Environmental Health Criteria 175
Anticoagulant Rodenticides

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Environmental Health Criteria 175

ANTICOAGULANT RODENTICIDES

First draft prepared by Dr M. Tasheva,
National Centre of Hygiene, Medical
Ecology and Nutrition, Sofia, Bulgaria

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the United Nations Environment Programme,
the International Labour Organisation,
and the World Health Organization

World Health Organization
Geneva, 1995
The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by the IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

* * *

A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Case postale 356, 1219 Châtelaine, Geneva, Switzerland (Telephone No. 9799111).

* * *

This publication was made possible by grant number 5 U01 ES02617-15 from the National Institute of Environmental Health Sciences, National Institutes of Health, USA, and by financial support from the European Commission.
Environmental Health Criteria

P R E A M B L E

Objectives

In 1973 the WHO Environmental Health Criteria Programme was initiated with the following objectives:

(i) to assess information on the relationship between exposure to environmental pollutants and human health, and to provide guidelines for setting exposure limits;

(ii) to identify new or potential pollutants;

(iii) to identify gaps in knowledge concerning the health effects of pollutants;

(iv) to promote the harmonization of toxicological and epidemiological methods in order to have internationally comparable results.

The first Environmental Health Criteria (EHC) monograph, on mercury, was published in 1976 and since that time an ever-increasing number of assessments of chemicals and of physical effects have been produced. In addition, many EHC monographs have been devoted to evaluating toxicological methodology, e.g., for genetic, neurotoxic, teratogenic and nephrotoxic effects. Other publications have been concerned with epidemiological guidelines, evaluation of short-term tests for carcinogens, biomarkers, effects on the elderly and so forth.

Since its inauguration the EHC Programme has widened its scope, and the importance of environmental effects, in addition to health effects, has been increasingly emphasized in the total evaluation of chemicals.

The original impetus for the Programme came from World Health Assembly resolutions and the recommendations of the 1972 UN Conference on the Human Environment. Subsequently the work became an integral part of the International Programme on Chemical Safety (IPCS), a cooperative programme of UNEP, ILO and WHO. In this manner, with the strong support of the new partners, the importance of occupational health and environmental
effects was fully recognized. The EHC monographs have become widely established, used and recognized throughout the world.

The recommendations of the 1992 UN Conference on Environment and Development and the subsequent establishment of the Intergovernmental Forum on Chemical Safety with the priorities for action in the six programme areas of Chapter 19, Agenda 21, all lend further weight to the need for EHC assessments of the risks of chemicals.

Scope

The criteria monographs are intended to provide critical reviews on the effect on human health and the environment of chemicals and of combinations of chemicals and physical and biological agents. As such, they include and review studies that are of direct relevance for the evaluation. However, they do not describe every study carried out. Worldwide data are used and are quoted from original studies, not from abstracts or reviews. Both published and unpublished reports are considered and it is incumbent on the authors to assess all the articles cited in the references. Preference is always given to published data. Unpublished data are only used when relevant published data are absent or when they are pivotal to the risk assessment. A detailed policy statement is available that describes the procedures used for unpublished proprietary data so that this information can be used in the evaluation without compromising its confidential nature (WHO (1990) Revised Guidelines for the Preparation of Environmental Health Criteria Monographs. PCS/90.69, Geneva, World Health Organization).

In the evaluation of human health risks, sound human data, whenever available, are preferred to animal data. Animal and in vitro studies provide support and are used mainly to supply evidence missing from human studies. It is mandatory that research on human subjects is conducted in full accord with ethical principles, including the provisions of the Helsinki Declaration.

The EHC monographs are intended to assist national and international authorities in making risk assessments and subsequent risk management decisions. They represent a thorough evaluation of risks and are not, in any sense, recommendations for regulation or standard setting. These latter are the exclusive purview of national and regional governments.
Content

The layout of EHC monographs for chemicals is outlined below.

- Summary - a review of the salient facts and the risk evaluation of the chemical
- Identity - physical and chemical properties, analytical methods
- Sources of exposure
- Environmental transport, distribution and transformation
- Environmental levels and human exposure
- Kinetics and metabolism in laboratory animals and humans
- Effects on laboratory mammals and in vitro test systems
- Effects on humans
- Effects on other organisms in the laboratory and field
- Evaluation of human health risks and effects on the environment
- Conclusions and recommendations for protection of human health and the environment
- Further research
- Previous evaluations by international bodies, e.g., IARC, JECFA, JMPR

Selection of chemicals

Since the inception of the EHC Programme, the IPCS has organized meetings of scientists to establish lists of priority chemicals for subsequent evaluation. Such meetings have been held in: Ispra, Italy, 1980; Oxford, United Kingdom, 1984; Berlin, Germany, 1987; and North Carolina, USA, 1995. The selection of chemicals has been based on the following criteria: the existence of scientific evidence that the substance presents a hazard to human health and/or the environment; the possible use, persistence, accumulation or degradation of the substance shows that there may be significant human or environmental exposure; the size and nature of populations at risk (both human and other species) and risks for environment; international concern, i.e. the substance is of major interest to several countries; adequate data on the hazards are available.

If an EHC monograph is proposed for a chemical not on the priority list, the IPCS Secretariat consults with the Cooperating Organizations and all the Participating Institutions before embarking on the preparation of the monograph.
Procedures

The order of procedures that result in the publication of an EHC monograph is shown in the flow chart. A designated staff member of IPCS, responsible for the scientific quality of the document, serves as Responsible Officer (RO). The IPCS Editor is responsible for layout and language. The first draft, prepared by consultants or, more usually, staff from an IPCS Participating Institution, is based initially on data provided from the International Register of Potentially Toxic Chemicals, and reference data bases such as Medline and Toxline.

The draft document, when received by the RO, may require an initial review by a small panel of experts to determine its scientific quality and objectivity. Once the RO finds the document acceptable as a first draft, it is distributed, in its unedited form, to well over 150 EHC contact points throughout the world who are asked to comment on its completeness and accuracy and, where necessary, provide additional material. The contact points, usually designated by governments, may be Participating Institutions, IPCS Focal Points, or individual scientists known for their particular expertise. Generally some four months are allowed before the comments are considered by the RO and author(s). A second draft incorporating comments received and approved by the Director, IPCS, is then distributed to Task Group members, who carry out the peer review, at least six weeks before their meeting.

The Task Group members serve as individual scientists, not as representatives of any organization, government or industry. Their function is to evaluate the accuracy, significance and relevance of the information in the document and to assess the health and environmental risks from exposure to the chemical. A summary and recommendations for further research and improved safety aspects are also required. The composition of the Task Group is dictated by the range of expertise required for the subject of the meeting and by the need for a balanced geographical distribution.

The three cooperating organizations of the IPCS recognize the important role played by nongovernmental organizations. Representatives from relevant national and international associations may be invited to join the Task Group as observers. While observers may provide a valuable contribution to the process, they can only speak at the invitation of the Chairperson.
EHC PREPARATION FLOW CHART

Commitment to draft EHC

Document preparation initiated

Draft sent to IPCS Responsible Officer (RO)

Possible meeting of a few experts to resolve controversial issues

Revision as necessary

Responsible Officer, Editor, check for coherence of text and readability (not language editing), preliminary reference cross check

1st Draft

International circulation to Contact Points (150+)

Comments to IPCS (RO)

Review of comments, reference cross-check, preparation of Task Group (TG) draft

Editor

Task Group meeting

Insertion of TG changes

Post-TG draft, detailed reference cross-check

Editor

Graphics

Word-processing

Camera-ready copy

Final editing

Approval by Director IPCS

WHO Publication Office

Printer

Proofs

Publication

French/Spanish translations of Summary/Evaluations

Library for CIP Data

--- routine procedure

--- optional procedure
Observers do not participate in the final evaluation of the chemical; this is the sole responsibility of the Task Group members. When the Task Group considers it to be appropriate, it may meet in camera.

All individuals who as authors, consultants or advisers participate in the preparation of the EHC monograph must, in addition to serving in their personal capacity as scientists, inform the RO if at any time a conflict of interest, whether actual or potential, could be perceived in their work. They are required to sign a conflict of interest statement. Such a procedure ensures the transparency and probity of the process.

When the Task Group has completed its review and the RO is satisfied as to the scientific correctness and completeness of the document, it then goes for language editing, reference checking, and preparation of camera-ready copy. After approval by the Director, IPCS, the monograph is submitted to the WHO Office of Publications for printing. At this time a copy of the final draft is sent to the Chairperson and Rapporteur of the Task Group to check for any errors.

It is accepted that the following criteria should initiate the updating of an EHC monograph: new data are available that would substantially change the evaluation; there is public concern for health or environmental effects of the agent because of greater exposure; an appreciable time period has elapsed since the last evaluation.

All Participating Institutions are informed, through the EHC progress report, of the authors and institutions proposed for the drafting of the documents. A comprehensive file of all comments received on drafts of each EHC monograph is maintained and is available on request. The Chairpersons of Task Groups are briefed before each meeting on their role and responsibility in ensuring that these rules are followed.
WHO TASK GROUP ON ENVIRONMENTAL HEALTH CRITERIA FOR ANTICOAGULANT RODENTICIDES

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ENVIRONMENTAL HEALTH CRITERIA FOR ANTICOAGULANT RODENTICIDES

A WHO Task Group on Environmental Health Criteria for Anticoagulant Rodenticides met in Geneva from 14 to 18 November 1994. Dr R. Pleština, IPCS, welcomed the participants on behalf of Dr M. Mercier, Director of the IPCS, and the three IPCS cooperating organizations (UNEP/ILO/WHO).

The first draft was prepared by Dr M. Tasheva of the National Centre of Hygiene, Medical Ecology and Nutrition, Sofia, Bulgaria. The second draft was prepared by Dr R. Pleština, incorporating comments received following the circulation of the first draft to the IPCS contact points for Environmental Health Criteria monographs. The Task Group reviewed and revised the draft document and made an evaluation of risks for human health and the environment from exposure to anticoagulant rodenticides. Dr R. Pleština and Dr P.G. Jenkins, both members of the IPCS Central Unit, were responsible for the overall scientific content and technical editing, respectively.

The efforts of all who helped in the preparation and finalization of the monograph are gratefully acknowledged.
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AAPCC</td>
<td>American Association of Poison Control Centers</td>
</tr>
<tr>
<td>DT&lt;sub&gt;50&lt;/sub&gt;</td>
<td>degradation time for 50% of a compound</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>median effect concentration</td>
</tr>
<tr>
<td>FD</td>
<td>fluorescence detection</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>I&lt;sub&gt;50&lt;/sub&gt;</td>
<td>concentration of an inhibitor causing 50% inhibition of an enzyme under given conditions</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>K&lt;sub&gt;a&lt;/sub&gt;</td>
<td>adsorption coefficient</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>median lethal dose</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MTD</td>
<td>maximum tolerated dose</td>
</tr>
<tr>
<td>NOAEL</td>
<td>no-observed-adverse-effect level</td>
</tr>
<tr>
<td>NOEL</td>
<td>no-observed-effect level</td>
</tr>
<tr>
<td>PT</td>
<td>prothrombin time</td>
</tr>
<tr>
<td>PTT</td>
<td>partial thromboplastin time</td>
</tr>
<tr>
<td>WISN</td>
<td>warfarin-induced skin necrosis</td>
</tr>
</tbody>
</table>
INTRODUCTION

The anticoagulants included in this review are those that are used as rodenticides. The development of coumarin anticoagulants occurred during the Second World War and they were introduced as effective antithrombotic agents for treatment of thromboembolic disease in humans. Warfarin has been used both as a drug and a rodenticide, and has been extensively evaluated. Several hydroxycoumarin and indandione derivatives have been synthesized and introduced as effective rodenticides. They act by interfering with the blood coagulation mechanism.

The appearance of rat strains resistant to warfarin and some other anticoagulants has stimulated the development of more potent, second-generation anticoagulants, some of which are also “single dose” anticoagulants or “superwarfarins”.

Many anticoagulant rodenticides are known, but it is not the aim of this monograph to include all available information on each compound. The purpose is to describe the general characteristics of anticoagulants, using suitable illustrations to indicate their impact on humans and the environment.

A distinction needs to be made between the characteristics of the technical compounds and those of their formulated products concerning the risks that their use poses to human health and the environment.
1. SUMMARY

1.1 General

The anticoagulants described in this monograph are those used mainly in agriculture and urban rodent control. Warfarin, the first widely used anticoagulant rodenticide, was introduced as an effective agent for treatment of thromboembolic disease in humans.

Based on their chemical structure, anticoagulant rodenticides may be grouped into two categories, hydroxycoumarins and indandiones, although their mechanisms of action are similar.

1.2 Properties and analytical methods

Anticoagulant rodenticides come in a solid crystalline or powder form, and are slightly soluble in water. Most of them are stable under normal storage conditions.

Most of the procedures for the determination of anticoagulant rodenticides are based on high-performance liquid chromatography.

1.3 Sources of human and environmental exposure

First-generation hydroxycoumarins were introduced as rodenticides in the late 1940s. The appearance of resistance to warfarin and other first-generation anticoagulants led to the development of more potent, second-generation anticoagulants. The concentrations of active ingredients in baits vary according to the efficacy of the rodenticides.

1.4 Environmental distribution, levels and exposures

Anticoagulant rodenticides are used mainly as bait formulations. Since their volatility is low, concentrations in the air will be negligible. As they are only slightly soluble in water, their use is unlikely to be a source of water contamination.

Since anticoagulant rodenticides are not intended for direct application to growing crops, no residues in plant foodstuffs are expected.
Non-target vertebrates are exposed to rodenticides primarily through consumption of bait and secondarily from consumption of poisoned rodents. Small pellets and whole grain baits are highly attractive to birds.

Warfarin is used as a therapeutic agent for thromboembolic disease.

There is a potential for occupational exposure to anticoagulant rodenticides during manufacture, formulation and bait application, but data on the levels of exposure are not available.

1.5 Mode of action and metabolism

Anticoagulant rodenticides are vitamin K antagonists. The main site of their action is the liver, where several of the blood coagulation precursors undergo vitamin-K-dependent post-translation processing before they are converted into the respective procoagulant zymogens. The point of action appears to be the inhibition of K1 epoxide reductase.

Anticoagulant rodenticides are easily absorbed from the gastrointestinal tract, and may also be absorbed through the skin and respiratory system. After oral administration, the major route of elimination in various species is through the faeces.

The metabolic degradation of warfarin and indandiones in rats mainly involves hydroxylation. However, the second-generation anticoagulants are mainly eliminated as unchanged compounds. The low urinary excretion precludes isolation of metabolites from the urine.

The liver is the main organ for accumulation and storage of rodenticide anticoagulants. Accumulation also occurs in the fat.

1.6 Effects on mammals and in vitro test systems

Signs of poisoning in rats and mice are those associated with increased bleeding tendency.

There is wide variation in the LD$_{50}$ of anticoagulant rodenticides, toxicity being greatest by the oral route. Dermal and inhalation toxicities of anticoagulants are also high.
Some anticoagulants show a similar range of acute toxicity for non-target mammals as for target rodents, but toxicity spectra for anticoagulants may vary between species.

Following repeated oral administration in rats, the main effects seen are those associated with the anticoagulant action.

There are few data available on repeated exposure of non-rodent species.

One study on warfarin in rats has indicated developmental effects. Otherwise, there is no convincing evidence that anticoagulants are teratogenic in experimental animals.

There is no evidence to suggest that any anticoagulant rodenticides are mutagenic, but there are insufficient data available on individual compounds to demonstrate an absence of mutagenicity. Strain, sex and diet are important factors modifying the toxicity of anticoagulants in rodents.

Poisoning incidents in domestic animals after consumption of anticoagulant baits have been reported. Fatalities and severe clinical syndromes are generally due to the second-generation anticoagulants. The major difference between warfarin and the other anticoagulants (both indandiones and second-generation hydroxycoumarins) is that they have a longer retention time in the body and consequently a more prolonged effect than warfarin. Therefore in cases of poisoning, antidote treatment with vitamin K₁ needs to be continued for a longer period.

1.7 Effects on humans

Many poisoning incidents (both intentional and unintentional) have been reported. A few cases of intoxications from occupational exposure to anticoagulants have also occurred. Symptoms of acute intoxication by anticoagulant rodenticides range from increased bleeding tendency in minor or moderate poisoning to massive haemorrhage in more severe cases. The signs of poisoning develop with a delay of one to several days after absorption.

Warfarin is associated in humans with the induction of developmental malformations when taken as a therapeutic agent during pregnancy. No cases of developmental defects following the use of anticoagulants as rodenticides have been reported.
The plasma prothrombin concentration is one guide to the severity of intoxication. This is a more sensitive indication than overall tests such as prothrombin time. In repeated occupational exposure, direct measurement of either trace amounts of circulating descarboxyprothrombin or circulating vitamin K 2,3-epoxide may provide a more sensitive assessment.

Treatment of anticoagulant poisoning is graded according to the severity of intoxication. Specific pharmacological treatment consists of parenteral administration of vitamin K, with, in serious cases, co-administration of blood components. Measurement of prothrombin time helps to determine the effectiveness and required duration of treatment.

1.8 Effects on other organisms in the laboratory and field

The possible effects of anticoagulant rodenticides on non-target organisms can be considered to fall into two categories: primary (direct poisoning through consumption of bait) and secondary (through consumption of poisoned rodents).

In the form of the technical product, anticoagulants are highly toxic to fish. As bait formulations they are unlikely to present any hazard because of their low water solubility. For this reason, they will not be available to fish unless misused.

Bird species vary in their susceptibility to anticoagulant rodenticides. It is difficult to assess the risks to birds resulting from direct consumption because most published studies consist of toxicity trials in laboratory conditions. The attractiveness of whole grain bait to small birds increases the risk in field conditions.

Secondary toxicity laboratory studies with wildlife have shown that captive predators can be intoxicated by no-choice feeding with anticoagulant-poisoned or -dosed prey. Some deaths of predators in the field have been reported.

1.9 Evaluation and conclusion

Anticoagulant rodenticides disrupt the normal blood-clotting mechanisms, resulting in increased bleeding tendency and, eventually, profuse haemorrhage.

Unintentional exposure of the general population to anticoagulant rodenticides is unlikely.
Occupational contact is a potential source of significant exposure. It may occur during manufacture and formulation as well as during bait preparation and application.

Anticoagulant rodenticide compounds are readily absorbed from the gastrointestinal tract, and through the skin and respiratory system. The liver is the major organ for accumulation and storage. The plasma prothrombin concentration is a suitable guide to the severity of acute intoxication and to the effectiveness and required duration of the therapy.

The specific antidote is vitamin $K_1$.

The major difference between first- and second-generation anticoagulant rodenticides is that the latter have longer body retention and therefore tend to lead to a longer period of bleeding.

Most anticoagulants are stable under conditions of normal use. Their low water solubility and low concentration in baits make them unlikely to be a source of water contamination. They also appear to bind quickly to soil particles, with very slow desorption and no leaching properties.

Non-target organisms are potentially at risk from direct consumption of baits (primary hazard) and from eating poisoned rodents (secondary hazard).
2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1 Identity

Based on their chemical structure, anticoagulant rodenticides may be grouped into two categories:

- hydroxycoumarins:

\[
\text{HO-} \quad \begin{array}{c}
\text{O} \\
\text{C}
\end{array}
\]

- indandiones:

\[
\begin{array}{c}
\text{O} \\
\text{C}
\end{array} \\
\text{O}
\]

The common and chemical names of the rodenticides are given in Table 1. Trade names, chemical structures, RTECS and CAS numbers, molecular formulae and relative molecular masses are listed in Table 2.

2.2 Physical and chemical properties

Anticoagulant rodenticides are solids (crystalline or powders), slightly soluble in water (Table 3) and readily soluble in acetone. Most of them are stable under normal storage conditions.
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<tr>
<th>Common name</th>
<th>CAS name</th>
<th>IUPAC name</th>
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<td><strong>First generation hydroxycoumarins</strong></td>
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<td>Coumachlor</td>
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<td>3-[1-(4-chlorophenyl)-3-oxobutyl]-4-hydroxycoumarin</td>
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<td>Coumatetrayl</td>
<td>4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthalenyl)-2H-1-benzopyran-2-one</td>
<td>4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl) coumarin</td>
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<td>Warfarin</td>
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<td>3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxy-2H-1-benzopyran-2-one</td>
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<td>Difethialone</td>
<td>3-[3-(4-bromo-1,1'-biphenyl)-4-yl]-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzothiopyran-2-one</td>
<td>3-[1RS,3RS;1RS,3SR]-3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl-4-hydroxy-1-benzothi-in-2-one</td>
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<td>4-hydroxy-3-[1,2,3,4-tetrahydro-3-[4-[(trifluoromethyl)phenyl]methoxy]phenyl-1-naphthalenyl]-2H-1-benzopyran-2-one</td>
<td>4-hydroxy-3-[1,2,3,4-tetrahydro-3-[4-(4-trifluoromethylbenzyloxy)phenyl]-1-naphthyl] coumarin</td>
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<td><strong>Indandione derivatives</strong></td>
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<td>Zoocoumarin</td>
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Table 3. Water solubility and vapour pressure of various anticoagulant rodenticides

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<th>Solubility</th>
<th>Vapour pressure</th>
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<tr>
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<td>in water (mg/litre)</td>
<td>at temperature (°C)</td>
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<tr>
<td>Brodifacoum</td>
<td>&lt; 10</td>
<td>20</td>
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<tr>
<td>Bromadiolone</td>
<td>19</td>
<td>20</td>
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<td>Coumachlor</td>
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<td>Coumatetrayl</td>
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<td></td>
<td>425</td>
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<td>Difenacoum</td>
<td>&lt; 10</td>
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<td>Difethialone</td>
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<td>Flocoumafen</td>
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<td>Pindone</td>
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<td>25</td>
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<tr>
<td>Warfarin</td>
<td>practically insoluble</td>
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2.3 Analytical methods

Most of the procedures for the determination of anticoagulant rodenticides are based on high-performance liquid chromatography (Hunter, 1983; Hoogenboom & Rammell, 1983; Murphy et al., 1989; O'Bryan & Constable, 1991; Chalermchaikit et al., 1993; Kelly et al., 1993).

Warfarin is an acid which, in its hydrogenated form, is practically insoluble in distilled water. At neutral or higher pH, however, it is ionized and as such it readily dissolves in water. In addition, compounds contaminating the water (such as proteins or detergents) may substantially increase the solubility of warfarin.

Hunter (1983) developed a multi-residue method for the determination of warfarin, coumatetralyl, bromadiolone, difenacoum and brodifacoum in animal tissues by high-performance liquid chromatography with fluorescence detection. A chloroform-acetone (1:1) mixture was significantly better than chloroform for the extraction of residues of these rodenticides from liver tissues. Detection limits in animal tissues of 2 µg/kg for coumatetralyl, difenacoum and brodifacoum, 10 µg/kg for bromadiolone, and 20 µg/kg for warfarin could be routinely achieved.

Felice et al. (1991) developed a reversed-phase liquid chromatographic method with fluorescence detection for multicomponent determination of the above-mentioned five rodenticides in blood serum with detection limits of 10 to 20 ng/ml. Acetonitrile was used for the extraction.

Braselton et al. (1992) developed a special method for confirming the presence of indandione rodenticides (diphacinone and chlorophacinone) in intoxicated domestic animals by using mass spectrometry/mass spectrometry with collision-activated dissociation. More details of analytical methods for individual rodenticides are given in Table 4.
<table>
<thead>
<tr>
<th>Sample type</th>
<th>Extraction</th>
<th>Analytical method</th>
<th>Limit of detection</th>
<th>Rodenticide</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Animal tissues</td>
<td>Chloroform-acetone (1:1)</td>
<td>HPLC/FD</td>
<td>2 μg/kg</td>
<td>coumatetralyl, difenacoum, brodifacoum</td>
<td>Hunter (1983)</td>
</tr>
<tr>
<td></td>
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<td>10 μg/kg</td>
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<td>Hunter (1983)</td>
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<td></td>
<td>20 μg/kg</td>
<td>warfarin</td>
<td>Hunter (1983)</td>
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<tr>
<td>Animal tissues</td>
<td>Chloroform-acetone (1:1)</td>
<td>HPLC/FD</td>
<td>10 μg/kg</td>
<td>warfarin</td>
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<td></td>
<td>2 μg/kg</td>
<td>other rodenticides</td>
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<tr>
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<td>HPLC</td>
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<td>1 μg/litre</td>
<td>brodifacoum</td>
<td>Felice &amp; Murphy (1989)</td>
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Table 4 (contd).

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<th>Sample type</th>
<th>Extraction</th>
<th>Analytical method</th>
<th>Limit of detection</th>
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<td>Plasma</td>
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<td>O'Bryan &amp; Constable (1991)</td>
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<td>Liver tissue</td>
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<td>60 µg/kg</td>
<td>protocol did not differentiate between brodifacoum and bromadiolone</td>
<td>Ray et al. (1989)</td>
</tr>
</tbody>
</table>

* Post-column pH-switching fluorescence detection
3. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

3.1 Natural occurrence

Anticoagulant rodenticides do not occur naturally in the environment, although some plants do contain coumarinic derivatives. Huebner & Link (1941), Overman et al. (1944) and Alstad et al. (1985) described the anticoagulant properties of dicumarol found in spoiled sweet clover and in connection with haemorrhagic disease in cattle.

3.2 Anthropogenic sources

Anticoagulant rodenticides are used worldwide, but figures for the total world production are not available.

First-generation hydroxycoumarins were introduced as rodenticides in the late 1940s. The appearance of resistance to warfarin and other early anticoagulant rodenticides stimulated the development of second-generation anticoagulants. About 95% of all commensal rodent control in the USA is carried out with anticoagulants (Marsh, 1985a). More than 50% of rodenticides used by professional pest controllers in the USA contain brodifacoum (Dubock, 1986).

Depending on the toxicity of the rodenticide, the concentration of the active ingredient varies from 0.005 to 0.05% for indandiones and second-generation hydroxycoumarins and from 0.025 to 0.05% for first-generation anticoagulants.

Anticoagulant rodenticides are available in a variety of different formulations, including paraffin wax blocks, whole grain baits, pelleted baits and tracking powder (FAO, 1979). Baits are the most widely used formulations for rodent control.

Some manufacturers have added bittering agents, such as Bitrex (denatonium benzoate), to anticoagulant baits. According to Kaukeinen & Buckle (1992), adult humans found wax-block and pelleted placebo baits containing denatonium benzoate (10 mg/kg) to be unpalatable. However, the concentration of Bitrex cannot be increased to levels that would make baits unpalatable to target rodents, and there is no evidence that concentrations of Bitrex that target rodents readily accept will deter bait-eating by non-target animals or by children under 14 months of age.
4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION AND TRANSFORMATION

4.1 Transport and distribution between media

4.1.1 Air, water and soil

Since anticoagulant rodenticides are generally used as bait formulations and have low volatility, increased levels in the air are unlikely. As mentioned in section 2.2, most anticoagulants are slightly soluble in water and therefore their use is unlikely to be a source of water pollution.

Newby & White (1978) studied the adsorption and desorption of $^{14}$C-brodifacoum in soil under laboratory conditions. Adsorption coefficients ($k_d$) for course sand (pH 6.6), sandy clay loam (pH 7.1) and calcareous sandy loam (pH 7.6) were 625, 1320 and 1180, respectively, indicating strong adsorption to soil particles. Adsorption equilibria were established fairly rapidly with the large water:soil ratios used and despite very low brodifacoum water solubility. Desorption was reported to be very slow and much less than that required for a reversible interaction.

Lewis (1992b) applied $^{14}$C-difenacoum at 0.2 mg/kg (dry weight) to a sandy soil with low humous content. After 142 days of incubation (the approximate half-life of difenacoum in this soil type), two soil samples were transferred to the top of soil columns. The columns were eluted with deionized water at a rate and amount equivalent to approximately 200 mm of rain falling onto the soil surface area (91.6 cm$^2$) for 50 h. The percentages of applied radioactivity present in the leachates were 0.41 and 0.47%, representing only a very small amount of leaching under these test conditions.

The leaching characteristics of aged soil residues of $^{14}$C-brodifacoum in four soil types were investigated. $^{14}$C-Brodifacoum was applied to soil at a nominal application rate of 0.4 mg/kg and incubated under aerobic conditions for 30 days. Samples were taken and transferred to soil columns. After leaching, most of the radioactivity applied to the soil was recovered in the top segment of each column. No detectable levels of $^{14}$C residues were found in the leachates. The results indicated that $^{14}$C-brodifacoum was effectively immobile in all the soils tested (Jackson & Hall, 1992).
A study was carried out with $^{14}$C-bromadiolone in four types of soil. With a soil rich in clay and organic compounds, bromadiolone stayed in the superficial layer and scarcely moved. However, in soil poor in clay and organic compounds, 67% of the added bromadiolone was eluted (Spare et al., 1980).

4.1.2 Vegetation and wildlife

Since anticoagulant rodenticides are not intended for direct application to growing crops, no residues in plant food stuffs are expected. Unlike conventional crop protection products, which must be applied over relatively large crop areas, rodenticides are applied to discrete sites in the form of low concentration baits. Even if the bait is spilled, it will not be taken up by plants.

Small pellets and whole grain baits are highly attractive to birds and other non-target vertebrates. The formulation in wax blocks consequently decreases the risk of primary poisoning of non-target species.

Rodenticides may present a risk not only of primary poisoning (from direct consumption of the bait) but also of secondary poisoning (from consumption of poisoned rodents), in spite of the fact that many of the target rodents die below ground in their burrows (Gorenzel et al., 1982). Commensal and wild rodents poisoned by anticoagulants may lead to the death of cats, pigs, foxes and birds of prey. The risk of secondary poisoning depends mainly on the extent to which predators feed on the target animals (Dubock, 1986).

4.2 Transformation

4.2.1 Biodegradation

Coveney & Forbes (1987) studied the degradation of flocoumafen in rat carcasses, rat faeces, loose grain and wax block baits placed on small soil plots. Overall losses of flocoumafen ranged from 85% to 95% over the 12-month study. The majority of the rodenticide present in samples collected after 4 months was found in the upper 15 cm of the soil. Only very small quantities were found in the lower soil layers.

The degradation of $^{14}$C-difenacoum was studied in two standard soils under controlled conditions for a period of 108 days. Degradation time ($DT_{50}$) values for the two soils were 146 and
439 days, indicating that difenacoum is a relatively long-lived compound in soils (Lewis, 1992a).

Hall & Priestley (1992) monitored the metabolism of $^{14}$C-brodifacoum in soil under aerobic conditions after applying it at a nominal rate of 0.4 mg/kg and incubating for up to 52 weeks. A mean total of 35.8% of the applied radioactivity was recovered as $^{14}$CO$_2$ within the test period. $^{14}$C-Brodifacoum was the major radionuclide component in the soil extracts throughout the 52 weeks. Under the conditions of the study the half-life of brodifacoum was calculated to be 157 days.

A study was carried out with $^{14}$C-bromadiolone in four types of soil. The rodenticide was degraded significantly with half-lives ranging from 1.8 to 7.4 days (Wölk & Galicia, 1992).

4.2.2 Abiotic degradation

4.2.2.1 Photolysis

A photolysis study was carried out with $^{14}$C-bromadiolone (1 mg/litre) in a solution at pH 7.3 (Spare, 1982). The rodenticide was very quickly degraded by exposure to artificial sunlight with a half-life of 2.1 h.

The photolytic stability of $^{14}$C-difenacoum was investigated in sterile buffered aqueous solutions of pH 5, 7 and 9 over a 24-h irradiation period. The photolytic half-lives for total difenacoum were calculated to be 3.26, 8.05 and 7.32 h at pH 5, 7 and 9, respectively (Hall et al., 1992).

4.2.2.2 Hydrolysis

Lewis (1992c) studied the stability of $^{14}$C-difenacoum in sterile buffered aqueous solutions of pH 5, 7 and 9. No hydrolysis was observed at pH 5, at pH 7 there was very slow hydrolysis (half-life estimated to be 847–1332 days), and at pH 9 the half-life was estimated to be 77–85 days.

Jackson et al. (1991) studied the hydrolytic stability of $^{14}$C-brodifacoum (0.04 mg/kg) in sterile buffered aqueous solutions at pH 5, 7 and 9 over a 30-day period. The hydrolytic half-life of brodifacoum at pH 7 and 9 was found to be much greater than 30 days, but precise calculation was not possible because the degradation seen after one day did not continue.
Spare (1992) demonstrated that $^{14}$C-bromadiolone was slowly hydrolysed in pH 5 buffer, with an estimated half-life of 392 days. No degradation was observed at pH 7 and 9.

In the absence of a co-solvent, bromadiolone has a half-life of 67 days at pH 7 and 20 °C (Morin, 1988). Degradation is more significant in the presence of $\text{H}_3\text{O}^+$ ions, in saline water and at increased temperatures.
5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 Environmental levels

There is no information available on concentrations of anticoagulant rodenticides in air, water and soil.

Since anticoagulant rodenticides are not intended for direct application to growing crops, no residues in plants are expected.

Residues of difenacoum and brodifacoum were detected in the bodies of 15 out of a total of 145 dead barn owls (*Tyto alba*) received from various parts of the United Kingdom during the period 1983-1989. Levels of difenacoum were in the range of 0.005-0.106 mg/kg body weight, whilst levels of brodifacoum were in the range 0.019-0.515 mg/kg body weight (Newton et al., 1990).

Merson & Byers (1984) analysed eastern screech owl (*Otus asio*) pellets following the application of 0.001% brodifacoum to an orchard for rodent control. The brodifacoum residues in pellet samples ranged from 0.06 to 0.09 mg/kg, indicating some exposure of the birds. Hegdal & Colvin (1988) analysed screech owl tissues up to 52 days after application of brodifacoum in an orchard. Brodifacoum was detected in livers (detection limit = 0.3 mg/kg) from 9 out of 16 birds, the concentrations ranging from 0.3 to 0.8 mg/kg. No detectable residues were found in the remainder of the carcasses (detection limit = 0.1 mg/kg).

Hegdal & Blaskiewicz (1984) sampled six barn owls of different ages in the vicinity of farm buildings treated with brodifacoum. Analysis of carcasses revealed only one with trace (< 0.05 mg/kg) levels of brodifacoum; the other carcasses did not contain detectable concentrations.

Brodifacoum residues in the liver, muscle and fatty tissue of rabbits poisoned during field trials with bait containing 0.005% active ingredient were 4.4, 0.26 and 0.86 mg/kg, respectively. During the same field trials, brodifacoum residues in seven poisoned birds of various species ranged from 0.12 to 8.1 mg/kg in the liver, < 0.05 to 0.14 mg/kg in muscle and < 0.05 to 0.25 mg/kg in fatty tissue (Rammel et al., 1984).
5.2 General population exposure

As mentioned in the previous section, residues are unlikely to be found in plant foods. The use of dry baits to protect grain stores can result in contamination of the stored food. Although on average the concentration of residues would be expected to be low, occasional areas of high concentration can occur.

With respect to residues in animals used for human food (pigs, sheep and birds), there are no residue data concerning animals that have survived anticoagulant poisoning. It should be emphasized, however, that in some countries rodents are used as food.

Warfarin is widely used as a therapeutic agent.

5.3 Occupational exposure

Exposure may occur during manufacture, formulation and bait application. The available information is discussed in section 8.2.
6. MODE OF ACTION AND METABOLISM

6.1 Vitamin K and its antagonists

Vitamin K is a collective name for a number of related compounds, which all may function as co-enzymes for the enzyme γ-glutamate carboxylase. They all contain the functional naphthoquinone ring structure, but differ in their aliphatic side chains. Vitamin K₁ (phytomenadione) contains a side chain composed of four isoprenoid residues, one of which is unsaturated. The vitamin K₂ compounds (menaquinones) have side chains which vary from 1 to 13 isoprenoid residues, all of which are unsaturated. They are generally referred to as MK-n, where n is the number of isoprenoid residues. Vitamin K₃ (menadione) has no side chain, but upon ingestion it is converted into MK-4 by a liver enzyme. The two products commercially available for human use are K₁ and MK-4. Both are equally active, but for some reason K₁ is almost exclusively used in Europe and North America, whereas MK-4 (also known as menatetrenone) is used in Asia, notably Japan. K₃ is not used any more for humans because of its adverse side effect, haemolysis, but is frequently added to animal food.

Both 4-hydroxycoumarin derivatives and indandiones (also known as oral anticoagulants) are antagonists of vitamin K. Their use as rodenticides is based on the inhibition of the vitamin K-dependent step in the synthesis of a number of blood coagulation factors. The vitamin K-dependent proteins involved in the coagulation cascade (Fig. 1) are the procoagulant factors II (prothrombin), VII (proconvertin), IX (Christmas factor) and X (Stuart-Prower factor), and the coagulation-inhibiting proteins C and S. All these proteins are synthesized in the liver. Before they are released into the circulation the various precursor proteins undergo substantial (intracellular) post-translational modification. Vitamin K functions as a co-enzyme in one of these modifications, namely the carboxylation at well-defined positions of 10-12 glutamate residues into γ-carboxyglutamate (Gla). The presence of these Gla residues is essential for the procoagulant activity of the various coagulations factors. Vitamin K hydroquinone (KH₂) is the active co-enzyme, and its oxidation to vitamin K 2,3-epoxide (KO) provides the energy required for the carboxylation reaction. The epoxide is then recycled in two reduction steps mediated by the enzyme KO reductase (Fig. 2). The latter enzyme is the target enzyme for coumarin
**INTRINSIC**

- Contact

  - XII → XIIa
  - XI → XIa
  - IX → IXa
  - X → Xa

**EXTRINSIC**

- VII
  - VIIa
  - Tissue protease

- V
  - Va
  - Vi

**Pathways**

- PC → APC
- Fibrinogen → Fibrin

**Legend**

- PS = protein S
- PC = protein C
- APC = activated PC
- Va = activated factor V
- Vi = inactivated Va
The formation of clotting factors is dependent on the conversion of vitamin K hydroquinone into vitamin K₁ epoxide. The K₁ epoxide is then converted firstly to vitamin K₁ quinone by the enzyme vitamin K epoxide reductase and then back to vitamin K₁ hydroquinone by the enzyme vitamin K reductase. The hydroxycoumarin-related compounds inhibit these reductases and thereby disrupt the recycling of vitamin K₁ (Buckle, 1994).

The vitamin K cycle is operational both for vitamin K₁ and for the vitamin K₂ group (Buitenhuis et al., 1990). All available data indicate that KO reductase and K reductase are two activities exerted by the same enzyme.
anticoagulants. Their blocking of the KO reductase leads to a rapid exhaustion of the supply of KH₂, and thus to an effective prevention of the formation of Gla residues. This leads to an accumulation of non-carboxylated coagulation factor precursors in the liver. In some cases these precursors are processed further without being carboxylated, and (depending on the species) may appear in the circulation. At that stage the under-carboxylated proteins are designated as descarboxy coagulation factors (Stenflo et al., 1974; Nelsestuen et al., 1974). Normal coagulation factors circulate in the form of zymogens, which can only participate in the coagulation cascade after being activated by limited proteolytic degradation (see Fig. 1). Descarboxy coagulation factors have no procoagulant activity (i.e. they cannot be activated) and neither they can be converted into the active zymogens by vitamin K action. Whereas in anticoagulated humans high levels of circulating descarboxy coagulation factors are detectable, these levels are negligible in warfarin-treated rats and mice. Reviews by Vermeer (1990) and Furie & Furie (1990) give further details.

Leek & Park (1981) compared the effects of warfarin and brodifacoum on vitamin K metabolism and blood-clotting factor activity in warfarin-susceptible and warfarin-resistant rats. In warfarin-susceptible rats both brodifacoum and warfarin induced a significant increase in the circulating KO (measured as the KO/K ratio using ³H-vitamin K₁), indicating that KO reductase is the target enzyme for both drugs. However, whereas warfarin (1 mg/kg) only inhibited the KO reductase in the susceptible strain, brodifacoum (1 mg/kg) produced the same decrease of plasma prothrombin concentration in both warfarin-susceptible and warfarin-resistant animals.

The KO/K ratio in warfarin-resistant rats is five times higher than in warfarin-susceptible animals. This is explained by the fact that the hepatic KO reductase in the resistant animals has not only a reduced affinity for warfarin, but also for KO. Hence the vitamin K requirement of warfarin-resistant animals is 5–10 times higher than that of warfarin-susceptible ones. Second-generation anticoagulants, if given in doses which cause anticoagulation, further increase the KO/K ratio (Leck & Park, 1981).

The much stronger potency of difenacoum and brodifacoum, as vitamin K-antagonists, was reported by Park & Leek (1982), who concluded that in the case of poisoning with these second-generation anticoagulants it will be necessary to give repeated and frequent doses of vitamin K to maintain clotting factor synthesis.
The potency of second-generation anticoagulants can be partly explained by their highly lipophilic nature, which enables them to bind strongly to membranes. Their target enzyme KO reductase is an integral membrane protein with, in addition, a highly lipophilic nature. It is to be expected that the dissociation of enzyme/inhibitor complexes will be extremely slow. Moreover, their effectiveness in warfarin-resistant rats demonstrates that the mutation leading to warfarin resistance does not significantly affect their interaction with the KO reductase.

Vermeer & Soute (1992) compared the inhibition of each of the three enzymes from the vitamin K cycle by four anticoagulants (warfarin, flocoumafen, difenacoum and brodifacoum). The studies were performed using in vitro enzyme systems prepared from rat, cow and human liver. It was shown that in all three species the inhibitor concentration required for 50% inhibition (I_{50}) was comparable for the KO reductase and K reductase activity, but that the I_{50} for γ-glutamylcarboxylase was 2-3 orders of magnitude higher. It was concluded that for all four anticoagulants the reductions of KO and K are the target reactions for inhibition. Moreover, it was found that there is no species specificity of the inhibitors, which means that they are equally active in cell-free systems derived from rat, cow and human liver. Any species-dependent differences which might be found in vivo will presumably be brought about by a different pharmacokinetic or pharmacodynamic behaviour in these species.

6.2 Metabolism

6.2.1 Absorption, distribution and elimination

Anticoagulant rodenticides are easily absorbed through the gastrointestinal tract, skin and respiratory system.

After a single oral dose of ^14C-flocoumafen (0.14 mg/kg body weight) to rats, the absorption into blood was rapid, reaching maximum concentrations (0.03-0.05 μg/ml) in plasma within 4 h (Huckle et al., 1989).

The major route of elimination in rats and sheep after oral administration of anticoagulants is through the faeces. The intestinal levels of brodifacoum in rats began increasing 24 to 72 h after an oral dose of 0.2 mg/kg body weight (Bachmann & Sullivan, 1983). Faecal elimination of radiolabelled flocoumafen following an oral dose of 0.14 mg/kg body weight accounted for
23-26% of the dose over the 7-day period; approximately half of this was recovered within the first 24 h. Less than 0.5% of the dose appeared in the urine within 7 days (Huckle et al., 1989).

After single oral administration of brodifacoum (0.2 and 2 mg/kg body weight) to sheep, about 20% and 30%, respectively, was excreted in the faeces within 8 days (Laas et al., 1985).

A larger proportion of a percutaneous dose of ¹⁴C-flocoumafen (0.17 mg/kg body weight) dissolved in acetone was found in the urine of rats (10%) than in the case of an equivalent oral dose (less than 0.5%) over a 7-day period. Faecal elimination accounted for 31% of the percutaneous dose (Huckle & Warburton, 1986b).

After oral ¹⁴C-flocoumafen doses of 0.02 mg/kg body weight or 0.1 mg/kg body weight were given to rats, once weekly for up to 14 weeks, approximately one-third of each weekly low dose was eliminated through the faeces within 3 days, mostly within the first 24 h. At the higher dose the elimination ranged from 18% after the first dose to 59% after the tenth dose (Huckle et al., 1988).

Following repeated oral administration of ¹⁴C-flocoumafen to rats at 0.02 mg/kg body weight per week for 14 weeks or 0.1 mg/kg body weight per week for 10 weeks, appreciable accumulation was seen in the liver. At both dose levels tissue concentrations were highest in the liver, followed by the kidney > skin > muscle > fat > blood. The hepatic residue in the low-dose group ranged from 0.1 mg/kg tissue after one week to 2.1 mg/kg by week 14 (Huckle & Warburton, 1986a).

Brodifacoum could not be detected in the omental fat of sheep 8 days after the oral administration of 0.2 and 2 mg/kg body weight (Laas et al., 1985).

### 6.2.2 Metabolic transformation

Warfarin is readily hydroxylated in vitro and in vivo by rat liver microsomal enzymes to form 6-, 8- and, especially 7-hydroxy-warfarin (Ullrich & Staundinger, 1968; Ikeda et al., 1986a,b). These inactive metabolites are to some extent conjugated with glucuronic acid, undergo enterohepatic recirculation, and are excreted in the urine and faeces (Ellenhorn & Barceloux, 1988).
The metabolic pattern of indandiones in rats also mainly involves hydroxylation (Yu et al., 1982).

The second-generation anticoagulants have mainly been found as unchanged compounds (Bachmann & Sullivan, 1983; Huckle et al., 1988). The low urinary elimination following oral dosing has precluded accurate isolation of metabolites in urine (Warburton & Hutson, 1985; Waburton & Huckle, 1986; Huckle & Warburton, 1986a).

Following administration of flocoumafen, liver residues in rats consisted mainly of unchanged flocoumafen, although in a repeat dose study a polar metabolite was detected. Eight urinary metabolites were detected after percutaneous exposure to 14C-flocoumafen (Huckle & Warburton, 1986b).

Studies in male Japanese quail have shown more rapid metabolism and elimination than in the rat following an oral dose of 14C-flocoumafen. Up to 12 radioactive components were detected in the excreta (Huckle & Warburton, 1986c).

Bromadiolone, brodifacoum and coumatetralyl were also found in rats as unchanged parent compounds, whereas in the case of difenacoum metabolites predominated (Parmar et al., 1987). The metabolism and elimination of the difenacoum trans isomer was more rapid than for the cis isomer (Bratt, 1987).

The suggestion that the anticoagulant effect in rats is mediated by the unchanged compound itself rather than by its metabolites has been confirmed by the effects of phenobarbital and SKF525A pretreatments on the general pattern of responses to warfarin and brodifacoum (Bachmann & Sullivan, 1983).

### 6.2.3 Retention and turnover

Metabolic studies of anticoagulant rodenticides show that the liver is the main organ of accumulation and storage. Liver concentrations of brodifacoum after a single oral dose of 0.2 mg/kg body weight to rats remained high and relatively constant for 96 h, with a maximum of 5.0 mg/kg after 50 h (Bachmann & Sullivan, 1983).

A high degree of body retention was found 7 days after a single oral dose of 0.14 mg/kg body weight 14C-flocoumafen (74-76% of the administered dose); approximately half the dose was found in the liver (Huckle et al., 1989).
Brodifacoum was detected in the liver of sheep 128 days after oral administration (0.2 and 2 mg/kg body weight) in concentrations of 0.64 and 1.07 mg/kg dry weight (equivalent to 0.22 and 0.36 mg/kg wet weight), respectively. The peak levels occurred at 2 days in the high-dose group and at 8 days in the low-dose group, being 6.50 and 1.87 mg/kg dry weight (2.21 and 0.64 mg/kg wet weight), respectively (Laas et al., 1985). Woody et al. (1992) observed an elimination half-life for brodifacoum in serum of 6 ± 4 days in four dogs.

The largest proportion of a percutaneous flocoumafen dose of 0.17 mg/kg body weight was located in the liver (25% of the dose at a concentration of 0.8 mg/kg), although this was 10 times lower than that following an oral dose (Huckle & Warburton, 1986b).

Parmar et al. (1987) found that elimination of radiolabelled brodifacoum, bromadiolone and difenacoum from the liver was biphasic, consisting of a rapid initial phase lasting from days 2 to 8 after dosing and a slower terminal phase when the elimination half-lives were 130, 170 and 120 days, respectively. Elimination of coumatetralyl was more rapid, with a half-life of 55 days.

Similar results for difenacoum were found by Bratt (1987). After a single oral ¹⁴C-difenacoum dose of 1.2 mg/kg body weight, the highest concentration of radioactivity (41.5% of the dose) was found in the rat liver 24 h after dosing. The elimination from the liver was biphasic. The half-life of elimination of the radioactivity during the first rapid phase was three days, and for the slower phase was 118 days. A similar biphasic elimination was also apparent in the kidney. In the pancreas the concentration declined more slowly than in any of the other tissues (182 days). The parent compound was the major component in the liver 24 h after dosing (42%).

Unchanged flocoumafen comprised the major proportion of the hepatic radioactivity in rats and was eliminated with a half-life of 220 days (Huckle et al., 1989). Veenstra et al. (1991) found retention of about 8% of an administered flocoumafen dose of 0.4 mg/kg in the liver of beagle dogs 43 weeks after dosing.

Despite the more rapid metabolism of flocoumafen in Japanese quail, a proportion of the administered dose is retained in the liver, with an elimination half-life of 115 days after oral dosing (Huckle & Warburton, 1986c).
Six Hereford heifers weighing approximately 230 kg each were dosed with diphacinone (1 mg/kg body weight) by injecting it into the rumen. The highest residue level of parent compound found in the liver was 0.15 mg/kg at days 30 and 90 after treatment. No detectable levels (> 0.01 mg/kg) could be found in any of the other tissues analysed (kidney, plasma, brain, heart, muscle and fat). The residues in the liver were almost constant from 30 to 90 days post-treatment (Bullard et al., 1976).

The plasma half-life of brodifacoum determined in three patients with severe bleeding disorders was found to be approximately 16 to 36 days (Weitzel et al., 1990).

The half-life for disappearance from the plasma of human volunteers given a single oral or intravenous warfarin dose of 1.5 mg/kg body weight varied from 15 to 58 h, with a mean of 42 h (O'Reilly et al., 1963).
7. EFFECTS ON LABORATORY MAMMALS AND IN VITRO TEST SYSTEMS

7.1 Acute effects

7.1.1 Rodent species

Wide variations exist in the literature for LD50 values of anticoagulant rodenticides. A particular reason for these variables is the use of single or repeated (5-day) doses. LD50 values also vary according to the animal strain and sex, but both have not always been indicated in reported data (Ashton et al., 1987) (Tables 5 and 6).

Table 5. Acute oral LD50 values for various rodenticides in albino Norway rats

<table>
<thead>
<tr>
<th>Rodenticide</th>
<th>LD50 (mg/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brodifacoum</td>
<td>0.26</td>
<td>Redfern et al. (1976)</td>
</tr>
<tr>
<td>Bromadiolone</td>
<td>1.125</td>
<td>Grand (1976)</td>
</tr>
<tr>
<td>Chlorophacinone</td>
<td>20.5</td>
<td>Thomson (1988)</td>
</tr>
<tr>
<td>Coumachlor</td>
<td>900.0</td>
<td>Thomson (1988)</td>
</tr>
<tr>
<td>Coumaruril</td>
<td>0.4</td>
<td>Wiswesser (1976)</td>
</tr>
<tr>
<td>Coumatetralyl</td>
<td>16.5</td>
<td>Thomson (1988)</td>
</tr>
<tr>
<td>Difenacoum</td>
<td>1.8</td>
<td>Bull (1976)</td>
</tr>
<tr>
<td>Difethialone</td>
<td>0.56</td>
<td>Lechevin &amp; Grand (1987)</td>
</tr>
<tr>
<td>Diphacinone</td>
<td>3.0</td>
<td>Thomson (1988)</td>
</tr>
<tr>
<td>Flocoumafen</td>
<td>0.46</td>
<td>Sharples (1983a)</td>
</tr>
<tr>
<td>Pindone</td>
<td>50.0</td>
<td>Tomlin (1994)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>58.0</td>
<td>Thomson (1988)</td>
</tr>
</tbody>
</table>

Reported oral LD50 values for warfarin in rats vary by a considerable magnitude. Values of 11 mg/kg body weight (Lund, 1982), 58 mg/kg body weight (Thomson, 1988) and 58 mg/kg body weight and 323 mg/kg body weight for female and male, respectively (Hagan & Radomski, 1953), have been reported.
Table 6. Five-day oral LD<sub>50</sub> (mg/kg body weight per day) of various anticoagulants for the Norway rat (*Rattus norvegicus*)

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Strain&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Male</th>
<th>Female</th>
<th>Both sexes</th>
<th>Number of animals&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.21 (0.70-2.11)</td>
<td>1.60 (0.83-3.08)</td>
<td>1.34 (0.87-2.06)</td>
<td>40</td>
</tr>
<tr>
<td>Pindone</td>
<td>SD</td>
<td>wild 7.60 (2.61-22.2)</td>
<td>25.60 (5.34-123)</td>
<td>12.80 (1.73-84.8)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.29 (0.14-0.57)</td>
<td>0.38 (0.22-0.66)</td>
<td>0.33 (0.22-0.50)</td>
<td>40</td>
</tr>
<tr>
<td>Warfarin</td>
<td>SD</td>
<td>wild 0.39 (0.16-0.91)</td>
<td>0.60 (0.23-1.09)</td>
<td>0.44 (0.25-0.76)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.19 (0.11-0.33)</td>
<td>0.23 (0.12-0.43)</td>
<td>0.21 (0.14-0.31)</td>
<td>40</td>
</tr>
<tr>
<td>Diphacinone</td>
<td>SD</td>
<td>wild 0.39 (0.15-0.84)</td>
<td>0.60 (0.22-0.57)</td>
<td>0.44 (0.23-0.54)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.18 (0.18-0.18)</td>
<td>0.20 (0.15-0.27)</td>
<td>0.19 (0.16-0.22)</td>
<td>40</td>
</tr>
<tr>
<td>Chlorophacinone</td>
<td>SD</td>
<td>wild 0.13 (0.10-0.19)</td>
<td>0.23 (0.14-0.36)</td>
<td>0.16 (0.12-0.22)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.13 (0.10-0.19)</td>
<td>0.10 (0.08-0.13)</td>
<td>0.12 (0.10-0.15)</td>
<td>40</td>
</tr>
<tr>
<td>Bromadiolone</td>
<td>SD</td>
<td>wild 0.06 (0.03-0.12)</td>
<td>0.09 (0.09-0.09)</td>
<td>0.07 (0.05-0.10)</td>
<td>16</td>
</tr>
</tbody>
</table>

* Modified from Ashton et al. (1987); figures in parentheses represent 95% confidence limits
<sup>b</sup> SD = Sprague-Dawley
<sup>c</sup> 50% males and 50% females in all tests
The second-generation anticoagulants are more toxic than the first-generation ones in the sense that a single feeding may be lethal. Apparent discrepancies between the single oral LD$_{50}$ values for Norway rats, as shown in Table 5, and the multiple dose oral LD$_{50}$ values, shown in Table 6, can be explained by the cumulative effects resulting from multiple dose (5 day) administration (Table 6) and characteristics of this family of compounds.

Reported oral LD$_{50}$ values for mice show similar variations, from less than 1 mg/kg body weight for second-generation rodenticides to 374 mg/kg body weight for warfarin (Hagan & Radomski, 1953; Redfern et al., 1976; Bull, 1976; Sharples, 1983a).

Signs of anticoagulant poisoning in rats and mice include lethargy, hunched posture and vein clearing in the ears. Blood around the eyes, mouth and anus, indicating internal haemorrhaging, appears prior to death (Sharples, 1984).

The percutaneous toxicity of anticoagulants to rats varied for different compounds from an LD$_{50}$ of 0.54 mg/kg body weight for flocoumafen (master mix in corn oil) to > 50 mg/kg body weight for brodifacoum and difenacoum (Price, 1985b; Tomlin, 1994). The signs of intoxication were identical to those observed after an oral dose.

Second-generation anticoagulants appeared to be highly toxic by inhalation. An acute inhalation study in Wistar rats exposed (nose only) for 4 h was conducted using the 0.5% manufacturing master mix specifically prepared to give a mass median aerodynamic diameter of less than 5 μm. The acute LC$_{50}$ values were between 0.16 and 1.4 mg/litre. Signs of intoxication, characteristic of an anticoagulant action, were observed within 3 days after exposure with deaths occurring between 4 and 9 days (Blair, 1984).

7.1.2 Non-target species

The acute toxicity of various anticoagulant rodenticides to non-target mammalian species is presented in Table 7.

From the data presented it appears that some anticoagulants show a similar range of acute toxicity for both non-target mammals and target rodents.
### Table 7. Acute oral toxicity (LD$_{50}$, mg/kg) of various anticoagulant rodenticides for non-target mammalian species

<table>
<thead>
<tr>
<th>Rodenticide</th>
<th>Guinea-pig</th>
<th>Rabbit</th>
<th>Dog</th>
<th>Cat</th>
<th>Sheep</th>
<th>Pig</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brodifacoum</td>
<td>2.78 (F)</td>
<td>0.29 (M)</td>
<td>0.25-1 (F)</td>
<td>~25 (F)</td>
<td>&gt; 25 (M)</td>
<td>0.5-2</td>
<td>Hadler (1975a,b); Parkinson (1975, 1976); Godfrey (1984); Godfrey et al. (1985)</td>
</tr>
<tr>
<td>Bromadiolone</td>
<td>2.8</td>
<td>1.0</td>
<td>10 (F) MTD</td>
<td>&gt; 25 MTD</td>
<td></td>
<td>3</td>
<td>Grand (1976)</td>
</tr>
<tr>
<td>Chlorophacinone</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pelfrène (1991)</td>
</tr>
<tr>
<td>Difenacoum</td>
<td>50 (F)</td>
<td>2 (M)</td>
<td>~50</td>
<td>100</td>
<td>100</td>
<td>80-100</td>
<td>Bull (1976); Tomlin (1994)</td>
</tr>
<tr>
<td>Diphacinone</td>
<td>35</td>
<td></td>
<td>3-7.5</td>
<td>14.7</td>
<td></td>
<td>150</td>
<td>Kosmin &amp; Barlow (1976)</td>
</tr>
<tr>
<td>Difethialone</td>
<td>0.75</td>
<td>5 MTD</td>
<td>&gt; 16 MTD</td>
<td></td>
<td></td>
<td>2-3</td>
<td>Lechevin &amp; Grand (1987)</td>
</tr>
<tr>
<td>Flocoumafen</td>
<td>&gt; 10 (M)</td>
<td>0.7 (M/F)</td>
<td>0.075-0.25 (M/F)</td>
<td>&gt; 10 (M/F)</td>
<td>&gt; 5</td>
<td>~60 (M,F)</td>
<td>Sharples (1983b); Price (1985a); Chesterman et al. (1984); Roberts et al. (1985a, 1986)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>800</td>
<td>20-50</td>
<td>6-40</td>
<td></td>
<td></td>
<td>1-5</td>
<td>Anonymous (1976)</td>
</tr>
</tbody>
</table>

* F = female; M = male; MTD = maximum tolerated dose
7.2 Short-term exposure

7.2.1 Rodent species

In a study by Hadler (1974), groups of Wistar rats (male and female) were given brodifacoum by gavage (0.01, 0.02, 0.05, 0.1 and 0.2 mg/kg body weight) for 5 consecutive days. All rats receiving the two lowest doses survived the 21-day experimental period, but all rats given the two highest doses died within 11 days of cessation of dosing. No abnormalities were detected in surviving animals sacrificed at the end of the observation period. In animals which died during the study, only massive internal haemorrhages, mainly in the peritoneum, were observed. The no-observed-effect level of brodifacoum for Wistar rats was 0.02 mg/kg body weight per day.

Wistar rats (male and female) were continuously given a diet containing brodifacoum at 0.1 mg/kg body weight, with no choice of other feed, for 12 weeks. Prothrombin time was increased and mortality occurred in 9 out of 20 males and 5 out of 20 females. Macroscopic examination of the major organs of surviving rats revealed no abnormalities other than those expected of anticoagulant action (Hadler, 1976).

Feeding Fisher-344 rats with diets containing flocoumafen at concentrations of 0.2 or 0.4 mg/kg feed for 5 days produced no toxicologically significant effects during the 15-day observation period (Price, 1985c).

In a 28-day feeding study in rats, fed on a diet containing 0, 0.01, 0.05, 0.1 or 0.2 mg-flocoumafen/kg, there were no overt signs of toxicity. Highest-dose females showed a slight but significant increase in mean prothrombin time (PT) and activated partial thromboplastin time (PTT), and a slight decrease in mean plasma total protein. No toxicological or pathological changes were observed in rats fed diets containing 0.01 or 0.05 mg/kg of diet (Price, 1985d).

No effect on prothrombin time was observed in a 12-week study with Wistar rats administered oral flocoumafen doses (by gavage) of 0.0125, 0.0625 and 0.125 mg/kg body weight once a week (Forsey, 1985).

Groups of six male and six female Sprague-Dawley rats were fed diphacinone in their diets at concentrations of 0 (control),
0.0313, 0.0625, 0.125, 0.25 and 0.5 mg/kg diet (equivalent to 0, 1.7, 3.3, 6.4, 13 and 27 µg/kg body weight per day) for 90 days (Elias & Johns, 1981). Additional satellite groups of one rat of each sex and each dose group were killed for gross pathological examination at 30 and 60 days. Mortality was unaffected by the treatment. In the survivors of the main groups and satellite groups, there were no gross pathological changes, but in the two males that died prematurely (in the 0.0625 and 0.25 mg/kg groups) there was subdural haemorrhage. Prothrombin time was unaffected by treatment. Routine haematological and clinical chemistry tests were performed on only two rats of each sex from each group, and no effects were observed apart from a reduced blood fibrinogen concentration in both sexes at the highest dose level.

As no clear NOAEL was indicated by the results of the 90-day study, a 21-day study was performed using two rats of each sex at diphacinone levels of 0 (control), 0.125, 0.25, 0.5, 1, 2 and 4 mg/kg diet. All the rats in the 2 and 4 mg/kg groups died within the first 14 days, but all others survived until the end of the study. The mean dosages received by the survivors were equivalent to 0, 9, 17, 34 and 67 µg/kg body weight per day. At 21 days, there was no effect on prothrombin time. Gross necropsy revealed haemorrhage in the thymus of one rat in the 0.5 mg/kg group, but no effects were seen in any other survivors. All the animals in the 2 and 4 mg/kg groups had massive and extensive internal haemorrhage (Elias & Johns, 1981).

7.2.2 Non-target species

There are few data available on the repeated exposure of non-rodent species to anticoagulant rodenticides.

Death followed five daily warfarin doses of 3 mg/kg body weight in cats and 1 mg/kg body weight in pigs (Tomlin, 1994). The route of administration was not specified.

The dermal administration of warfarin (188 mg/kg per day) to female baboons caused profuse bleeding in 5 days followed by death (Dreyfus et al., 1983).

When it became known that vitamin K was not an antagonist for warfarin action in bone, it became possible to study the long-term effects of this anticoagulant in bones without the risk of inducing haemorrhages and bleeding. By feeding lambs with high doses of warfarin (up to 150 mg/kg body weight per day) under
the protection of 4 mg/kg body weight per day of vitamin K, Pastoureau et al. (1993) showed that within 3 months the lambs had developed a marked osteopenia that resulted from a decrease in resorption and a much more pronounced decrease in bone formation. As a result the bone density in the warfarin-treated animals was substantially lower than that in control animals. This is in agreement with earlier studies in rats (Price et al., 1982) where it was found that, under the protection of vitamin K, warfarin induced excessive mineralization and growth plate closure, due to which bone growth came to a halt.

The maximum tolerated 5-day oral dose of bromadiolone was considered to be 25 mg in pigs (Large White strain) weighing 25 kg. After 45 oral daily doses of 0.5 mg no change in the prothrombin time was observed (Grand, 1976).

Woody et al. (1992) studied the effect of a cumulative dose of 1.1 mg brodifacoum/kg body weight administered orally to dogs over a 3-day period. Signs of coagulopathic effects appeared within 24 h (greater PT and PTT) and increased over a 10-day period. Treatment with Vitamin K, at 10 days post-exposure reduced the effects.

Six horses were treated by gavage with brodifacoum containing bait at a dosage of 0.125 mg/kg body weight (Boermans et al., 1991). Four of the horses became anorexic and depressed, one requiring K, therapy. Peak plasma concentration occurred 2–3 h after administration. Pharmacokinetic evaluation indicated that brodifacoum has a plasma half-life of 1.22 days. An increase in clotting time was observed as early as 24 h after dosing, returning to the pre-treatment level by day 12.

Male pigs (five per group) were fed difenacoum in the diet for 14 days at dose levels of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/kg diet. With the exception of the lowest-dose group, all groups showed a marked increase of prothrombin time values. Extensive subcutaneous, inter- and intra-muscular haemorrhage and oedema was observed in animals dosed at levels of 0.5 mg/kg or more (Ross et al., 1979).

7.3 Long-term exposure

No data on long-term exposure are available.

Studies with second-generation anticoagulants are difficult to carry out for more than a few weeks due to the rapid acute
effects, and NOEL values for second-generation rodenticides and longer exposure periods have not been established. In any chronic study approaching two years in length, the dose level would have to be less than the analytical limit of detection.

### 7.4 Skin and eye irritation; sensitization

Brodifacoum is a slight skin irritant and a mild eye irritant in the rabbit (Hadler, 1975c). No skin or eye irritation was observed in New Zealand white rabbits treated with flocoumafen (Forsey, 1983a,b). Neither of these rodenticides was a skin sensitizer when tested in the guinea-pig maximization test (Parkinson, 1979; Price, 1986).

### 7.5 Reproductive toxicity and teratogenicity

Brodifacoum was given by oral gavage to female rats at daily dose levels of 0.001, 0.01 or 0.02 mg/kg body weight during days 6–15 of pregnancy. There was no evidence of adverse effects on the fetus at termination. Higher daily doses (above 0.05 mg/kg) caused an anticoagulant effect in the dams which resulted in a high incidence of abortion (Hodge et al., 1980a).

Pregnant female rabbits were given oral gavage doses of 0.001, 0.002 or 0.005 mg brodifacoum/kg body weight per day from days 6–18 of pregnancy. At the highest dose level a high proportion of maternal deaths occurred as a result of haemorrhage. Although the survivors showed signs of haemorrhage, there were no effects on the developing fetus. No effects were observed at either of the other dose levels used (Hodge et al., 1980b,c).

Bromadiolone was given orally to four groups of 25 female rats from day 6 to 15 of pregnancy at doses of 0, 17.5, 35 and 70 µg/kg body weight per day. Maternal toxicity occurred at the higher dose levels. There was no evidence of embryotoxicity or teratogenic effects at any dose level (Monnot et al., 1981). A similar absence of effects was reported in a study on rabbits treated orally with daily doses of either 2, 4 or 8 µg/kg body weight per day on days 6–18 of pregnancy, although there was maternal toxicity at the highest dose level (Virat, 1981).

Groups of 18 pregnant F-344 rats were given daily oral doses of 0, 0.01 or 0.04 mg flocoumafen/kg body weight from day 8 to 17 of gestation. Eight animals in the 0.04 mg/kg body weight group either died or were killed with signs of anticoagulant poisoning.
In contrast, Mirkova & Antov (1983) found warfarin to be embryotoxic and teratogenic to Wistar rats when administered by gavage in single doses or repeatedly throughout the periods of pre-implantation (1-7 days of gestation) and organogenesis (8-16 days), and also throughout the whole gestation (1-21 days) at a wide range of dose levels (0.04-8 mg/kg body weight). At these dose levels and treatment regimens, warfarin induced substantially increased rates of embryolethality, subcutaneous and internal haemorrhage and gross structural malformations (pes varus, internal hydrocephalus and anomalies of skeletal ossification).

7.6 Mutagenicity

Various in vitro and in vivo studies have been undertaken to assess the genotoxic potential of brodifacoum. No mutagenic activity was detected in the Salmonella reverse mutation assay in any of the five tester strains employed (TA98, TA100., TA1535, TA1537 and TA1538) either in the presence or absence of Arochlor 1254-induced rat liver S9 fraction at brodifacoum concentrations ranging from 1.6 to 5000 µg/plate (Callander, 1984). Brodifacoum showed no activity in a forward mutation assay using L5178 mouse lymphoma cells, either with or without metabolic activation, at concentrations of 47.5, 63.3 and 84.4 mg/litre (Cross & Clay, 1984).

Brodifacoum caused no significant chromosomal aberrations in cultured human lymphocytes (concentrations 1, 10, 100 and 1000 mg/litre), either with or without metabolic activation, and did not induce unscheduled DNA synthesis in cultured HeLa cells at the same range of concentrations (Mellano, 1984a,b). Difenacoum did not induce unscheduled DNA synthesis in rat hepatocytes in vivo at either dose level or time-point (Kennelly, 1990).

An in vivo micronucleus test, in which mice were given single brodifacoum intraperitoneal doses of 0.187 or 0.30 mg/kg body weight, showed no induction of micronuclei in bone marrow polychromatric erythrocytes (Sheldon et al., 1984).

Bromadiolone was tested in the Salmonella reverse mutation assay at concentrations ranging from 10 to 3330 µg per plate on strains TA1535, TA1537 and TA1538. No evidence of mutagenic effect was found either with or without Aroclor metabolic activation (Lawlor, 1992).
Bromadiolone did not induce forward mutations in Chinese hamster ovary cells either with or without metabolic activation (Cifone, 1993).

In a mouse micronucleus test at four dose levels from 50 to 400 mg/kg, bromadiolone did not induce micronuclei in bone marrow polychromatic erythrocytes (Murli, 1993).

Flocoumafen did not induce reverse gene mutation in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538 nor in Escherichia coli WP2uvrA pkm 101 either with or without metabolic activation. Flocoumafen was tested at concentrations ranging from 31 to 2000 μg/plate, beyond which precipitation from suspension occurred (Brooks et al., 1984).

Flocoumafen did not increase the frequency of mutation to 6-thioguanine resistance in Chinese hamster V79 cells either in the presence or absence of an Arochlor-induced rat liver S9 fraction. Doses ranging from 5 to 150 mg/litre were used, beyond which cytotoxicity occurred (Clare & Wiggins, 1986).

Flocoumafen did not induce in vitro cell transformation in C3H10T1/2 mouse fibroblasts, either in the presence or absence of a rat S9 metabolizing system, at concentrations ranging from 12.5 to 100 μg/litre (Meyer & Wiggins, 1986).

Flocoumafen did not induce mitotic gene conversion in liquid suspension cultures of Saccharomyces cerevisiae JD1, either in the presence or absence of a rat liver S9 fraction, at concentrations ranging from 0.01 to 2 g/litre (Brooks et al., 1984).

When incubated at concentrations ranging from 5 to 25 mg/litre for 24 h in monolayer cultures of rat liver RL4 cells, flocoumafen did not induce in vitro chromosomal damage (Brooks et al., 1984).

Oral administration of flocoumafen to rats at doses of 0.25 mg/kg or 1000 mg/kg body weight (a dose 4000 times the acute oral LD₅₀ for rats) did not produce chromosomal damage (Allen et al., 1986).

The cytogenic effect of chlorophacinone was investigated in vivo in metaphase bone marrow cells taken at 48 and 96 h after oral dosing of male CFLP mice with 20 mg/kg body weight. No induction of chromosomal aberrations was observed (Nehez et al., 1985).
In the same study, chlorophacinone was investigated in spermatocytes taken from male CFLP mice at 1, 2, 3 and 4 weeks after a single oral dose of 20 mg/kg body weight. The spermatocytes were analysed in the diakinesis phase of meiosis. There was no increased incidence of chromosomal aberrations (Nehez et al., 1985).

Rabbits were treated orally with 20 mg chlorophacinone/kg body weight and chromosomal analysis was performed in bone marrow and spermatocytes taken at 48 h post-dosing. No increase in the incidence of chromosomal aberrations was seen (Selypes et al., 1984).

7.7 Factors modifying toxicity

Phenobarbital pretreatment of rats followed by a single administration of brodifacoum or warfarin decreased the anticoagulant effects of both compounds, more markedly in the case of warfarin (Bachmann & Sullivan, 1983).

The non-steroid anti-inflammatory drugs ibuprofen and phenylbutazone potentiated the anticoagulant effects of brodifacoum and bromadiolone in rats (Sridhara & Krishnamurthy, 1992).

Strain and sex are important factors modifying the toxicity of anticoagulants in rodents (see Table 5, section 7.1.1). Winn et al. (1987) observed greater sensitivity of male rats and mice to difenacoum, compared with female rats and mice. The possible explanation of different responses to difenacoum was the greater turnover of plasma proteins in male rats or the marked inter-individual variation of vitamin K₁ level in male rat liver.

Large amounts (relative to farm animals’ dietary requirements) of vitamin K₃ (menadione and its salts) are sometimes added to animal feedstuffs. This gives rodent pests ready access to a substance which acts as an antidote to anticoagulant rodenticides, and thus can reduce the efficacy of these rodenticides.

Differences in metabolism to inhibitors of vitamin K synthesis among strains of mice and rats has been attributed to a number of different factors (Misenheimer et al., 1994). Among these are: 1) reduced sensitivity of vitamin K epoxide hydrolase to inhibition; 2) greater reversibility of inhibition of epoxide hydrolase; and 3) faster clearance of the rodenticide. The mechanism of resistance differs between strains.
A single flocoumafen dose of 0.5 mg/kg body weight resulted in clear signs of anticoagulation in five out of eight beagle dogs (Veenstra et al., 1991). Administration of a second dose 5 weeks later resulted in clinical evidence of anticoagulation in two out of the three remaining dogs. Vitamin K treatment (2 to 5 mg/kg subcutaneously) reversed the effects in all cases.

### 7.8 Adverse effects in domestic and farm animals

#### 7.8.1 Domestic animals

**7.8.1.1 Poisoning incidents**

The main cause for accidental poisoning of domestic animals is direct consumption of anticoagulant baits. Secondary poisoning through the consumption of rats and mice killed with anticoagulants may occur in dogs and cats in urban situations, but is more likely in farm situations (Marsh, 1985b). The majority of fatalities and severe clinical syndromes are connected with the second-generation anticoagulants (Dodds & Frantz, 1984). Du Vall et al. (1989) studied 10 cases of second-generation anticoagulant rodenticide poisoning in dogs and cats. The presence of anticoagulants (brodifacoum, bromadiolone or diphacinone) in serum or liver was confirmed by HPLC or GC/MS. Several other cases of brodifacoum poisonings in dogs have been reported and some of them were fatal (Mc Sporran & Phillips, 1983; Stowe et al., 1983; Dodds & Frantz, 1984).

Until 1983, only first-generation anticoagulants were available in New Zealand and, between 1977 and 1983, warfarin, coumatetralyl and diphacinone were involved in 45 reported incidents of accidental poisoning of domestic and farm animals. Brodifacoum and flocoumafen were introduced after 1983. From 1983 to 1994, 40 cases of poisoning of domestic and farm animals resulted from the use of second-generation compounds and 17 cases were due to the ingestion of first-generation anticoagulants (Hoogenbroom, 1994).

Schulman et al. (1986) reported five cases of coagulopathy in dogs caused by consumption of diphacinone-containing baits. Lethargy and respiratory distress, associated with pulmonary interstitial haemorrhage and pleural and/or pericardial effusion, were the most consistent signs. The prothrombin time and the activated partial thromboplastin time were moderately to markedly prolonged.
Effects on Laboratory Mammals and In Vitro Test Systems

7.8.1.2 Diagnosis and treatment of poisoning

The major difference between warfarin and the other anticoagulant rodenticides is that the latter have longer body retention and a tendency to induce bleeding for a longer period of time. It is therefore necessary to continue the treatment for weeks rather than days.

Signs of poisoning occur after a latent period of 12 h to several days and may include:

- bruising easily with occasional nose or gum bleeds
- blood in stools or urine
- excessive bleeding from minor cuts or abrasions
- laboured breathing
- pale mouth and cold gums
- anorexia and general weakness

A reliable indication of an anticoagulant effect is the determination of prothrombin time.

Vitamin K₁ (phytomenadione) is the antidote of choice. The recommended dosage is 2–5 mg/kg body weight in dogs. Intravenous injection is the quickest route but it is not recommended because of reported anaphylaxis. Vitamin K₁ is much safer if given subcutaneously for the initial 2–3 treatments. A dose of 0.25 to 2.5 mg/kg body weight is recommended for use in small animals (Mount et al., 1982). The recommended period of treatment with vitamin K₁ is 3–6 weeks (Braithwaite, 1982; Mackintosh et al., 1988).

Mount & Feldman (1983) observed that therapy effective for warfarin poisoning was ineffective against diphacinone toxicosis due to inadequate vitamin K₁ dosage and duration of therapy. Oral therapy was ineffective.

In a study on dogs, Mount & Kas (1989) found that the serum concentration of vitamin K epoxide was significantly higher in diphacinone-treated dogs than in controls, the difference being significant as early as 1–4 h after vitamin K application. The authors suggested that this phenomenon could be used as diagnostic information.

In cases of severe blood loss, fresh plasma should be infused every 6 h to the extent of 5–10% of total blood volume, assuming
this to be 90 ml/kg in the dog and 70 ml/kg in the cat (Mount et al., 1982).

Vitamin K₁ (phytomenadione) is the antidote for treating dogs exposed to anticoagulant rodenticides. The therapeutic dosages and duration of treatment needed vary with the compound involved. Mount & Feldman (1983) reported that dogs poisoned with diphacinone require treatments for at least three weeks, while treatment for 4-6 days is sufficient for treating warfarin poisonings. Initial treatments are by subcutaneous injection, subsequent therapy being given orally.

For animals exposed to the second-generation rodenticides brodifacoum, bromadiolone, difenacoum and flocoumafen, the treatment regimen that has been recommended includes one or more injections of vitamin K₁ at 2-5 mg/kg body weight until prothrombin times return to normal levels. Once this has been achieved, daily oral doses of vitamin K₁ are recommended for a period of 3-4 weeks. Oral doses of 2-5 mg/kg body weight are recommended initially, with some reduction being possible over time if the animal is observed closely for signs of recurrence of symptoms. If prothrombin times increase following withdrawal of vitamin K₁, the oral dosing should be resumed for 2-3 weeks. If blood loss was severe, transfusions (10-15 ml/kg body weight) with fresh whole blood may be needed (Anonymous, 1988).

7.8.2 Farm animals

Feinsod et al. (1986) reported an outbreak of abortions and haemorrhages in sheep and goats in Egypt caused by brodifacoum intoxication. Clinical signs appeared within 3-7 days after exposure to the rodenticide and included epistaxis, haematochezia, recumbency, subcutaneous haemorrhage, flank ecchymosis, lameness, abdominal bloating and abortion of various stages of gestation. The signs resembled epidemic Rift valley fever, but there was no fever, icterus or loss of appetite in affected animals.
8. EFFECTS ON HUMANS

8.1 General population exposure

Incidents of human exposures to rodenticides are reported to poison control centres in countries where such facilities exist. In 1988, for example, the American Association of Poison Control Centers (AAPCC) received accounts of 10,626 cases of human exposures to rodenticides. These incidents represented 17% of reported exposures involving pesticides and 0.8% of the total number of cases reported in the AAPCC system. The rodenticide incidents included 4,190 cases involving "anticoagulants" (principally warfarin) and 5,133 involving "long-acting anticoagulants" (second-generation anticoagulants plus the indandione compounds). More than 95% of the rodenticide cases were classified as "accidental". Most of the remainder were classified as "intentional" and included attempted suicides. Of the 10,540 rodenticide incidents for which the ages of victims were reported, 9,406 (89%) involved children under 6 years of age (Litovitz et al., 1994).

Victims in nearly 32% of the rodenticide exposure incidents reported to the AAPCC in 1988 were treated in health care facilities. However, the medical outcome "none" was reported in more than 93% of the 5,708 incidents for which information regarding outcomes was reported. The remaining 380 cases included 333 with "minor" medical effects, 41 with "moderate" effects, 4 with "major" effects, and two deaths (Litovitz et al., 1994).

In 1993, the Swedish Poison Information Centre received 338 enquiries concerning exposures to anticoagulant rodenticides. This number represented 0.6% of all enquiries to the centre and 37% of the enquiries concerning pesticides. Of the anticoagulant rodenticide enquiries, 202 pertained to warfarin and 136 to "superwarfarin" compounds (Persson, 1994).

Human exposure to second-generation and indandione anticoagulants produces symptoms consistent with anticoagulation effects (e.g., haematomas, haematemesis, haematuria, easy bruisability). Treatment of cases of exposure, particularly of substantial and repeated exposure, may require vitamin K₁ therapy and monitoring of prothrombin times for periods of many months (Rauch et al., 1994).
Suicide and/or unintentional poisonings with anticoagulant rodenticides have occurred in many countries. Thus, Ungvary (1994) reported 70 cases, mostly involving children, that occurred in Hungary between 1988 and 1993.

Warfarin is widely used as a therapeutic and preventive agent in the treatment of thromboembolic disease. Patients have been maintained for years on this treatment with control of the prothrombin level, which should be kept between 10 and 30% of normal.

Diphacinone has also been used as a drug because of its long-lasting action (the half-life in humans is 15–20 days). It ceased to be listed in the American Medical Association Drug Evaluations, (AMA, 1980) because of its structural relation to phenindion, which had been reported to have adverse effects.

8.1.1 Acute poisoning

Typical features of poisoning result from increased bleeding tendency and include:

- minor poisoning: coagulation disturbance detected only by laboratory analyses;

- moderate poisoning: coagulation disturbance resulting in haematomata, haematuria, blood in faeces or excessive bleeding from minor cuts or abrasions, gum bleeding;

- severe poisoning: retroperitoneal haemorrhage, severe gastrointestinal bleeding, cerebrovascular accidents, massive haemorrhage (internal bleeding) resulting in shock.

If anaemia or liver disease is present then the above features may be more severe and persistent and the poisoning may be more difficult to control (Anonymous, 1988).

The onset of the signs of poisoning may not be evident until a few days after ingestion.

8.1.2 Poisoning incidents

Cases of human poisoning with “superwarfarins” were reviewed by Katona & Wason (1989).
Fourteen members of a family in the Republic of Korea were poisoned by eating warfarin-containing maize meal. The first symptoms appeared 7-10 days after the beginning of exposure and were followed by massive bruises or haematomata on the buttocks in all cases (Lange & Terveer, 1954).

Pribilla (1966) reported a total dose of about 1000 mg of warfarin to be fatal after 13 days of consumption.

Out of a total of 741 infants, 177 died after the use of warfarin-contaminated talc in Viet Nam. The concentrations of warfarin in the powder varied from 1.7 to 6.5% (Martin-Bouyer et al., 1983).

A 73-year-old woman suffered from recurrent episodes of hypoprothrombinaemia. Clotting tests and further investigation showed that this was due to a warfarin rodenticide intentionally mixed in the woman's cough syrup by her daughter-in-law. As the patient had as many as seven relapses, it was possible to compare different types of therapy. Menadione had no effect (Nilsson, 1957).

Several suicidal attempts with chlorophacinone have been reported. Murdoch (1983) reported a case of ingestion of 625 mg chlorophacinone (250 ml of a 0.25% concentrate formulation) by a 37-year-old woman. The prolonged anticoagulant action of chlorophacinone persisted for at least 45 days even though treatment was given. It was found that menadiol, the synthetic analogue of vitamin K₁, was ineffective. The natural form, phytomenadione, was effective only when given at high dosage (20 mg daily) 30 days after the ingestion of chlorophacinone.

In a case reported by Dusein et al. (1984), the amount of ingested chlorophacinone was unknown. After adequate therapy, the prothrombin level became normal within 4 weeks.

Vogel et al. (1988) reported the case of an 18-year-old woman hospitalized 3 days after ingesting approximately 100 mg chlorophacinone. Under high-dose vitamin K₃ therapy (160 mg) the prothrombin time was normalized, but it increased again following withdrawal of vitamin K₃. After prolonged vitamin K₃ administration, the prothrombin time finally became normal after 7 weeks.

Brodifacoum poisoning has occurred in South Sumatra, Indonesia. Some of the villagers used a 0.005% brodifacoum rice
grain bait as a food source even though they knew it was poisonous and unfit for human consumption. They attempted to remove the rodenticide by repeated washing, rinsing and cooking before eating the rice. Because of the delay in the appearance of poisoning symptoms it appeared that they had been successful, thus encouraging further attempts to purify the rice baits. As a result, deaths occurred before appropriate remedial treatment could be initiated (Anonymous, 1985).

Jones et al. (1984) reported the first case of human brodifacoum poisoning in a 17-year-old boy who attempted suicide by ingesting approximately 7.5 mg (0.12 mg/kg) of brodifacoum in Canada. He was initially seen with gross haematuria, followed by epistaxis and gum bleeding. The prothrombin time and the activated partial thromboplastin time were notably prolonged. He was treated for 56 days with either parenteral or oral vitamin K, and either fresh or stored plasma until coagulation values remained normal and stable.

Lipton & Klass (1984) reported a similar case in a 31-year-old mentally disturbed woman who ingested over a 2-day period approximately thirty 50-g packages of Talon-G (approximately 75 mg of brodifacoum). Prothrombin time and activated partial thromboplastin time were considerably prolonged (respectively 6-fold and 4-fold above normal values). After 4 days of therapy with high doses of vitamin K, (up to 125 mg/day), partial correction in the prothrombin time occurred. Vitamin K, therapy continued with interruptions for 8 months until normal prothrombin time levels were found.

Chong et al. (1986) reported a case of suicidal poisoning after ingestion of 10 mg brodifacoum (as 0.05% Klerat). The coagulation test became normal after large doses and prolonged use of vitamin K, over 6 months.

A case of intentional ingestion of brodifacoum (200 g of Talon G, 0.005% brodifacoum) was reported by Hoffman et al. (1988). A profound decrease in the levels of factors II, VII, IX and X, lasting 43 days after ingestion, was observed. Treatment with subcutaneous vitamin K, in doses up to 100 mg per day was effective.

Weitzel et al. (1990) described three patients with severe bleeding disorders due to deficiency of the vitamin K-dependent blood clotting proteins after ingestion of an anticoagulant.
Although the patients denied any ingestion, brodifacoum was detected in their serum at concentrations of 7.6 nmol/litre, 270.7 nmol/litre and 2759 nmol/litre, respectively. The anticoagulant effect was found to persist long after brodifacoum was no longer detectable in the serum. A half-life of approximately 16-36 days was determined for brodifacoum in the plasma.

Kruse & Carlson (1992) reported the case of a 25-year-old man who attempted suicide by consuming a brodifacoum rodenticide. He developed a severe coagulopathy that was treated with vitamin K_1 and fresh frozen plasma and he was discharged from hospital with oral phytomenadione. Fifteen weeks later the man presented again with a history of further brodifacoum ingestion. He suddenly became comatose and computer tomography revealed a subarachnoid haemorrhage that led to brain death 24 h later.

Wallace et al. (1990) described the clinical course of a patient poisoned with brodifacoum in a suicide attempt. He developed microhaematuria and melaena. His clotting factors were depressed and were poorly responsive to vitamin K treatment.

Barlow et al. (1982) reported a case of attempted suicide with 25 mg of difenacoum (500 g of rat bait) followed several months later by 1800 g of rat bait. The patient was treated with vitamin K_1 (phytomenadione) for 48 and 42 days, respectively, until the pharmacological effect of difenacoum ceased.

Nighoghossian et al. (1990) reported an unusual coagulopathy after accidental exposure to a diphenacoum rodenticide. A 59-year-old man developed subacute tetraparesis following severe sudden neck pain, which on clinical examination was shown to be due to a subdural cervical haematoma. Prothrombin complex activity was low and diphenacoum was present in the plasma. Specific medical management led to a complete recovery.

Greeff et al. (1987) reported accidental bromadiolone poisoning in two children, resulting in prolonged anticoagulation. Descarboxyprothrombin levels were increased in both cases by 27% and 29.9%, respectively (normal, non-detectable level). The first child rapidly recovered after treatment with high-dose intravenous factor IX-prothrombin complex and vitamin K_1. The clotting profile became normal on the third day after admission. The second child gave a poor response to 10 mg intravenous vitamin K_1, and the dose was increased to 20 mg.
8.1.3 Controlled human studies

Single oral doses of 60, 70, 80 or 120 mg warfarin decreased the prothrombin concentrations in volunteers to zero by the third day. After the administration of 50 mg vitamin K\textsubscript{1}, the prothrombin concentrations returned by the sixth day to 60, 70, 55 and 63\%, respectively, of the normal value (Anonymous, 1965).

When a single oral dose of 20 mg chlorophacinone was given to three volunteers, the lowest prothrombin times were 35, 34 and 38\% of the pretreatment value on days 2, 4 and 2, respectively. Eight days after administration without any treatment the values were 80, 100 and 90\%, respectively (Anonymous, 1965).

8.2 Monitoring of biological effects

8.2.1 Effects of short- and long-term exposure

Two cases of occupational exposure to brodifacoum and difenacoum were reported by Park et al. (1986). The exposure was of a chronic nature (2 and 4 years, respectively). Plasma analysis in the first patient revealed the presence of both difenacoum and brodifacoum in the range of 30-50 $\mu$g/litre. In both patients unexpectedly high concentrations of vitamin K\textsubscript{1}, 2,3-epoxide were found in the presence of normal clotting factor activities and antigen levels suggesting the presence of coumarin anticoagulants in the liver.

A case of poisoning in a 23-year-old man resulting from prolonged skin contact during the process of preparing and distributing warfarin baits has been reported (Fristedt & Sterner, 1965).

8.2.2 Epidemiological studies

During a production run preparing ready-to-use flocoumafen bait (0.005\% in baits) in a formulation plant, the effect of the rodenticide on blood coagulation factors was monitored in 12 subjects, using the classical prothrombin time test, a modified prothrombin time technique and measurement of prothrombin (factor II) concentration in blood. No adverse health effects were observed in any subject involved in formulation operations. No changes were observed in any of the three tests that could be ascribed to absorption of flocoumafen into the body (Tuinman & Van Sittert, 1986).
8.3 Developmental effects

Developmental effects have been reported when anticoagulants, particularly warfarin, have been administered as therapeutic agent during pregnancy. Hall et al. (1980) reviewed 418 reported pregnancies in which coumarin and indan-1,3-dione derivatives were used, and found that one-sixth resulted in abnormal liveborn infants, one-sixth in abortion or stillbirth, and two-thirds in apparently normal infants.

According to Hall (1976), there are two types of defects associated with anticoagulants, dependent upon the time of administration during pregnancy. The first, a characteristic embryopathy described by the terms "warfarin embryopathy" or "fetal warfarin syndrome", occurs from early, first-trimester use. Fetal wastage and other abnormalities, especially central nervous system anomalies, result from treatment later during gestation (usually the second or third trimesters). Clotting factors are not present in first-trimester embryos and this may explain the differences in fetal abnormalities.

The most consistent feature of warfarin embryopathy is nasal hypoplasia. Choanal stenosis has been observed, and respiratory difficulty is typical because of the narrowed nasal passages (Smith & Cameron, 1979; Pauli & Hall, 1979).

The other common feature is bone abnormalities of the axial and appendicular skeleton. Laryngeal and tracheal-thyroid calcification has also been noted (Schardein, 1985).

Other non-skeletal abnormalities reported include ophthalmological malformations of several types, including defects leading to blindness, developmental delay, low birth weight (premature birth), mental retardation, hypotonia and ear anomalies (Carson & Reid, 1976; Schardein, 1985).

No cases of embryopathy from anticoagulants in their use as rodenticides have been reported.

8.4 Other adverse effects

One of the more common side-effects of coumarin therapy is skin necrosis (Brooks & Blais, 1991; Eby, 1993; Locht & Lindström, 1993). Although this effect has been associated with a number of different agents, it is commonly referred to as
warfarin-induced skin necrosis (WISN). The frequency of this effect has been estimated to be between 1 in 100 and 1 in 10,000, with the majority of cases occurring in women. Symptoms of WISN typically appear 3–6 days after the initiation of warfarin therapy, and the areas of skin involved are most frequently areas with subcutaneous fat, particularly the breasts, thighs, and buttocks. There is some evidence that deficiencies in vitamin K-dependent anticoagulant proteins (protein C and protein S) may underlie the susceptibilities of at least some individuals to WISN (Anderson et al., 1992). The occurrence of WISN may be avoided in at least some cases by the co-administration of heparin.

Warfarin therapy has been noted to result in increases in the incidence of haematomas, including intraspinal epidural haematoma (Murphy & Nye, 1992), liver haematoma (Erichsen et al., 1993), and intramural haematoma of the small intestine (Avent et al., 1992).

Bone contains three vitamin-K-dependent proteins, i.e. osteocalcin, matrix Gla-protein and protein S. It has been shown that oral anticoagulants (phenprocoumon, acenocoumarol) reduce both the plasma antigen level as well as the Gla content of osteocalcin (Van Haarlem et al., 1988). Furthermore it was found that a poor vitamin K status was associated with a high urinary calcium loss (Knapen et al., 1993). These data are consistent with the observation that the bone mass in patients on long-term anticoagulant treatment was significantly lower than in a group of age- and sex-matched controls (Fiore et al., 1990; Resch et al., 1991). Vitamin-K-dependent proteins have also been identified in calcified atherosclerotic plaques, where they were suggested to contribute to the prevention of vascular calcification (Gijsbers et al., 1990). In this respect it is remarkable that warfarin treatment has been shown to increase rather than to reduce atherosclerosis in an animal model (Antov et al., 1985). Vitamin-K-dependent proteins not related to blood coagulation are still discovered regularly (Manfioletti et al., 1993), and it is important to remain alert for unexpected side-effects of vitamin K antagonists, notably after long-term exposure.

8.5 Methods for assessing absorption and effects of anticoagulant rodenticides

The laboratory control of orally administered coumarin derivatives has been carried out using the classical one-stage prothrombin time test (Quick, 1935) or modified techniques such
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as “Thrombotest” (Owren, 1959). However, these tests have been designed for clinical monitoring of circulating clotting factors during anticoagulant therapy. The monitoring of occupational exposure to rodenticides requires the prothrombin time test to be of sufficient sensitivity to measure changes in the normal range (Tuinman & Van Sittert, 1985).

Repeated occupational exposure to low levels of anticoagulant rodenticides could gradually deplete vitamin-K-dependent coagulation factors in the blood. To detect unwanted exposure of humans in an early state, a careful screening of those at risk is recommended. The question is which screening method is the most suitable to monitor low levels of rodenticide ingestion. Prothrombin time and related tests are “overall” clotting tests, which were developed for monitoring patients under deep anticoagulation. These tests are easy to perform and do not require complicated equipment, but they are relatively insensitive when used for monitoring milder anticoagulation states (Tuinman & Van Sittert, 1985; Ross et al., 1992; Travis et al., 1993). If possible, specific and more sensitive tests should be used. The most sensitive test, applicable over a wide range of anticoagulation states, is the direct detection of descarboxy-prothrombin using a monoclonal antibody specifically recognizing the descarboxy form of prothrombin (Widdershoven et al., 1987). Another marker for monitoring poor vitamin K status at an early stage is descarboxy-osteocalcin (Knapen et al., 1993), but the commercial test kits presently available need to be substantially improved and simplified before they can be recommended for this purpose in routine laboratories.

Another method was suggested by Park et al. (1986), who repeatedly injected 10 mg of vitamin K, into factory workers who had been exposed to brodifacoum. The authors observed that 2-4 h after injection the circulating KO/K ratios were significantly elevated even 18 months after the prothrombin times had returned to normal values. This suggests that very low liver concentrations of brodifacoum can be detected from the altered KO/K ratio rather than from tests based on blood coagulation parameters. Because this method requires vitamin K administration shortly before blood sampling, it is only applicable in cases of anticoagulant poisoning, and not for the routine control of plant workers.

Methods for the direct detection of coumarin anticoagulants in plasma and serum have been reported, all of which are based on
the extraction of plasma and pre-purification of the sample, followed by HPLC analysis with fluorescence detection (Hunter, 1983; Murphy et al., 1989; Felice & Murphy, 1989; Felice et al., 1991; O'Bryan & Constable, 1991). However, such facilities will not be available in most routine laboratories. Moreover, the blood sampling should be performed within a reasonably short period after ingestion of the coumarins, because these drugs are rapidly cleared by the liver. This places severe restrictions on the applicability of these techniques, particularly for the second-generation anticoagulants.

Plasma chlorophacinone determinations were performed in three cases of intoxication. The risk of bleeding was minimal when the plasma level was below 1 mg/litre (Burcuoa et al., 1989).

Brodifacoum was detected in a case of self-ingestion by a 25-year-old woman who denied any intake of anticoagulants but was in need of vitamin K₁ treatment for 8 months. Factor assays revealed a marked reduction in the levels of the vitamin K₁-dependent factors II, VII, IX and X and normal levels of factor V and VIII:C (Exner et al., 1992).

The diagnosis in a case of difenacoum intoxication was confirmed by analysis of difenacoum in serum (0.6 mg/litre) (Butcher et al., 1992).

Bromadiolone was analysed in the serum of a 27-year-old female with a history of bleeding. She denied any contact with a rodenticide but the bromadiolone concentration in serum was 40 mg/litre (Chow et al., 1992).

Hollinger & Pastoor (1993) reported results of comparison of plasma brodifacoum concentrations and prothrombin levels over time in a case of brodifacoum poisoning. Brodifacoum was eliminated according to a two-compartment mode, with an initial half-life of 18 h and a terminal half-life of 24.2 days. On admission, the brodifacoum level was 731 µg/litre. Treatment with large doses of phytonadione lasted for 4 months.

8.6 Treatment of anticoagulant rodenticide poisoning

All suspected poisoned patients should receive medical attention immediately. Rapid determination of prothrombin time and search for evidence of bleeding is essential and may have to be maintained for several weeks.
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8.6.1 Minimizing the absorption

Gastric lavage or induction of emesis are indicated in all cases of superwarfarin rodenticide ingestion if it was recent and the amount is possibly lethal or uncertain. Repeated administration of activated charcoal is useful. Cathartics could also be administered (Sullivan et al., 1989; Smolinske et al., 1989; Donovan et al., 1990).

8.6.2 Specific pharmacological treatment

8.6.2.1 Vitamin K₁ (phytomenadione)

Vitamin K₁, is the specific antidote of choice. Depending on whether the poisoning is due to warfarin or superwarfarin, the dosage may differ as well as the duration of treatment. Dosage is dependent on coagulation parameters, mainly prothrombin time.

If the patient is bleeding severely, 25 mg of vitamin K₁ (phytomenadione) should be given by slow intravenous injection. Prothrombin time should be checked at 3-hourly intervals in severe cases and after 8–10 h in less severe cases. If no improvement occurs, vitamin K₁ injection should be repeated. Doses of up to 125–200 mg/day have been given without adverse effects (Lipton & Klass, 1984; Sheen et al., 1994).

In moderate to minor cases of poisoning, vitamin K₁ may be given in lower doses.

After initial parenteral vitamin K₁ administration, oral treatment can be continued for a prolonged period of time. Oral treatment can also be sufficient in minor cases.

The major difference between warfarin and second-generation rodenticides is that the latter can cause increased bleeding for a longer period of time than warfarin, as they have a much longer half-life in the body. Therefore vitamin K₁ should be given for months rather than weeks. It is also prudent to monitor prothrombin time for some time after cessation of this treatment to ensure that there is no regression.

In warfarin-resistant individuals, 10 times the normal dose of warfarin is required to achieve a reduction in the plasma prothrombin level. However, these individuals also respond more strongly to the effect of vitamin K (O'Reilly et al., 1963; O'Reilly et al., 1964).
8.6.2.2 Blood components

The following products could be considered, subject to availability:

- whole blood
- fresh frozen plasma and fresh blood should be used in cases of acute severe bleeding in order to rapidly restore the blood clotting factors
- factor concentrate may be considered in severe cases, especially if the amount of plasma would be too great (volume overload)

8.6.2.3 Phenobarbital

In the cases reported by Lipton & Klass (1984) and Jones et al. (1984), phenobarbital therapy was included. It was presumed that anticoagulants had been metabolized by the mixed function oxidase system based on animal experiments (Bachmann & Sullivan, 1983) and that, by inducing this enzyme system, phenobarbital might increase their metabolism.

Robinson & Mac Donald (1966) found that the pharmacological activity of warfarin in humans decreased when it was combined with phenobarbital.

8.6.3 Response to therapy

The patient should be kept in hospital until the prothrombin time has remained normal for 3 days. It is suggested that oral treatment with 10 mg vitamin K, twice daily may be necessary for up to 60 days with close monitoring of prothrombin time.

According to Hoffman et al. (1988), factor analysis allows for a detailed evaluation of the course of toxicity, and the response to therapy. Monitoring the prothrombin time alone could offer a false sense of confidence and delay effective treatment.
9. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

9.1 Laboratory experiments

9.1.1 Microorganisms

No data are available for the effects on microorganisms in water and in soil.

9.1.2 Aquatic organisms

The acute toxicity of technical flocoumafen to planktonic algae (*Selenastrum capricornutum*) was determined in a 4-day growth test. The 96-h EC\textsubscript{50}, based on cell counts on day 4, was calculated to be 1.1 mg/litre (Pearson & Wallace, 1984).

Pearson (1984) reported static acute toxicity tests for flocoumafen on *Daphnia magna*. The 48-h EC\textsubscript{50} values, based on immobilization, were 1.4 mg/litre for technical grade flocoumafen and 280 mg/litre for 0.5% flocoumafen. Pearson & Wallace (1984) found a 48-h EC\textsubscript{50} for *Daphnia magna* of 0.66 mg/litre for technical grade flocoumafen.

Anticoagulants are highly toxic to fish when tested as technical formulations (Hill et al., 1976; Pearson & Wallace, 1984) (Table 8).

9.1.3 Terrestrial organisms

9.1.3.1 Acute toxicity

Bird species vary in their susceptibility to anticoagulant rodenticides. The acute oral LD\textsubscript{50} of brodifacoum for the mallard duck (*Anas platyrhynchos*) is 2.0 mg/kg (Ross et al., 1978). Symptoms of poisoning became apparent approximately 7 days after dosing and included lethargy, weakness and lack of muscular coordination. Prolonged bleeding occurred from any small wounds and extensive bruising and subcutaneous haemorrhage were noted. Blood was observed in the faeces. Deaths occurred principally during the period of 7-14 days after administration. No deaths occurred more than 4 weeks after administration.

The acute toxicity of flocoumafen to the mallard duck appears to be dependent on the age of birds. The LD\textsubscript{50} values for 12- and
<table>
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<th>Rodenticide</th>
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<th>LC₅₀ (mg/litre)</th>
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* stat = static conditions (water unchanged for duration of test)
18-week-old birds are 24 mg/kg and 94 mg/kg, respectively (Roberts et al., 1985b,c).

Sex differences were observed in the acute toxicity for bobwhite quail (Colinus virginianus) of difenacoum. LD$_{50}$ values for male and female birds were 140 mg/kg body weight and 56 mg/kg body weight, respectively (Ross et al., 1980c).

Birds seem to be relatively tolerant to diphacinone, the LD$_{50}$ for mallard duck being 3158 mg/kg (Kosmin & Barlow, 1976).

The acute toxicity of brodifacoum for various species of birds is presented in Table 9.

<table>
<thead>
<tr>
<th>Species</th>
<th>Acute oral LD$_{50}$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black-backed gull (Larus dominicans)</td>
<td>&lt; 0.75</td>
</tr>
<tr>
<td>Black-billed gull (L. bulleri)</td>
<td>&lt; 5.0</td>
</tr>
<tr>
<td>Canada goose (Branta canadensis canadensis)</td>
<td>&lt; 0.75</td>
</tr>
<tr>
<td>Pukeko (Porphyris porphyris melanotus)</td>
<td>0.95</td>
</tr>
<tr>
<td>Mallard duck (Anas platyrhynchos)</td>
<td>4.6</td>
</tr>
<tr>
<td>California quail (Lophortyx californica)</td>
<td>3.3</td>
</tr>
<tr>
<td>Blackbird (Turdus merula)</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>House sparrow (Passer domesticus)</td>
<td>&gt; 6</td>
</tr>
<tr>
<td>Hedge sparrow (Prunella modularis occidentalis)</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>Wax-eye (Zosterops lateralis)</td>
<td>&gt; 6</td>
</tr>
<tr>
<td>Harrier hawk (Circus approximans)</td>
<td>10.0</td>
</tr>
<tr>
<td>Ring-necked pheasant (Phasianus colchicus)</td>
<td>10.0</td>
</tr>
<tr>
<td>Paradise duck (Tadorna variegata)</td>
<td>&gt; 20</td>
</tr>
</tbody>
</table>

9.1.3.2 Primary toxicity

The subacute (5-day) dietary toxicity (LC$_{50}$) of difenacoum for the bobwhite quail was found to be between 0.25 and 7.00 mg/kg
diet (Ross et al., 1980a). The 5-day LC$_{50}$ of difenacoum for the mallard duck was calculated to be 18.9 mg/kg diet (Ross et al., 1980b).

Similarly to the acute toxicity studies, diphacinone was found to be less toxic in 8-day dietary studies. The LC$_{50}$ for the bobwhite quail was 4485 mg/kg diet and for the mallard duck was > 10 000 mg/kg diet (Kosmin & Barlow, 1976).

White Leghorn hens (4 per group) were fed warfarin, coumatetralyl, bromadiolone, difenacoum or brodifacoum in baits as a choice to non-poisonous chicken food for 15 days. No symptoms appeared at a total warfarin intake of up to 171 mg/kg body weight. Coumatetralyl and brodifacoum killed all hens after intakes of 79-137 mg/kg body weight of active ingredient and 7.1-15 mg/kg body weight of active ingredient, respectively. Both bromadiolone and difenacoum killed two of the hens, at 5.9 and 15.9 mg/kg body weight of active ingredient for bromadiolone, and at 18.9 and 26.3 mg/kg body weight active ingredient for difenacoum within 10-15 days (Lund, 1981).

Christopher et al. (1984) dosed male hybrid leghorn chickens with anticoagulant rodenticides. No signs of poisoning were observed in chickens fed 183.7 mg warfarin/kg (mean a.i. ingested) for 3 days. No mortality occurred after the ingestion of a total dose of 36.9 mg bromadiolone/kg over a 3-day period. Birds fed a diet containing brodifacoum for 3 days ingested a mean of 28.9 mg/kg (active ingredient). One bird died on day 4 while the other five birds exposed had died by day 16.

Two-day no-choice tests in which chukar partridges (Alectoris graeca cypriotes Hartert) were fed 0.005% bromadiolone or difenacoum resulted in no mortality. Ten-day no-choice feeding tests resulted in 6 out of 12 and 8 out of 12 dead partridges for bromadiolone and difenacoum, respectively. The highest quantity of bait consumed by an individual bird without any lethal effects was 411 g of bait with bromadiolone (34.8 mg a.i./kg body weight) and 326.2 g of bait with difenacoum (29.1 mg a.i./kg body weight) (Krambias & Hoppe, 1987).

9.1.3.3 Secondary toxicity

Barn owls (Tyto alba) were fed rats poisoned with diphacinone, chlorophacinone, coumafuryl, difenacoum, bromadiolone or brodifacoum. Five out of six owls died of haemorrhaging after
feeding on rats killed with brodifacoum after 8 to 11 days. Sublethal haemorrhaging, but no mortality, occurred in owls fed rats killed with difenacoum. One owl died following 10 days of treatment with bromadiolone-poisoned rats, while five showed no symptoms. No abnormalities were observed in two owls fed rats killed with diphenacine, coumafuryl or chlorophacinone. Owls that died behaved normally until 24 h or less before death, when they became lethargic and stopped eating (Mendenhall & Pank, 1980).

Mice (weighing 35 g) were fed for 1 day (no choice) on a mixture containing either 0.005% difenacoum or 0.002% brodifacoum. The mean mass of residue on the day of death in a whole mouse was estimated to be 10.17 µg for difenacoum and 15.36 µg for brodifacoum. Difenacoum- and brodifacoum-poisoned mice were fed to captive barn owls for successive periods of 1, 3 and 6 days. All of the owls fed on difenacoum-poisoned mice survived the treatments and none showed external bleeding. In contrast, four of the six owls fed brodifacoum-poisoned mice died within 6-17 days after a 1-day dose. The estimated lethal dose of brodifacoum was 0.15-0.18 mg/kg. After death these owls had 0.63-1.25 mg brodifacoum/kg in their livers (Newton et al., 1990).

Newton et al. (1994) fed barn owls flocoumafen-dosed mice for consecutive periods of 1, 3 and 6 days. Four of the birds survived; the fifth bird died from haemorrhaging 5 days after the final dose. During the feeding trial birds received cumulative flocoumafen doses ranging from 0.78 to 1.25 mg/kg.

Gray et al. (1992) investigated the toxicity of brodifacoum, difenacoum and flocoumafen for barn owls fed poisoned mice. For each rodenticide, the owls survived a cumulative dose of at least 1.9 mg/kg owl weight over 15 days of treatment. All owls with cumulative doses in excess of 1.9 mg/kg body weight showed multiple treatment-related effects. The three rodenticides had approximately the same order of magnitude of toxicity to barn owls. Gray et al. (1994) developed a non-invasive method for monitoring the exposure of these birds to second-generation rodenticides by measuring compounds in regurgitated owl feed.

Radvanyi et al. (1988) fed American kestrels (*Falco sparverius*) on meadow voles that had been maintained on 2% chlorophacinone. Voles consumed approximately 53 mg of 2% chlorophacinone (1.14 mg a.i.) before dying within 6 days. No
Kestrels fed poisoned mice, for up to 21 consecutive days, died. Haematomas were observed on the pectoral muscles, lungs, liver and heart of exposed birds.

In a study by Townsend et al. (1981), captive-bred tawny owls (Strix aluco) were given warfarin-treated mice for 3 months. No behavioural changes were observed. Prothrombin levels decreased to less than 10-12% of normal but returned to normal after 9 days.

Townsend et al. (1984) found that daily warfarin intakes approaching 0.3 mg/kg body weight or 30 μg of warfarin/day can cause the death of list weasels (Mustela nivalis) by secondary poisoning. Lethal exposure occurred if mice were contaminated with 1.0-1.5 mg warfarin/kg, an amount reached following exposure to 0.005 or 0.02% sodium warfarin for about 3 days.

9.2 Field observations

9.2.1 Primary poisonings

There are few data available on the non-target hazard of anticoagulant rodenticides in field conditions.

Greig-Smith et al. (1988) reported incidents in England and Wales with barn owls and foxes. There were three incidents in which barn owls were found to have residues of the anticoagulant rodenticide brodifacoum in their livers. In two birds, residues were thought to represent lethal poisoning (0.61 and 0.29 mg/kg) but in the third the level was only 0.099 mg/kg. There were six separate incidents in which dead foxes were found to contain residues of brodifacoum, bromadiolone, coumatetralyl, difenacoum or warfarin. Residue levels showed a wide range from 0.009 to 0.46 mg/kg tissue. Three of the foxes contained more than one rodenticide, one animal revealing residues of difenacoum, bromadiolone and warfarin.

Poisonings of pheasants and partridges by chlorophacinone used against Microtus arvalis have been reported (Giban, 1974).

Reece et al. (1985) reported that, a few days after bromadiolone was placed out for control of rodents, three pea fowl (Pavo cristatus), a little raven (Corvus mellori) and an eastern swamp hen (Porphyrio porphyrio melanotus) died. Necropsy showed that the birds had been in good condition. One of the pea fowl and the swamp hen had massive intra-abdominal
haemorrhage without evidence of external trauma. All had severe congestion of the liver and lungs. The birds had negligible gut contents, so analysis for bromadiolone could not be performed.

9.2.2 Secondary poisonings

Difenacoum and brodifacoum were detected in 15 of a total of 145 dead barn owls in Britain, and on postmortem the cause of death for one owl was diagnosed as rodenticide poisoning (Newton et al., 1990, see section 5.1).

A study of 35 active nests and the radio-telemetry of 34 barn owls in an area where brodifacoum baits (0.005 %) were used in and around farm buildings in New Jersey, USA, showed no adverse effects of the rodenticide on the owls studied. In this study, rats and house mice were the target organisms and barn owls were found not to feed on these animals to any extent. Chicks were fledged from at least eight nests where poisoned rodents had been available during part of the nesting and feeding period, but no rodenticide-induced mortality was observed. Traces of brodifacoum (less than 0.05 mg/kg) were found in a barn owl that had been accidentally electrocuted (Hegdal & Blaskiewicz, 1984).

Merson & Byers (1984) monitored eastern screech owls (Otus asio) using radio-transmitters following the use of 0.005% brodifacoum for rodent control in a commercial orchard. Brodifacoum was detected in screech owl pellets (see section 5.1). There were no owl deaths which could be attributed to brodifacoum poisoning. Two owls were analysed at the end of the study; one showed signs of haemorrhage and contained 0.21 mg brodifacoum/kg and the other owl contained no brodifacoum. In a larger study, Hegdal & Colvin (1988) monitored 38 eastern screech owls and several other species of owl following brodifacoum application in an orchard. Minimum mortality was 58% among screech owls for which more than 20% of the home range was treated, as compared with 17% among those for which less than 10% of the home range was treated. Secondary brodifacoum poisoning was the most probable cause of death in six screech owls. Six radio-equipped owls were collected 1-2 months post-treatment, and four contained detectable concentrations of brodifacoum.
10. EVALUATION OF HUMAN HEALTH RISKS AND EFFECTS ON THE ENVIRONMENT

10.1 Evaluation of human health risks

Anticoagulants, both hydroxycoumarins and indandiones, known also as vitamin K antagonists, are widely used in urban rodent control and against rodent pests in agriculture. They act by inhibiting the vitamin K\textsubscript{1} epoxide cycle, thereby depleting the active form of vitamin K\textsubscript{1}, necessary for producing blood-clotting factors.

Since anticoagulant rodenticides are used in general as low-concentration bait formulations and have low volatility, increased levels in the air are unlikely. Being only slightly soluble in water, their use could not be a major source of water contamination. Anticoagulant rodenticides are not intended for direct application to growing crops. Hence no residues in plant foodstuffs are expected.

The controlled medicinal use of warfarin exposes more people to higher concentrations over a longer period than would be expected to occur as a result of accidental human exposure due to its use as a rodenticide.

Occupational exposure may occur during manufacture, formulation and bait application, but figures indicating the levels of exposure are not available.

Anticoagulant rodenticides are readily absorbed through the gastrointestinal tract, skin and respiratory system. The major route of elimination in various species after oral administration is through the faeces. The urine is a very minor route of elimination. The liver is the major organ for accumulation and storage of anticoagulants.

The metabolism pattern of warfarin and indandiones mainly involves hydroxylation. The second-generation hydroxycoumarins are found principally as unchanged parent compounds. Their elimination from the liver is slow with a biphasic rate, where a rapid initial phase is followed by a prolonged second phase.

Most of the anticoagulants have high acute toxicity by oral, percutaneous and inhalation routes of exposure. The second-generation anticoagulants are more toxic than the first-generation.
one in the sense that a single feeding may be lethal. Signs of poisoning in all species, including humans, are associated with increased bleeding tendency.

Many poisoning incidents (both intentional and unintentional) have been reported. A few cases of intoxication from occupational exposure to anticoagulants have also been observed.

The level of prothrombin concentration is a satisfactory guide to the severity of acute intoxication and the effectiveness and duration of the therapy. The specific antidote is vitamin K₁. The high retention of the second-generation anticoagulants in the liver means that any treatment of intoxication should be prolonged to ensure that anticoagulant activity does not recur.

The addition of bittering agents to anticoagulant rodenticides is aimed at discouraging human consumption.

Warfarin has been found to be teratogenic in both rats and humans. Second-generation anticoagulant rodenticides do not demonstrate teratogenicity in laboratory animals. Developmental effects in humans are observed when warfarin has been taken as a therapeutic agent during pregnancy. No cases of embryopathy from anticoagulants in their use as rodenticides have been reported. Second-generation anticoagulants are not intended to be used as therapeutic agents, and the risks associated with, for example, warfarin will not apply.

There is no evidence to suggest that any anticoagulant rodenticides are mutagenic, but there are insufficient data available on some compounds to demonstrate an absence of mutagenicity.

There is experimental evidence that coumarin anticoagulants not only inhibit the vitamin K cycle in the liver, but also in other tissues such as bone. In humans it has been demonstrated that even low levels of vitamin K deficiency form a risk factor for developing osteoporosis (Hart et al., 1985; Szulc et al., 1993). Therefore, it appears that long-term exposure to low levels of anticoagulant may have an adverse effect on bone metabolism.

10.2 Evaluation of effects on the environment

Unlike conventional crop protection products, which must be applied over relatively large crop areas, anticoagulant rodenticides are applied to discrete sites in the form of low concentration baits.
Most of the anticoagulants are stable under normal conditions. They are slightly soluble in water, and as bait formulations their use is unlikely to be a source of water contamination. They appear to bind rapidly in the soil, with very slow desorption and no leaching. In general, anticoagulants are highly toxic to aquatic organisms when tested as technical material.

Non-target organisms are potentially at risk in two ways: from direct consumption of baits (primary hazard) and through eating poisoned rodents (secondary hazard).

Bird species vary in their susceptibility to anticoagulant rodenticides. Small pellets and whole grain baits are highly attractive to birds. Wax block formulation appear to decrease the attractiveness to the birds and so reduce the possibility of poisoning incidents. It is difficult to assess the risks to birds due to direct consumption of baits because most of the published studies are toxicity trials in laboratory conditions.

The main cause for poisoning of domestic animals is through direct consumption of anticoagulant baits.

Some of the anticoagulants show a similar range of acute toxicity for non-target non-rodent mammals as for target rodents. The primary hazard is usually expressed by the amount of finished bait which must be consumed to approach the lethal dose. The bait concentration of second-generation rodenticides is usually around 0.005%, whereas the concentration of the first-generation rodenticides in the bait may be 5 to 10 times higher. Thus, to reach the toxic or lethal dose, non-target animals must consume comparatively large amounts of bait with low concentrations of active ingredient.

Secondary poisoning through the consumption of rats and mice killed with anticoagulants may occur in dogs and cats in urban situations but is more likely in farm situations.

Some secondary toxicity laboratory studies with wildlife have shown that captive predators could be intoxicated by no-choice feeding of anticoagulant-poisoned or dosed prey. The significance of these results in terms of hazard under field conditions is difficult to assess because the predators would not be expected to eat only poisoned animals. However, where they occur, predators may take poisoned, but not dead, small mammals preferentially. In areas close to baiting, poisoned rodents may represent a high
proportion of the diet for individual birds. However, only a few individuals will be affected except in situations of very widespread and constant use of baits. Therefore, some kills of owls will be expected but no severe population effects. This agrees with observed field effects with small numbers of poisoned owls.
11. CONCLUSIONS AND RECOMMENDATIONS
FOR PROTECTION OF HUMAN HEALTH AND
THE ENVIRONMENT

11.1 Conclusions

a) Exposure of the general population to anticoagulant rodenticides via food and drinking-water is unlikely and does not constitute a significant health hazard.

b) Poisoning incidents may occur in cases of massive intentional or unintentional ingestion or prolonged skin contact during manufacture and formulation.

c) Anticoagulant rodenticides are relatively persistent in the environment, but their specific use as low-concentration bait formulations limits the potential for environmental contamination.

d) Direct and secondary poisoning of birds, domestic and farm animals, and wildlife may occur.

e) The major difference between the first- and second-generation anticoagulant rodenticides is that the latter have longer body retention, resulting in an increased tendency for bleeding over a longer period of time.

f) The mode of action of anticoagulant rodenticides is known and an effective antidote is available, i.e. vitamin K₁.

g) There is no available evidence that this class of compound is mutagenic or carcinogenic.

h) Only warfarin has been shown to possess some teratogenic potential in both rats and humans.

11.2 Recommendations for protection of human health and the environment

a) Exposed workers should receive appropriate biomonitoring and health evaluation.

b) The inclusion of a bittering agent in formulations at appropriate concentrations may reduce accidental ingestion.
c) For preventing primary poisoning, baits less attractive to birds and domestic animals, as well as pulsating baiting, should be used.

d) The location of bait placement should be carefully selected.

e) Killed rodents should be burned or buried to reduce the risk of secondary poisoning of predators.

f) Training in the safe handling of rodenticides is essential.
12. FURTHER RESEARCH

a) Studies of exposed humans are required, particularly with regard to possible teratogenic and embryotoxic effects.

b) More information is needed in order to evaluate the risks of occupational exposure to anticoagulant rodenticides.

c) Methods more sensitive than prothrombin time determination need to be developed for routinely assessing the absorption and effects of anticoagulants.

d) More data are required to assess secondary effects on non-target organism populations.

e) The extent to which second-generation anticoagulant rodenticides are transferred across the placenta should be evaluated.

f) More information should be collected concerning the tissue distribution of anticoagulant rodenticides after their ingestion and their effects on physiological processes other than blood coagulation, notably calcium and bone metabolism. This is particularly necessary for long-term, low-level exposure to these compounds.
13. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES

In the WHO Recommended Classification of Pesticides by Hazard (WHO, 1994), anticoagulant rodenticides were classified, according to their acute oral LD$_{50}$, as follows:

<table>
<thead>
<tr>
<th>Class Ia (Extremely hazardous)</th>
<th>Oral LD$_{50}$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brodifacoum</td>
<td>0.3</td>
</tr>
<tr>
<td>Bromadiolone</td>
<td>1.12</td>
</tr>
<tr>
<td>Chlorophacinone</td>
<td>3.1</td>
</tr>
<tr>
<td>Difenacoum</td>
<td>1.8</td>
</tr>
<tr>
<td>Difethialone</td>
<td>0.56</td>
</tr>
<tr>
<td>Diphacinone</td>
<td>2.3</td>
</tr>
<tr>
<td>Flocoumafen</td>
<td>0.25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Class Ib (Highly hazardous)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coumachlor</td>
<td>33</td>
</tr>
<tr>
<td>Coumatetra1yl</td>
<td>16</td>
</tr>
<tr>
<td>Warfarin</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Class II (Moderately hazardous)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pindone</td>
<td>50</td>
</tr>
</tbody>
</table>

A Poison Information Monograph for brodifacoum has been issued (IPCS, 1992), and one on warfarin is in preparation.
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References


1. Généralités

Les anticoagulants qui sont décrits dans la présente monographie sont ceux que l'on utilise principalement en agriculture et pour la destruction des rongeurs en milieu urbain. La warfarine, premier rodenticide anticoagulant à connaître une large utilisation, était à l'origine un médicament efficace pour le traitement des thromboembolies chez l'homme.

Selon leur structure chimique, les rodenticides anticoagulants peuvent se répartir en deux catégories, les hydroxycoumarines et les indane-diones, mais leur mode d'action est analogue.

2. Propriétés et méthodes d’analyse

Les rodenticides anticoagulants se présentent sous la forme de solides cristallins ou de poudres et sont légèrement solubles dans l'eau. La plupart d'entre eux sont stables dans les conditions normales de conservation.

La plupart des méthodes de dosage des rodenticides anticoagulants reposent sur la chromatographie en phase liquide à haute performance.

3. Sources d’exposition humaine et environnementale

Les hydroxycoumarines de la première génération ont commencé à être utilisées comme rodenticides vers la fin des années 1940. Par la suite, l'apparition d'une résistance à la warfarine et aux autres anticoagulants de la première génération a conduit à mettre au point des produits de deuxième génération, plus actifs. La concentration du principe actif dans les appâts varie selon l'efficacité du rodenticide.

4. Distribution, concentration et exposition dans l'environnement

Les rodenticides anticoagulants sont principalement utilisés sous la forme d'appâts. Comme ils sont peu volatils, leur concentration dans l'air est négligeable. De même n'étant que légèrement solubles dans l'eau, il est peu probable que leur utilisation conduise à la contamination des eaux.
Etant donné que les rodenticides anticoagulants ne sont pas destinés à être appliqués directement sur les cultures, il n'y a pas lieu de s'attendre à en trouver sous forme de résidus dans les aliments d'origine végétale.

L'exposition aux rodenticides des vertébrés non visés peut se produire directement par la consommation d'appâts empoisonnés et indirectement par celle de rongeurs contaminés. Les petits granulés et les appâts constitués de grains entiers attirent fortement les oiseaux.

La warfarine est utilisé dans le traitement des thrombo-embolies.

Il existe une possibilité d'exposition professionnelle aux rodenticides anticoagulants lors de leur fabrication ou formulation ou encore lors de la pose d'appâts empoisonnés, mais on ne dispose pas de données sur l'ampleur de cette exposition.

5. Mode d'action et métabolisme

Les rodenticides anticoagulants sont des antagonistes de la vitamine K. Leur action est principalement localisée dans le foie où plusieurs précurseurs de l'hémostase subissent un processus post-traductionnel dépendant de la vitamine K avant d'être convertis en zymogènes procoagulants. L'action proprement dite consiste dans l'inhibition de la K$_1$-époxyde-réductase.

Les rodenticides anticoagulants sont facilement absorbés au niveau des voies digestives et pourraient l'être également à travers la peau et dans les voies respiratoires. Après administration par voie orale, c'est principalement dans les matières fécales que ces composés sont éliminés chez diverses espèces.

Il est possible que la décomposition métabolique de la warfarine et des indane-diones chez le rat s'effectue principalement par l'intermédiaire d'une hydroxylation. En revanche, les anticoagulants de la deuxième génération s'éliminent essentiellement tels quels. Le faible taux d'excrétion urinaire empêche d'isoler les métabolites de l'urine.

Le foie est le principal organe où s'accumulent les rodenticides anticoagulants. Cette accumulation a lieu également dans les graisses.
6. **Effets sur les mammifères et les systèmes d'épreuve in vitro**

Chez le rat et la souris, les signes d'intoxication consistent en une tendance accrue au saignement.

La DL_{50} est très variable, et c'est par la voie orale que les composés sont les plus toxiques. La toxicité est également élevée par la voie percutanée et par la voie respiratoire.

Certains anticoagulants présentent une toxicité aiguë du même ordre pour les mammifères non visés que pour les rongeurs à détruire, toutefois leur spectre toxique peut varier d'une espèce à l'autre.

Après administration répétée par voie orale à des rats, les principaux effets observés sont ceux qui résultent de l'activité anticoagulante.

On ne possède que peu de données sur les expositions répétées aux anticoagulants chez les espèces n'appartenant pas à l'ordre des rongeurs.

Une étude relative aux effets de la warfarine sur le rat a fait ressortir une action sur le développement de ces animaux. En dehors de cela, rien n'indique que les anticoagulants aient une action tératogène sur les animaux d'expérience.

Rien n'indique non plus que les rodenticides anticoagulants soient mutagènes, toutefois les données relatives aux différents composés sont insuffisantes pour qu'on puisse en conclure à l'absence de mutagenicité. La souche, le sexe et le régime alimentaire sont des facteurs importants qui influent sur la toxicité des anticoagulants chez les rongeurs. On a fait état d'épisodes d'intoxication chez des animaux domestiques qui avaient consommé des appâts additionnés d'anticoagulants. Lorsqu'il y a issue fatale ou du moins un tableau clinique grave, c'est généralement qu'il y a eu consommation d'anticoagulants de la deuxième génération. La principale différence entre la warfarine et les autres anticoagulants (qu'il s'agisse des indane-diones ou des hydroxycoumarines de la deuxième génération), c'est que ceux-ci persistent plus longtemps dans l'organisme et ont par conséquent un effet plus durable que la warfarine. Dans ces conditions, devant une intoxication, il faut poursuivre plus longtemps l'administration de l'antidote, c'est-à-dire de la vitamine K₁.
7. Effets sur l'homme

De nombreuses intoxications (qu'elles soient intentionnelles ou non) ont été signalées. Il y a eu également quelques cas d'intoxication imputables à une exposition professionnelle. En cas d'intoxication aiguë par des rodenticides anticoagulants, les symptômes vont de l'accroissement de la tendance au saignement en cas d'intoxication minime ou modérée, à une hémorragie massive dans les cas plus graves. Les signes cliniques se manifestent un à plusieurs jours après l'absorption.

Chez l'homme, on attribue certaines malformations congénitales à des traitements par la warfarine pendant la période de grossesse. Aucune malformation de ce type n'a été observée par suite de l'utilisation d'anticoagulants comme rodenticides.

La concentration de la prothrombine plasmatique donne une indication de la gravité de l'intoxication. Elle est plus sensible que des épreuves globales telles que le temps de Quick. En cas d'exposition professionnelle répétée, le dosage direct des traces de carboxyprothrombine circulante ou du 2,3-époxyde de la vitamine K peut permettre un bilan plus sensible.

Le traitement d'une intoxication par des anticoagulants doit être adapté à la gravité de l'intoxication. Il est basé sur l'action pharmacologique spécifique de la vitamine K, que l'on administre par voie parentérale avec, dans les cas graves, administration simultanée de constituants sanguins. La mesure du temps de Quick permet d'apprécier l'efficacité du traitement et de déterminer sa durée.

8. Effets sur les autres êtres vivants au laboratoire et dans leur milieu naturel

On peut répartir en deux catégories les effets possibles de rodenticides anticoagulants sur les organismes non visés: les effets directs (par consommation d'appâts) et les effets indirects (par consommation de rongeurs empoisonnés).

Sous forme de produit technique, les anticoagulants sont extrêmement toxiques pour les poissons. Incorporés à des appâts, il est peu probable qu'ils présentent un danger, du fait de leur faible solubilité dans l'eau. C'est pourquoi, à moins d'une erreur de manipulation, ils ne devraient pas parvenir jusqu'aux poissons.
Les différentes espèces d'oiseaux sont d'une sensibilité variable aux rodenticides anticoagulants. Il est difficile d'apprécier les risques que représente, pour les oiseaux, la consommation directe d'appâts car la plupart des travaux publiés sont des études toxicologiques effectuées au laboratoire. Le caractère attractif des appâts constitués de grains entiers pour les petits oiseaux en accroît le danger dans la nature.

Des études de toxicité indirecte effectuées en laboratoire sur des animaux sauvages ont montré que des prédateurs captifs pouvaient s'intoxiquer si on leur donnait de la nourriture empoisonnée sans autre choix ou des proies empoisonnées. On a également constaté la mort de prédateurs dans le milieu naturel.

9. Evaluation et conclusion

Les rodenticides anticoagulants bloquent le mécanisme normal de l'hémostase, d'où une tendance accrue au saignement qui peut déboucher sur une forte hémorragie.

Il est peu probable que la population générale puisse être exposée involontairement à des rodenticides anticoagulants.

Il peut y avoir une exposition non négligeable par suite de contacts lors de l'activité professionnelle. Ces contacts peuvent se produire au cours des opérations de production et de formulation aussi bien que lors de la préparation et de la pose des appâts.

Les rodenticides anticoagulants sont facilement absorbés au niveau des voies digestives ainsi qu'à travers la peau et les voies respiratoires. C'est principalement dans le foie qu'ils sont retenus et s'accumulent. La concentration de prothrombine plasmatique est un bon indicateur de la gravité d'une intoxication aiguë et permet d'avoir une idée de l'efficacité et de la durée nécessaire du traitement.

L'antidote spécifique est la vitamine K₁.

La principale différence entre les rodenticides anticoagulants de première et de deuxième génération tient au fait que ces derniers séjournent plus longtemps dans l'orgasme et ont donc tendance à prolonger l'effet hémorragique.

La plupart des anticoagulants sont stables dans les conditions normales d'utilisation. Comme ils sont peu solubles dans l'eau et
que les appâts n’en contiennent qu’une faible quantité, il est peu probable qu’ils puissent contaminer les étendues d’eau. Par ailleurs, ils se fixent rapidement aux particules du sol, ne s’en désorbent que très lentement et ne sont pas lessivés.

Aucun organisme non visé ne court de risque d’intoxication directe par consommation d’appâts, ni de risque d’intoxication indirecte par consommation de rongeurs contaminés.
RESUMEN

1. Generalidades

Los anticoagulantes descritos en esta monografía son los utilizados principalmente en agricultura y en la lucha contra los roedores urbanos. La warfarina, el primer rodenticida anticoagulante de uso generalizado, se introdujo como agente eficaz para el tratamiento de la tromboembolia en el ser humano.

De acuerdo con su estructura química, los rodenticidas anticoagulantes pueden agruparse en dos categorías, las hidroxicumarinas y las indandonas, aunque su mecanismo de acción es similar.

2. Propiedades y métodos analíticos

Los rodenticidas anticoagulantes se presentan en forma cristalina sólida o en polvo, y son ligeramente solubles en agua. La mayoría de ellos son estables en condiciones de almacenamiento normales.

La mayoría de los procedimientos para la determinación de los rodenticidas anticoagulantes se basan en cromatografía líquida de alta resolución.

3. Fuentes de exposición humana y ambiental

Las hidroxicumarinas de primera generación se introdujeron como rodenticidas a finales de los años cuarenta. La aparición de resistencia a la warfarina y a otros anticoagulantes de primera generación dio lugar a la elaboración de anticoagulantes más potentes, de segunda generación. Las concentraciones de componentes activos en los cebo varían en función de la eficacia de los rodenticidas.

4. Distribución, niveles y exposición ambientales

Los rodenticidas anticoagulantes se utilizan principalmente como formulaciones para cebo. Dada su baja volatilidad, las concentraciones en el aire son insignificantes. Como son muy poco solubles en agua, es improbable que su uso sea fuente de contaminación del agua.
Como los rodenticidas anticoagulantes no están pensados para su aplicación directa a cosechas en pie, no son de prever residuos en alimentos vegetales.

Los vertebrados no destinatarios están expuestos a los rodenticidas principalmente por medio del consumo del cebo y de forma secundaria por el consumo de roedores envenenados. Los cebos en bolitas y de grano entero son muy atractivos para las aves.

La warfarina se utiliza como agente terapéutico para la tromboembolía.

Hay un potencial de exposición ocupacional a los rodenticidas anticoagulantes durante la fabricación, formulación y aplicación del cebo, pero no se dispone de datos sobre los niveles de exposición.

5. Modo de acción y metabolismo

Los rodenticidas anticoagulantes son antagonistas de la vitamina K. Su lugar principal de acción es el hígado, donde varios de los precursores de la coagulación de la sangre sufren un procesamiento post-traslación dependiente de la vitamina K antes de convertirse en los zimógenos procoagulantes respectivos. Parece que el mecanismo de acción es la inhibición de la reductasa epoxidica K₁.

Los rodenticidas anticoagulantes se absorben fácilmente por el tracto intestinal, y también pueden absorberse por la piel y el sistema respiratorio. Tras la administración oral, la principal vía de eliminación en diversas especies son las heces.

La degradación metabólica de la warfarina y las indandonas en ratas es principalmente la hidroxilación. Sin embargo, los anticoagulantes de segunda generación se eliminan principalmente como compuestos inalterados. El bajo nivel de excreción urinaria impide aislar los metabolitos a partir de la orina.

El hígado es el órgano principal para la acumulación y almacenamiento de anticoagulantes rodenticidas. La acumulación también tiene lugar en la grasa.
6. Efectos en los mamíferos y en los sistemas de prueba \textit{in vitro}

Los signos de envenenamiento en ratas y ratones son los asociados a una mayor tendencia a la hemorragia.

Hay una gran variación en la DL$_{50}$ de los rodenticidas anticoagulantes, siendo máxima la toxicidad por via oral. También es alta la toxicidad cutánea y por inhalación.

Los márgenes de toxicidad aguda de algunos anticoagulantes son similares en el caso de los mamíferos no destinatarios y de los roedores de destino, pero los espectros de toxicidad para los anticoagulantes pueden variar entre las especies.

Tras una administración oral repetida en ratas, los principales efectos observados son los asociados a la acción anticoagulante.

Se dispone de pocos datos sobre la exposición repetida de especies distintas de los roedores.

Un estudio sobre la warfarina en ratas ha indicado efectos sobre el desarrollo. Por lo demás, no hay pruebas convincentes de que los anticoagulantes sean teratogénicos en animales de experimentación.

No hay prueba que sugiera que los rodenticidas anticoagulantes son mutagénicos, pero se dispone de datos insuficientes sobre los compuestos individuales para demostrar la inexistencia de mutagenicidad. La sobrecarga, el sexo y la alimentación son importantes factores modificadores de la toxicidad de los anticoagulantes en los roedores.

Se han dado casos de envenenamiento de animales domésticos tras la ingestión de cebos anticoagulantes. Las muertes y los síndromes clínicos graves se deben por lo general a anticoagulantes de segunda generación. La diferencia principal entre la warfarina y los demás anticoagulantes (tanto las indandonas como las hidroxicumarinas de segunda generación) es que éstos tienen mayor tiempo de retención en el organismo y por consiguiente un efecto más prolongado que la warfarina. Por ello, en los casos de envenenamiento, el tratamiento antidoto con vitamina K$_1$ debe proseguir durante un periodo más largo.
7. Efectos en el ser humano

Se han notificado muchos casos de envenenamiento (tanto intencionados como no intencionados). También se han producido unos pocos casos de intoxicación por exposición ocupacional a los anticoagulantes. Los síntomas de intoxicación aguda por rodenticidas anticoagulantes van desde una mayor tendencia a la hemorragia en el envenenamiento leve o moderado a una hemorragia masiva en casos más graves. Los signos de envenenamiento aparecen con un retraso de uno a varios días después de la absorción.

La warfarina va asociada en el ser humano a la producción de malformaciones del desarrollo cuando se toma como agente terapéutico durante el embarazo. No se han notificado casos de defectos de desarrollo tras el uso de anticoagulantes como rodenticidas.

La concentración de protrombina plasmática orienta sobre la gravedad de la intoxicación. Es una indicación más sensible que pruebas generales como el tiempo de protrombina. En la exposición ocupacional repetida, la medición directa de cantidades ínfimas de descarboxiprotrombina en circulación o de 2,3-epóxido de vitamina K en circulación pueden constituir una evaluación más sensible.

El tratamiento del envenenamiento con anticoagulantes se gradúa de acuerdo con la gravedad de la intoxicación. El tratamiento farmacológico específico consiste en la administración parenteral de vitamina K, con administración simultánea, en los casos graves, de componentes sanguíneos. La medición del tiempo de protrombina ayuda a determinar la eficacia y la duración de tratamiento necesaria.

8. Efectos en otros organismos en el laboratorio y sobre el terreno

Los posibles efectos de los rodenticidas en organismos no destinatarios pueden dividirse en dos categorías: primarios (envenenamiento directo por consumo de cebo) y secundario (por consumo de roedores envenenados).

En la forma del producto técnico, los anticoagulantes son muy tóxicos para los peces. Como formulaciones para cebo es improbable que planteen riesgos en razón de su baja solubilidad en
agua. Por esta razón, a menos que se utilicen de forma indebida, no están al alcance de los peces.

La susceptibilidad de las aves a los rodenticidas anticoagulantes es variable. Es difícil evaluar los riesgos para las aves que entraña el consumo directo porque la mayoría de los estudios publicados consisten en ensayos de toxicidad en condiciones de laboratorio. El atractivo del cebo de grano integral para las aves pequeñas aumenta el riesgo en las condiciones de campo.

Los estudios en laboratorio de la toxicidad secundaria con la fauna silvestre han mostrado que los predadores cautivos pueden intoxicarse mediante alimentación sin otra elección con presas que se han envenenado con anticoagulante o a las que se ha administrado este producto. Se tiene noticias de algunas muertes de predadores en su medio natural.

9. Evaluación y conclusión

Los rodenticidas anticoagulantes perturban los mecanismos normales de coagulación de la sangre, determinando una mayor tendencia a la hemorragia y, por último, una hemorragia abundante.

La exposición no intencionada de la población general a los rodenticidas anticoagulantes es improbable.

El contacto ocupacional es una fuente potencial de exposición significativa. Puede tener lugar durante la elaboración y formulación, así como durante la preparación y aplicación del cebo.

Los compuestos de rodenticida anticoagulante se absorben fácilmente por el tracto intestinal, y por la piel y el sistema respiratorio. El hígado es el órgano principal de acumulación y almacenamiento. La concentración de protrombina plasmática es una buena orientación de la gravedad de la intoxicación aguda y de la eficacia y la duración necesaria de la terapia.

El antidoto específico es la vitamina K₁.

La principal diferencia entre los rodenticidas anticoagulantes de la primera generación y los de la segunda es que éstos últimos tienen una mayor retención en el organismo y por ello suelen dar lugar a un periodo de hemorragia más prolongado.
La mayoría de los anticoagulantes son estables en condiciones de uso normal. Su baja solubilidad en agua y baja concentración en los cebos hace improbable que sean una fuente de contaminación del agua. Parece que se asocian rápidamente a las partículas del suelo, con desorción muy lenta y nula propiedad de lixiviación.

Los organismos no destinatarios pueden correr al riesgo de consumir directamente cebos (riesgo primario) y de ingerir roedores envenenados (riesgo secundario).
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