledge of products are not adequate to surround the risk for non target species. Trials very similar to fight conditions must be added; lethal doses administered, where relevant to wild rodent strains.

It is also classical to express the result of kinetic studies by calculating the half-lives and in particular the hepatic half-life. This way of calculating is not appropriate to the knowledge of risk for non target species and moreover varies according to different factors. So, residue levels must be taken into account and toxicological consequences involved must be examined. These various points are going to be developed through some examples published in the literature.

## STUDIES ON KINETICS AND METABOLISM—RELATION BETWEEN RESIDUE LEVELS AND TOXICITY

Several studies were dedicated to the kinetics and metabolism of flocoumafen in different species among which the rat (Huckle et al. 1989). The oral administration of a subtoxic dose: 0.14 mg/kg ($LD_{50}$ 0.25 mg/kg) of $^{14}C$ flocoumafen shows that the behaviour of the product is the same as other anticoagulant rodenticides, in particular a preferential storage in the liver: about 50 percent of the administered dose. The hepatic concentration is about 1.2 µg/g of liver during the first 7 days and then slowly decreases and remains higher than 0.5 µg of liver 200 days after the administration. Assuming that the rats' liver of this experiment weighs 7 g on average, the maximum residual flocoumafen dose is 8.4 µg. This concentration may not have any toxic effect on non target species even on the most sensitive ones like the dog: $LD_{50}$ between 0.075 and 0.25 mg/kg (Johnson et al. 1986 mentioned in Lund 1988). In the worst case, $LD_{50}$: 0.075 mg/kg corresponds to a single absorption by a 10-kg dog of about 10 kg of poisoned rodents assuming that the liver contains 50 percent of the administered flocoumafen dose. This event is impossible (Figure 3).

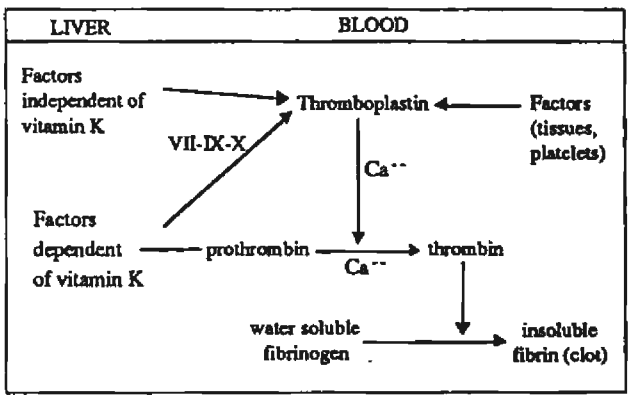


Figure 1. Simplified diagram of blood coagulation.

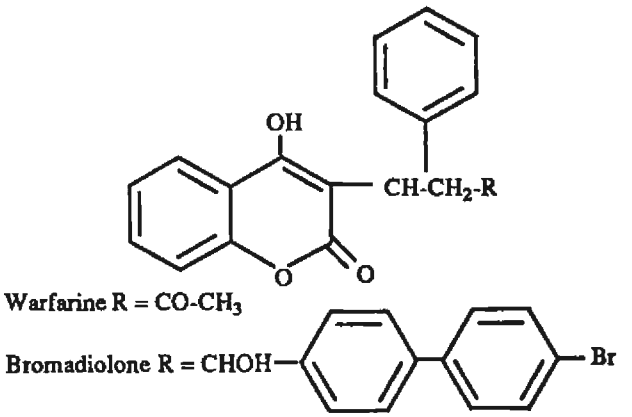


Figure 2. Comparison of the structures of warfarin and bromadiolone side chains emphasizing the more lipophilic nature of bromadiolone.

| | |
|---|---|
| Rat 228 g (liver 7 g) | maximum residue 8.4 µg |
| Dog 10 kg $LD_{50}$ | flocoumafen 0.75 to 2.5 mg equivalent of about 10 kg of rats |

Figure 3. Liver flocoumafen residues and toxicity for the dog.

Rat 180–240 g (liver 7 g) residues 7 to 11.2 µg

Dog MTD brodifacoum 0.5 mg/kg (any dog)
bromadiolone 2 mg/kg (Beagle dog)

Figure 4. Brodifacoum, bromadiolone: liver residues and toxicity for the dog (Brodifacoum – Godfrey et al. 1981, Bromadiolone – Poché 1988).

The same conclusion may be drawn from the comparative study of liver storage carried out with 4 anticoagulant rodenticides: brodifacoum, bromadiolone, coumatetralyl, and difenacoum $^{14}C$ (Parmar et al. 1987). The authors indicate that the elimination of products from liver is biphasic, an initial, rapid phase lasting up to 8 days followed by a second very slower phase lasting up to several months depending on products. During the second phase, the concentrations for brodifacoum and bromadiolone for which the metabolism is low are 2–3 nmoles equivalent/g of liver, that is to say 1 to 1.6 µg/g. It is not specified in this summarized publication to which study time these values correspond to. If as previously seen, it is assumed that a rat's liver weighs 7 g, the total quantity of products: 7 to 11.2 µg may have no toxic consequences for the dog, for instance when the single maximum tolerated doses by this species are taken into account (Figure 4).

These 2 examples show very clearly that the studies on kinetics and metabolism carried out with no toxic doses give residue levels which do not allow to anticipate the toxicity risk for non target species.

## RESIDUE LEVELS AFTER ADMINISTRATION OF TOXIC DOSES

The assay of anticoagulant rodenticide residues in rodents poisoned with lethal doses, therefore under normal conditions of struggle, is preferably made in the whole animal.

For bromadiolone, in order to study the secondary toxicity for stoat (*Mustela erminea*) and common buzzard (*Buteo buteo*), water voles (*Arvicola terrestris*) were poisoned under different conditions and in particular under conditions similar to those of struggle: a 3-day carrot bait consumption titrated at 100 mg/kg of active ingredient (Grolleau et al. 1989). After a 3-day bait consumption, the quantity of bromadiolone found in the whole animal is quite significant: 10.93 mg/kg and then quickly decreases: 1.29 mg/kg in the following 2 days. Analyses made on rodents found killed after fields trial confirm the residue levels (Poché 1988). So, for a trial performed in Chino, California, the average residual content in bromadiolone was 1.92 mg/kg for the roof rat and 1.17 mg/kg for the mouse. These results are summarized in Table 1.

A similar quantity of residues (2.2 to 5.2 mg/kg) was found for voles (*Microtus pinetorum*) poisoned for 3 days with a bait titrated at 50 mg/kg of brodifacoum and then sacrificed on day 4 (Kaukeinen 1982).

Table 1. Bromadiolone residues in different target species after the administration of toxic doses.

| Target species | Residues in mg/kg (time limits in days after the administration) |
|---|---|
| Water vole (*Arvicola terrestris*) | 10.93 (3)–1.29 (5) |
| Roof rat (*Rattus rattus*) | 1.92 |
| Mouse (*Mus domesticus*) | 1.17 |

Table 2. Study of liver storage of difethialone. R correlation coefficient between study time and deviations between recorded concentration and assessed concentration.

| Model | R correlation coeffieient | Relative standard deviation of the parameters of the exponential equation |
|---|---|---|
| 1 exponential | 0.92 | 15 to 24 percent |
| 2 exponentials | 0.94 | 68 to 333 percent |

The administration of toxic doses gives low but quite significant residuary levels of brodifacoum and bromadiolone of about some ppm. In the kinetic study carried out with flocoumafen, assuming that 50 percent of the product is concentrated in the liver, the maximum residual concentration only represents a small proportion of ppm (0.07 mg/kg).

## STUDY ON KINETICS AND METABOLISM— RESULTS EXPRESSED

The elimination curve is an exponential and must be, for the calculation of half-life, drawn in a semi-logarithmic system (log of concentrations).

Choosing a mathematical model requires a lot of attention as shown by the following example from an unpublished study on liver storage with difethialone (Vigie 1989). The mathematical parameters calculated through the SIPHAR/PC software are summarized in Table 2.

Although the correlation coefficient is slightly better in the model with 2 exponentials, the system with 1 exponential for which the relative standard deviations are the lowest ones must be chosen. This choice has some consequences on the half-life: 73 days (model with 1 exponential) instead of 159 days (model with 2 exponentials, second half-life).

The number of experimental points, therefore the study time may also act upon the value of half-life. So, in the previous trial, the 73-day value corresponding to a 6-month study is shortened to 51 days if the study is stopped 3 months after.

Finally, the only half-life values taken into account may lead to wrong conclusions. So, in the study carried out by Parmar et al. (1987), it is mentioned that brodifacoum and bromadiolone have hepatic half-lives of 130 and 170 days respectively, which may make believe that the hepatic fixation of bromadiolone is higher. It is actually the contrary, as shown by a comparative study carried out on a lab rat strain

Table 3. Secondary toxicity of bromadiolone.

| Non target species | Poisoned voles: Residual bromadiolone | Adminstered number | Mortality |
|---|---|---|---|
| Common buzzard (*Buteo buteo*) | 10.93 mg/kg | 1/day | no |
| | 6.75 mg/kg | 1/day/3 days | 2/10 |
| Stoat (*Mustela erminea*) | 6.5 mg/kg | 1/day/ 3 days | no |
| | 6.5 mg/kg | 1/day/5 days | 1/3 |

which was given a $LD_{50}$ of each product (Ray et al. 1989). For the 3 rats administered 1.25 mg/kg of bromadiolone (dose slightly higher than $LD_{50}$: 1.125 mg/kg) and sacrificed 5 days later, the hepatic content is lower than 0.06 µg/g of liver (detection limit). For brodifacoum ($LD_{50}$ 0.28 mg/kg), the contents 7 days after the administration are 0.4 and 2.2 µg/g of liver for 2 rats out of 3. It is therefore very clear that the value of half-life is not a sufficient accurate way for expressing results to allow to anticipate the secondary toxicity risk. Only the taking into account of residue levels allows it.

## RESIDUE LEVELS AFTER THE ADMINISTRATION OF TOXIC DOSES—TOXICOLOGICAL CONSEQUENCES

The studies measuring residues in association with the assay of secondary toxicity allow to precisely determine the toxicological consequences for non target species. Table 3 summarizes some of the secondary toxicity results of bromadiolone for the buzzard and stoat in the study carried out by Grolleau et al. (1989). Mortality is only recorded in case of repeated administration of rodents.

The absence of bromadiolone toxicity with a single ingestion of poisoned rodent was also noted for the fox, the supposed predator of the coypu (Morin 1988). We must be under severe experimental conditions - administration of coypus poisoned by bromadiolone bait until death that is to say for 5 to 6 days - in order to record mortality.

Other studies on secondary toxicity, without assaying residues in poisoned rodents, allowed to confirm the low toxicity of bromadiolone and to put forward differences between products. So, in the study on toxicity for the owl (*Tyto alba*) carried out by Mendenhall et al. (1980), the 6-day administration of poisoned rodents by bromadiolone does not induce any sign of poisoning. Rodents must be given for 10 days before noting the death of 1 bird out of 2. Under the same conditions, difenacoum which has a half-life similar to bromadiolone in classical kinetic studies (Parmar et al. 1987) induces haemorrhages without mortality after a 6-day administration. For brodifacoum, the mortality of the 2 birds is recorded after a 3-day administration of poisoned rodents. In the same way, the stone martens' consumption (*Martes foina*) of 7 to 8 field mice a day during 4 days (total 31), poisoned during 4 days by a bromadiolone bait titrated at 50 mg/kg shows no consequence. Stone martens do not show any sign of poisoning (Lund et al. 1986). As a result, these experiments show that the toxicity risk for non target species is more significant in the case of repeated consumption of poisoned rodents. Different sensitivities must be noted among non target species. On another hand, products for which studies on kinetics and metabolism may have made believed a similar toxicity have in fact quite different behaviours, very probably related to different residue levels.

## CONCLUSION

It is important to make a clear difference of the usefulness of studies on kinetics in target rodents according to the administered dose. Low but no lethal doses are used to know absorption, distribution and elimination of products. They cannot be used to foresee the risk for non target species; the residuary contents represented by traces having no toxicological meaning. In order to know the risk for non target species, residuary contents in poisoned rodents by lethal doses must be known. Only the values of the first days of intoxication are important from a toxicological point of view.

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