The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NOTE FOR GUIDANCE:

ENVIRONMENTAL RISK ASSESSMENT FOR VETERINARY MEDICINAL PRODUCTS OTHER THAN GMO-CONTAINING AND IMMUNOLOGICAL PRODUCTS

FINAL APPROVAL BY THE CVMP	**14-16 January 1997**
DATE FOR COMING INTO OPERATION[1]	1 January 1998

[1] Applications for new active substances will have to comply with this Note for Guidance as from 01.01.98 onwards. However, companies are advised to take this Note for Guidance into consideration as of its date of approval when planning and preparing their Environmental Risk Assessment.

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NOTE FOR GUIDANCE: ENVIRONMENTAL RISK ASSESSMENT FOR VETERINARY MEDICINAL PRODUCTS OTHER THAN GMO[1]-CONTAINING AND IMMUNOLOGICAL PRODUCTS

[1] Genetically Modified Organisms

1. GENERAL CONSIDERATIONS

Directive 81/852/EEC as amended by Directive 92/18/EEC states that *the purpose of the study of environmental safety of a veterinary medicinal product is to assess the potential harmful effects which the use of the product may cause to the environment and to identify any precautionary measures which may be necessary to reduce such risks*[1]. Such an assessment shall be included in applications for marketing authorization for veterinary medicinal products other than those submitted in accordance with Article 5, second paragraph point 10 of Directive 81/851/EEC concerning abridged applications. This assessment should normally be conducted in two phases.

The first phase (Phase I) shall assess the potential of exposure of the environment to the product, its ingredients or relevant metabolites.

This Note for Guidance presents, as a guide for the conduct of Phase I, a straightforward decision tree to identify products which can be exempted from further testing because they are unlikely to result in significant exposure of the environment and will consequently be of low environmental risk.

In a second phase (Phase II), having regard to of the extent of exposure of the environment to the product, and the available information about the physical/chemical, pharmacological and/or toxicological properties of the product obtained during the conduct of the other tests required by Directive 81/852/EEC, the investigator shall then consider whether further specific investigation of the effects of the product on particular ecosystems is necessary. In this Note for Guidance, Phase II is divided in two parts, Tier A and Tier B.

Tier A begins an evaluation of the possible fate and effects of the drug and/or its major metabolites which is more detailed than the evaluation performed in Phase I. There may further be a need for the determination of the degradation half-life of the active substance and/or the relevant metabolites in the environmental compartments of interest.

If within Tier A, no hazard is detected or the risk management strategy proposed by the applicant is taking care of any potential hazard, thus avoiding harmful effects of the product on the environment, there would be no need to proceed to Tier B, which involves studies on the effects on fauna/flora within the environmental compartments that are likely to be affected.
If the applicant is unable to demonstrate that exposure is minimised to a level of no concern to the environment, then the effects in the relevant compartments must be adequately investigated. However, the applicant is advised to contact the competent authorities prior to commencing any programme of testing at Tier B. Nevertheless, it is expected that for most veterinary medicinal products entering Phase II, the assessment will be completed at Tier A.

For the purpose of assessing the environmental fate and effect of the product, test protocols from the following guidance documents may be used:

- Test protocols listed in Annex II and III of this Note for Guidance;
- Test protocols listed in Annex V of Directive 67/548/EEC as amended on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances;
- Tests protocols from OECD Guidelines on Testing of Chemicals;
- Testing methods and calculation models published in the Technical Guidance Documents in support of Directive 93/67/EEC on risk assessment for new notified

[1] This Note only provides guidance on the assessment of potential harmful effects which the use of the product may cause to the environment. It does not impact on any precautionary measures that Competent Authorities may further require to be indicated in the SPC, the package insert and the labelling of any veterinary medicinal product to prevent exposure of the environment to unused product or waste materials, in order to comply with EU waste management legislation, including Council Directive 75/442/EEC on waste (25) and Council Directive 91/689/EEC on hazardous waste (28).

substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances (36);

- The principles for evaluation of plant protection product as laid down in Annex VI to Directive 91/414/EEC concerning the placing of plant protection product on the market (29).

Other tests protocols recommended by other Authorities such as the European Pharmacopeia, the US Food and Drug Administration (FDA) or the US Environment Protection Agency (EPA) may also be acceptable.

The environmental risk assessment should take into consideration other possible use of the active substance contained in the product, in particular when the active substance is used as a pesticide or as an additive to animal feeding stuffs.

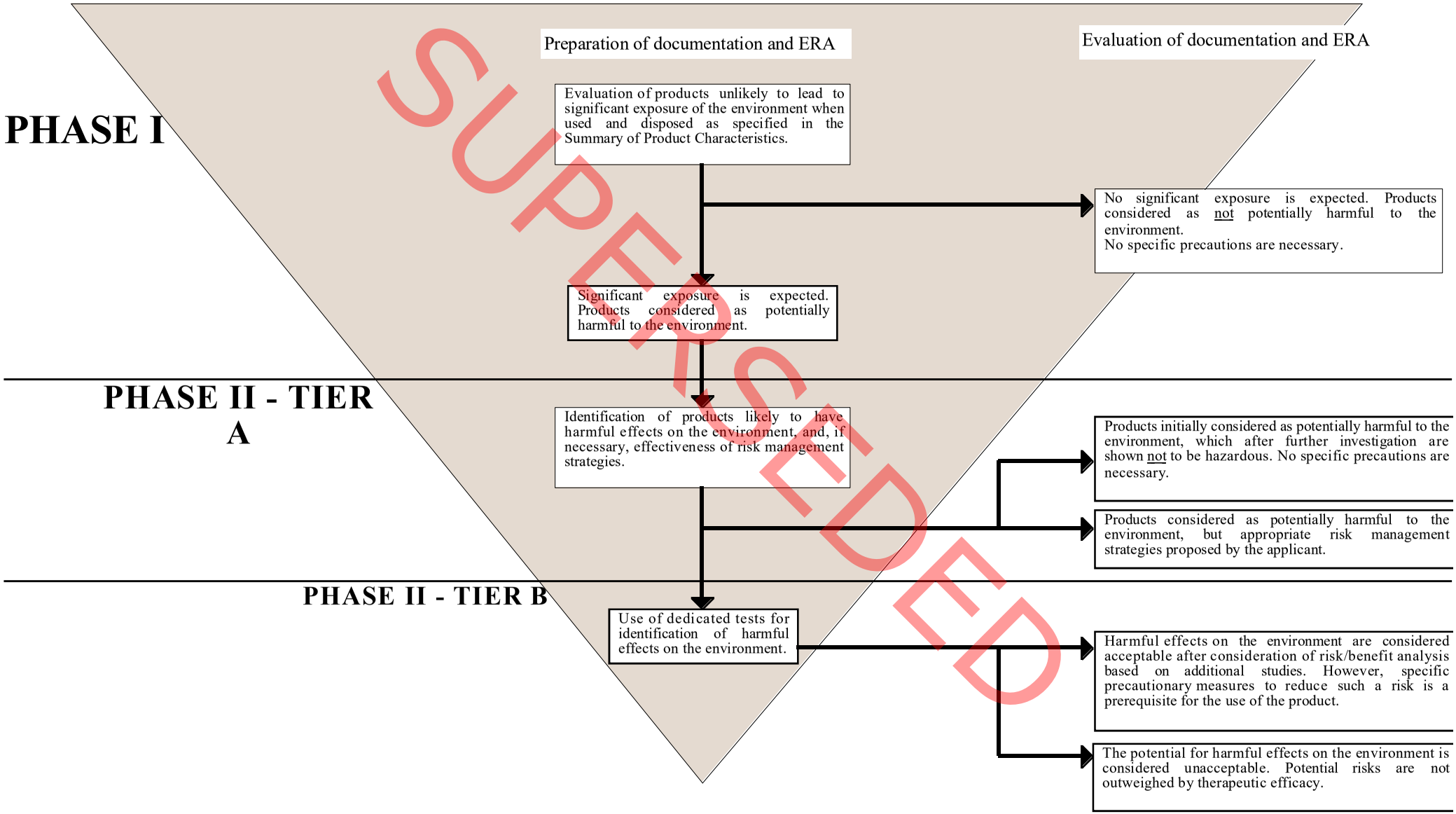
In such cases, data available from previous evaluations may be cited in the application, including in particular the recommendations/conclusions from other relevant EU bodies (Scientific Committee for Animal Nutrition (SCAN), European Environmental Agency).

Applicants are required to submit a complete report which would conclude with an environmental risk assessment (ERA) based on the characteristics of the product, its potential environmental exposure, environmental fate and effects, and risk management strategies as appropriate. The report should take into account the pattern of use and administration of the product, the excretion of active substance and major metabolites and the disposal of the product as set out in Directive 81/852/EEC.

Finally, the environmental risk assessment made should be subject to an expert report. This should constitute part of the Safety expert report (Part III), and its conclusions should be based on sound scientific reasoning supported by adequate experimental studies or other data.

Figure 1.

SCHEMATIC FLOW DIAGRAM FOR PREPARATION AND EVALUATION OF DOCUMENTATION AND ENVIRONMENTAL RISK ASSESSMENT (ERA)



2. PHASE I

For the purpose of Phase I, a straightforward decision tree can be followed (see figure 2). This decison tree is based on whether the use and disposal of a veterinary medicinal product is likely to lead to significant environmental exposure. It is as the basis for deciding if the product should enter or not Phase II for evaluation of environmental risk.

Should the applicant conclude that possible environmental exposure is insignificant, the applicant would then be required to submit a statement to this effect, which should be supported by an assessment based on the characteristics and use pattern of the product and its potential exposure to the environment. The criteria laid down in the Phase I decision tree are to be viewed as a guidance to prepare the ERA.

External application is defined as pour-ons, dips, fumigation etc. However, if there is appreciable absorption through the skin leading to systemic effect, the pathway for internal application should be followed.

Exemption from further testing is in principle acceptable for:

- Physiological substances such as vitamins, electrolytes, natural amino acids and herbs
- Substances intended for administration to companion animals (not including horses)
- Substances intended for individual treatment of a small number of animals (as opposed to mass medication)
- Substances that will be present in manure or slurry, for spreading on land, in concentrations lower than 100 µg/kg[1]
- Substances used for animals kept on pasture and that will be present in the fresh dung excreted in concentrations lower than 10 µg/kg[1]
- Substances likely to be rapidly degraded in manure (DT_{50} in manure less than 30 days)[2]
- Substances that have a predicted environmental concentration (PEC) in soil below 10 µg/kg[3]
- Substances that have a predicted environmental concentration (PEC) in ground water below 0.1 µg/L[4]

However, not withstanding the above possible exemptions, if adverse environmental effects are still anticipated from the use of such products, the further assessment of possible exposure to the environment may be necessary and risk management procedures proposed (see Phase II Tier A).

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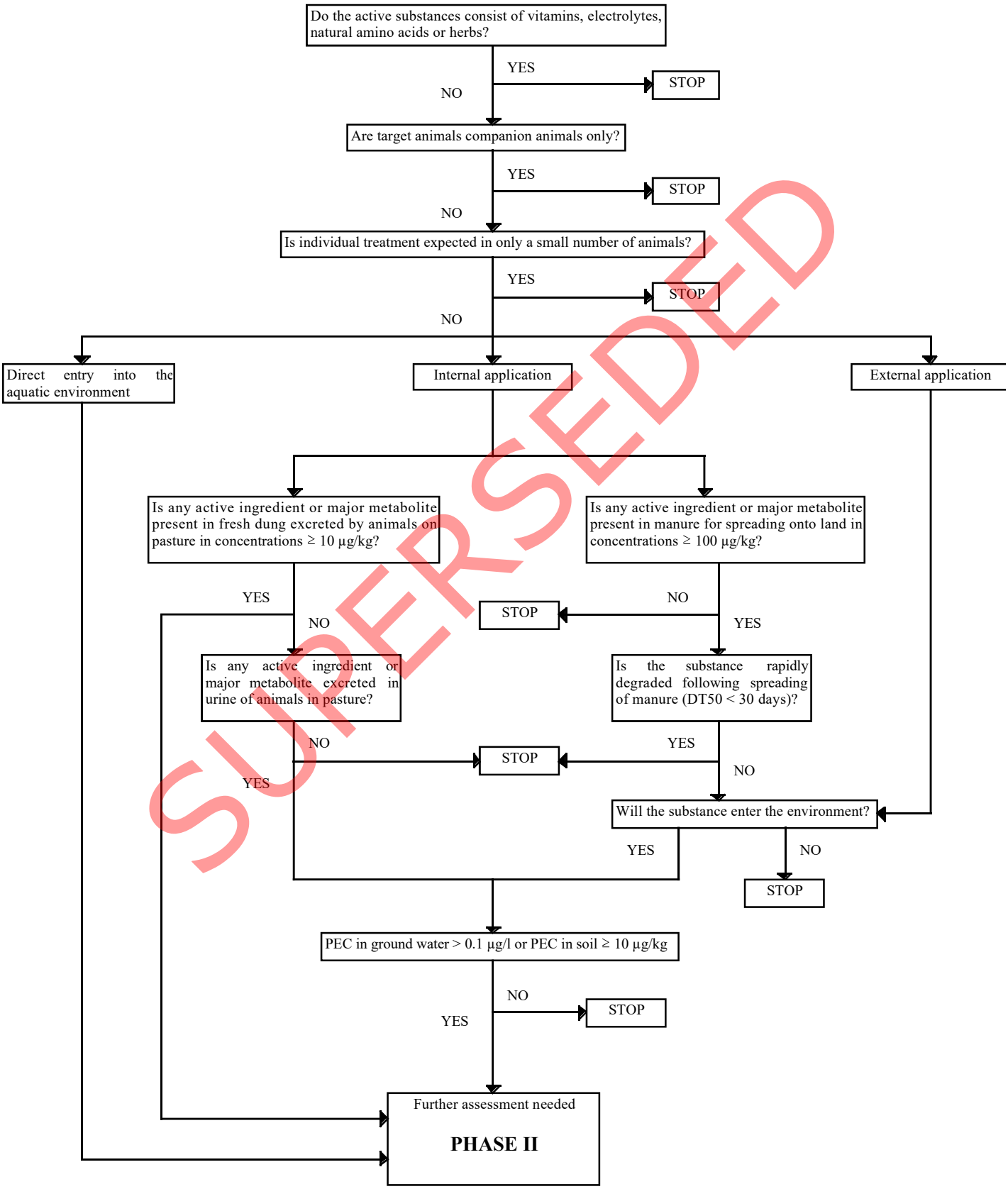
1 The excretion of the active substance and metabolites by the treated animals gives an indication of the extent of the environmental risk. Metabolites which represent less than 20% of the applied dose are not considered relevant in this respect and therefore Phase I can be limited to the parent compound for drugs weakly metabolised.

2 The conclusion 'rapidly degradable' can be based on theoretical calculations or experimental studies in relevant compartments. The presence or degradation of relevant residues can also be shown in bioassays involving relevant target organisms.

3 This should be demonstrated by a 'worst case' calculation.

Figure 2. **PHASE I DECISION TREE**



3. PHASE II

At this point, it is important to make use of all available documentation relevant to the environmental risk assessment of the product. This includes physico-chemical data, relevant pharmacological-toxicological and toxicokinetic studies and information on degradability or persistence of the active ingredient and metabolites under various conditions. To simplify the evaluation procedure a schematic but comprehensive decision tree has been elaborated. Apart from the EEC Directives indicated under general considerations, studies performed to satisfy the requirements of environmental risk assessment posed by other authorities may be used. Specifically, the guidelines and test protocols issued by the European Commission and OECD for testing of chemicals are to be followed whenever possible (Annex I). Only valid and plausible test results should be used in the environmental risk assessment and the principles of Good Laboratory Practices (26 and 27) should apply whenever possible. Definitions and methods of calculation are found in these guidelines. However, the basic definitions are included below.

K_{oc}: Partition coefficient for adsorption/desorption of a substance onto soil (oc stands for organic carbon). OECD Guideline 106 is applicable.

P_{ow} or K_{ow}: Partition coefficient octanol/water. EU testing method A.8. or OECD Guideline 107 are applicable.

LC_{50} or EC_{50}: A concentration lethal to or effective in 50% of the number of organisms or animals included in the test.

MIC: Minimal inhibitory concentration of the most sensitive of the 5 microorganisms tested

PEC/PNEC: Predicted Environmental Concentration/Predicted No Effect Concentration

DT_{50}: Time to degradation to 50% of original concentration of the compound in the tested soils

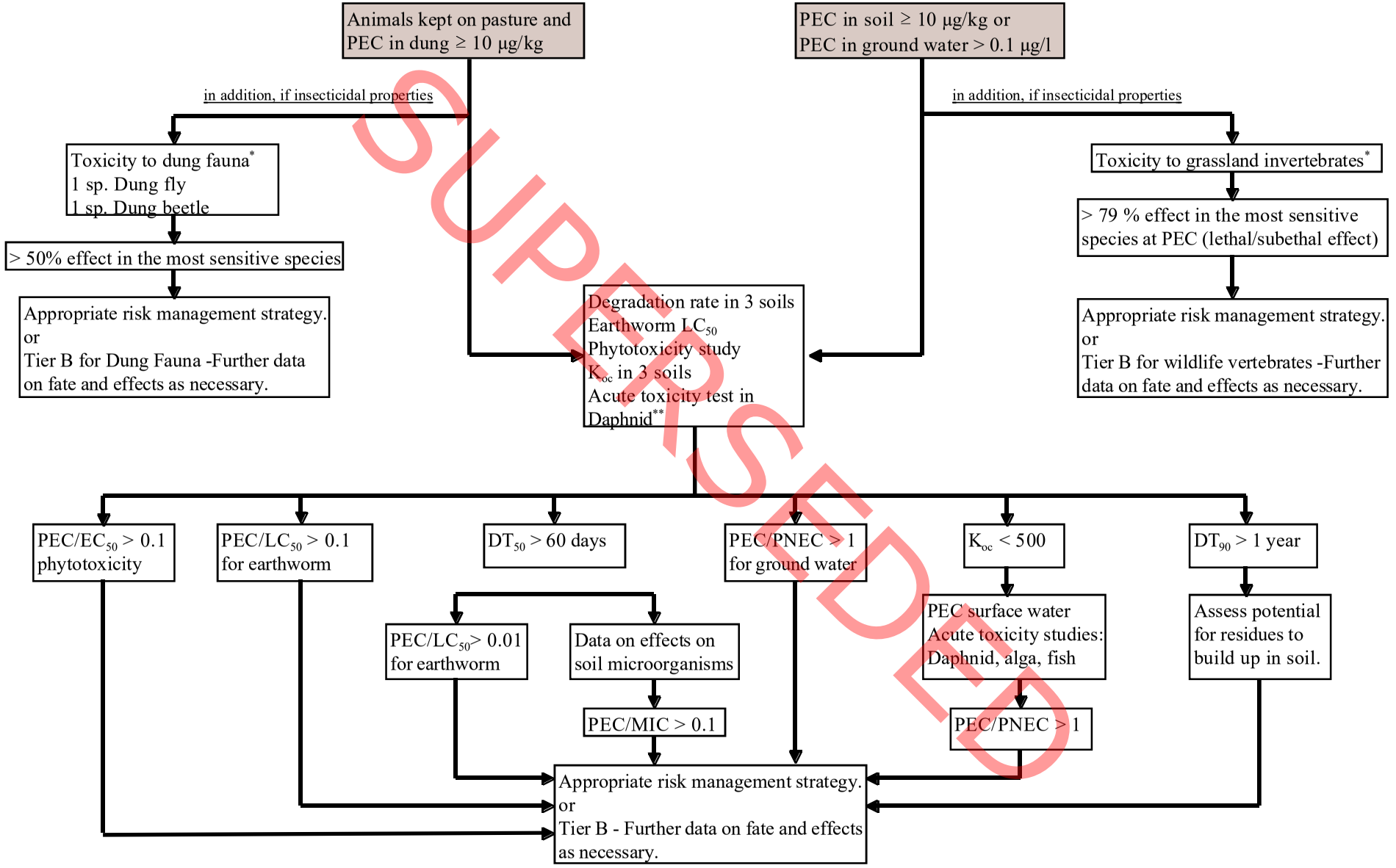
DT_{90}: Time to degradation to 90% of original concentration of the compound in the tested soils

The value of DT_{50} or DT_{90} used should be the average of the values found in the 3 soils tested. The degradation curves should be fitted and the best model should be taken into account for further calculations.

Figure 3.

PHASE II - TIER A
DECISION TREE FOR MEDICINES OTHER THAN FISH MEDICINES

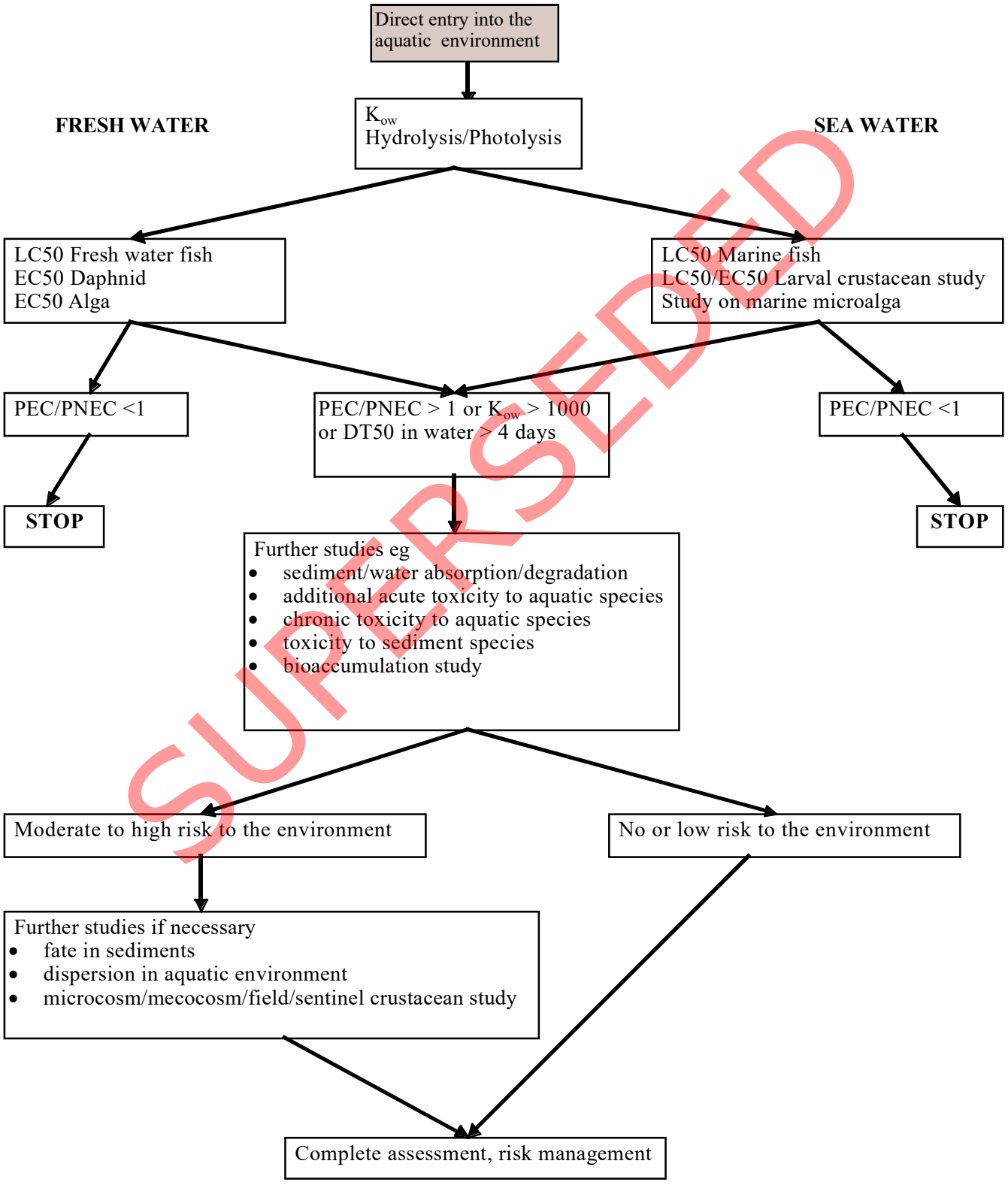
Phase I trigger values in grey boxes



* This should include toxicity test to earthworm in dung if toxicity to earthworm in soil is PEC/LC_{50} > 0.1.
** This test is to be performed if PEC in ground water is > 0.1 µg/L.

Figure 4.

PHASE II - TIER A and B
DECISION TREE FOR FISH MEDICINES
Phase I trigger value in grey box



3.1 Tier A - explanatory notes

The second phase of the environmental risk assessment inevitably begins with a more detailed evaluation of the possible fate and effects of the drug and/or its major metabolites (all metabolites which may reach a concentration of 20% or above of the applied dose) in relevant environmental compartments (Tier A). These include slurries and farm yard manure; other litter such as dirty water as defined in Annex II, section 2); soil, taking into account the possible exposure to manure spread on land and run-off from the soil; aquatic environment, taking into account run-off from soil, possible contamination of ground water and direct exposure in fish farming areas (both running and still water). Examples of estimation of the predicted environmental concentration (PEC) in relevant compartments are found in Annex II.

There may further be a need for the determination of the degradation half-life of the active substance and/or the relevant metabolites in the environmental compartments of interest. During this part of the evaluation, the physico-chemical properties of the active substance and/or the relevant metabolites and the influence of light, pH, humidity and other factors should be taken into account. The kinetics of the elimination of the active substance and/or the relevant metabolites from the environmental compartments of interest may also give valuable information about the environmental fate of the veterinary drug.

There may also be a need to determine the immediate effect of the excreted residues of a veterinary drug and/or the relevant metabolites from animals kept on pasture on dung fauna, the acute effects on terrestrial wild-life, specifically dung-feeding birds and the possible long-term effects on dung fauna and biodegradability of the excreted dung.

The applicant should take all the above indicated factors into account when assessing the possible accumulation and subsequent effects of the veterinary drug in relevant environmental compartments. The environmental risk assessment can be concluded at Phase II Tier A if the exposure calculations demonstrate that the compound does not present any significant risk to the environment or that appropriate risk management strategies are proposed by the applicant to ensure that no environmental risk is associated with the use and disposal of the product.

Emission into the air will be negligible for the vast majority of substances. Nevertheless, in Phase II the applicant should consider whether there is indication of significant air exposure. In such cases, consultation with the competent authority regarding suitable testing is advisable.

3.2. Tier B - explanatory notes

In the case where the assessment indicates that the use of the drug may represent a definite risk to certain species in the environment, a second phase of evaluation will be necessary. In phase II Tier B, or the final stage of the evaluation of the risks to the environment of the veterinary medicinal product, specific tests are required. It should be noted that there may be considerable variation in receptor specificity/sensitivity between species, which may influence the environmental impact. Further guidance on specific test scenarios for the various environmental compartments can be found in Annex III. The applicant is adviced to liaise with the competent authority prior to commencing testing in Tier B.

3.3. Tier B Fish medicines - explanatory notes

For veterinary medicinal products to be used in freshwater or marine aquacultures, the decision tree in figure 4 applies, i.e. basic acute toxicity studies in the relevant species indicated must be supplied in the documentation. Specific guidance is found in Annex IV. If the veterinary drug poses a risk to certain species of aquatic flora or fauna, the applicant may suggest risk management procedures to be followed when using the product for the treatment or prevention of disease. Such procedures should clearly show that the environmental hazard of the veterinary drug is minimised. The applicant is required to submit a statement to this effect, including a risk assessment based on the characteristics of the product and its fate in the environment in relation to the proposed precautionary measures for its use and disposal.

ANNEX I
GUIDELINES FOR TESTING OF CHEMICALS

OECD Guidelines (TG) and corresponding EU Testing Methods (TM)

PHYSICAL-CHEMICAL PROPERTIES

A	OECD TG No.	OECD Testing Guidelines	EU TM No.	EU Testing Methods in Annex V of Directive 67/548
1.	101	UV-VIS Absorption Spectra		None
2.	102	Melting Point/Melting Range	A.1.	Melting/Freezing Temperature
3.	103	Boiling Point/Boiling Range	A.2.	Boiling Temperature
4.	104	Vapour Pressure Curve	A.4.	Vapour Pressure
5.	105	Water Solubility	A.6.	Water Solubility
6.	106	Adsorption/Desorption		None
7.	107	Partition Coefficient (n-octanol/water)	A.8.	Partition Coefficient
8.	108	Complex Formation Ability in Water		None
9.	109	Density of Liquids and Solids	A.3.	Relative Density
10.	110	Particle Size Distribution/Fibre Length and Diameter Distributions		None
11.	111	Hydrolysis as a Function of pH	C.7.	Degradation: Abiotic Degradation: Hydrolysion of pH
12.	112	Dissociation Constants in Water		None
13.	113	Screening Test for Thermal Stability and Stability in Air		None
14.	114	Viscosity of Liquids		None
15.	115	Surface Tension of Aqueous Solutions	A.5.	Surface Tension
16.	116	Fat Solubility of Solid and Liquid Substances		None
17.	117	Partition Coefficient (n-octanol/water). HPLC Method	A.8.	Partition coefficient
18.			A.9.	Flash Point
19.			A.10.	Flammability (Solids)
20.			A.11.	Flammability (Gases)
21.			A.12.	Flammability (Contact with Water)
22.			A.13.	Phyrophoric Properties of Solids and Liquids
23.			A.14.	Explosive Properties
24.			A.15.	Auto-Ignition Temperature (Liquid and Gases)
25.			A.16.	Relative Self-Ignition Temperature for Solids
26.			A.17.	Oxidizing Properties (Solids)
27.	118	Det. Number Average M W and M W Distribution Polymers by Gel Perm. Chromatogr.	A.18.	Number - Average Molecular Weight and Molecular Weight Distribution of Polymers (Draft)
28.	119	Det. Low M W Content Polymers by GPC	A.19.	Low Molecular Weight Content of Polymers (Draft)
29.	120	Solution/Extraction Behaviour of Polymers in Water	A.20.	The same title (Draft)

ENVIRONMENTAL EFFECTS

C	OECD TG No.	OECD Testing Guidelines	EU TM No.	EU Testing Methods in Annex V of Directive 67/548
1.	201	Alga Growth Inhibition Test	C.3.	Algal Inhibition Test
2.	202 Part A Part B	- *Daphnia* sp. Acute Immobilisation Test and - Reproduction Test	C.2.	- Acute Toxicity for *Daphnia* - None
3.	203	Fish, Acute Toxicity Test	C.1.	Acute Toxicity for Fish
4.	204	Fish, Prolonged Toxicity Test: 14-Day Study		None
5.	205	Avian Dietary Toxicity Test		None
6.	206	Avian Reproduction Test		None
7.	207	Earthworm, Acute Toxicity Tests	C.8.	Toxicity for Earthworms: Artificial Soil Test
8.	208	Terrestrial Plants, Growth Test		None
9.	209	Activated Sludge, Respiration Inhibition Test	C.11.	Biodegradation: Activated Sludge Respiration Inhibition Test
10.	210	Fish, Early-Life Stage Toxicity Test		None
	301	**Ready Biodegradability**	C.4.	**Biodegradation: Determination of the 'ready' Biodegradability**
11.	301 A	DOC Die-Away Test	C.4-A	Dissolved Organic Carbon (DOC) Die-Away
12.	301 B	$C0_2$ Evolution Test	C.4-C	Carbon Dioxide (CO_2) Evolution
13.	301 C	Modified MITI (I) Test	C.4-F	MITI (Ministry of International Trade and Industry - Japan)
14.	301 D	Closed Bottle Test	C.4-E	Closed Bottle
15.	301 E	Modified OECD Screening Test	C.4-B	Modified OECD Screening Test
16.	301 F	Monometric Respirometry Test	C.4-D	Manometric Respirometry
		Inherent Biodegradability		**Biodegradation:**
17.	302 A	Modified SCAS Test	C.12.	Modified SCAS Test
18.	302 B	Zahn-Wellens/EMPA Test	C.9.	Zahn-Wellens Test
19.	302 C	Modified MITI (II) Test		None
20.	303 A	Simulation Test - Aerobic Sewage Treatment: Coupled Units Test	C.10.	Biodegradation: Activated Sludge Simulation Tests
21.	304 A	Inherent Biodegradability in Soil		None
22.	305 A	Bioaccumulation: Sequential Static Fish Test		None
23.	305 B	Bioaccumulation: Semi-Static Fish Test		None
24.	305 C	Bioaccumulation: Degree of Bioaccumulation in Fish		None
25.	305 D	Static Fish Test		None
26.	305 E	Flow-Through Fish Test		None
27.	306	Biodegradability in Seawater		None
28.			C.5	Degradation: Biochemical Oxygen Demand
29.			C.6.	Degradation: Chemical Oxygen Demand

ANNEX II
EXPOSURE SCENARIOS

A series of exposure scenarios has been produced to help users of the guidance document in estimating the PEC (Predicted Environmental Concentration). These are not intended to be prescriptive, but are to inform users of the types of assumptions that may be made and to bring a consistent approach to exposure assessment. However, alternative exposure scenarios may be equally acceptable if submitted as part of the dossier.

The following exposure scenarios are included for guidance:

1. Estimation of PEC in soil from spreading of drug residues in slurry;
2. Estimation of PEC in soil from disposal of drug residues in dirty water, where this is disposed of separately to slurry;
3. Estimation of PEC in soil resulting from drug residues excreted outdoors;
4. Estimation of PEC in water (surface and ground water);
5. Products with direct entry to the aquatic environment.

1. ESTIMATION OF PEC IN SOIL FROM SPREADING DRUG RESIDUES IN SLURRY

1.1. Calculation of PEC in soil

A method for estimating the PEC_{soil} for intensive livestock treatments which are excreted in urine or faeces and the slurry is to be spread onto land may be based on the following components:

- total dose/animal/year, i.e. the total dose given to the number of animals that occupy a defined space in the farm during a year;
- excreta production/animal/year;
- percentage of animals treated;
- degradation during storage;
- maximum slurry application rates/year;
- plough depth (an estimate should also be made based on slurry not being ploughed into soil, assuming even distribution of residues to 5cm);
- soil density (1.5 g/cm^3).

If information is available, the estimate should take account of the percentage of the dose that is excreted. If there is a specific interval at which the slurry is spread onto land, this should be taken into account in the 'worst case' scenario.

1.2. Degradation of compound in manure during storage

From the estimate of the initial concentration in slurry an assessment is required of likely residues in slurry at the time of spreading on land. Information is required on the nature and concentration of residues in excreta, and the persistence and kinetics of elimination of these residues in slurries, farm yard manure and litter. If residues degrade significantly in slurry during storage, producing a PEC_{soil} estimate for active substance or metabolites of less than 10µg/kg, there will be no need to assess the risk to the wider environment. Some indication of the rate of breakdown of a veterinary medicine or its transformation products in slurry may be determined from basic data on chemical and physical properties.

2. ESTIMATION OF PEC IN SOIL: DISPOSAL OF DRUG RESIDUES IN DIRTY WATER

Dirty water is waste, generally less than 3% dry matter, made up of water contaminated by manure, urine, crop seepage, milk, other dairy products or cleaning materials. Although dirty water may be dealt with separately to slurry, it is defined as slurry. With respect to contamination of dirty water by veterinary medicines, this exposure scenario will generally only be applicable to products used in dairy cows. Use of a veterinary medicine may result in the product being found in washings from dairy parlours, or where milk is discarded into the dirty water system. Discarded milk may alternatively be fed to calves or disposed of in slurry.

The maximum application rate for spreading or irrigating dirty water onto land is generally 50,000 L/ha. Dirty water may be irrigated directly onto land or stored for a period up to a few months. The equipment used for spreading can generally handle volumes of 4,000-10,000 L at a time. Dirty water is not ploughed into land the assessment therefore assumes even distribution of residues to 5cm depth, and a soil density of 1.5 g/cm^3. The following table provides information on amounts of water used in different operations:

Livestock type	Cleaning system	Amount of water used, L/animal/day (range)	Amount of water used, L/animal/day (typical)
Dairy cows	cleaning milking parlour equipment, washing udders, etc without a power hose	14 - 22	18
	with a power hose	27 - 45	35
Pigs	cleaning out pens after each batch (10 pigs per pen)	16 - 24	18

3. ESTIMATION OF PEC IN SOIL FROM DRUG RESIDUES EXCRETED OUTDOORS

Some treatments to outdoor-reared animals may be excreted directly onto pasture in either urine or faeces. PEC estimates for residues in soil from urine may assume direct entry to soil and even distribution in the upper 5cm. However, residues in soil resulting from leaching from dung are more difficult to estimate and should take account of whether the residue is likely to be strongly adsorbed to dung, and therefore unlikely to leach into soil (see also insectidical activity). These estimates should be made for whole herd/flock treatments where residues will be excreted by grazing animals. The estimate is based on the following:

- dose/animal based on mg/kg bw and body weight of animal
- percentage of dose excreted by treated animals, if there are no data assume 100%
- stocking density of treated animals/ha
- assumption that residues are evenly distributed in top 5cm of soil, throughout field
- soil density is 1.5 g/cm^3

Examples of stocking densities are given below:

Sheep 10 (upland) - 15 (lowland)/ha
Dairy cows 2 - 3.33/ha
Beef cattle, 200kg at turnout, grass grazed without cutting 3.5 - 9.5/ha
Beef cattle, 350kg at turnout, grass grazed without cutting 2.4 - 6.4/ha
Sows 14 - 19 /ha

Typical animal weights are:

Dairy cow	450 - 650 kg
Beef bullock	200 - 450 kg
Sow	90 - 120 kg
Pig	45 - 75 kg
Fattening lamb	45 kg
Mature sheep	60 - 80 kg

4. ESTIMATION OF PEC IN WATER

4.1. Surface water

When estimating the concentrations that may reach surface waters by run-off the influence of normal agricultural waste disposal methods, according to national or European regulations should be taken into account. These may place restrictions on disposal, such as the types of land that may be used, or stipulate the use of buffer zones between areas used for spreading and surface waters.
There are few public domain models available to enable an estimate to be made, and these generally have been developed for pesticides or for industrial chemicals. If used, the procedure of deriving an exposure level by applying model calculations should be made transparent within the dossier. The choice of model should be explained, and the parameters and default values used for the calculations must be documented.
Adsorption to solid surfaces is the main partitioning process which drives distribution between soil and surface waters. The K_{oc} may be measured by adsorption studies (see Annex I) or by Quantitative Structure Activity Relationships (QSARs) using the octanol/water partition coefficient K_{ow}. This type of calculation can only be used when the substance fulfills certain prerequisites as follows: organic substance, MW<500, solubility in water >2 mg/L and non-ionizable.
Substances not adsorbed to soil particles may be present in the soil water (interstitial pore water) and thus be prone to run-off during rainfall events. The concentration in surface waters will be influenced by the amount of rainfall relative to interstitial pore water, and subsequent dilution by the receiving water. The concentration of substance in the interstitial pore water can be estimated using the formula:

$Ciw = Cs/Koc * foc$ where Ciw = concentration in interstitial water (µg/l)
Cs = concentration in soil (µg/kg dry soil)
foc = fraction of organic carbon in soil (kg oc/kg dry soil)

If the PEC/PNEC ratio is greater than one then the PEC should be further refined, using appropriate dilution factors, as indicated below. PNEC is derived from acute toxicity studies in fish, *Daphnia magna* and algae as referred to in figure 3 (see Annex I and Annex III Tier A, 2.3.).
It is assumed that catchment areas tend to be proportional in size to the receiving stream therefore no account is taken of the size of the catchment or receiving water.
Run-off will occur after a rainfall event thus diluting soil water. It could be assumed that soil moisture increases by 10% when run-off occurs.
Further dilution occurs on entry of run-off water into the receiving water. It can be assumed that one part run-off water will be diluted by two parts receiving water.

4.2. PEC in ground water

For the estimation of the PEC in ground water there are several useful models available for the evaluation of pesticides. The models are summarised in the Commission's Guidance Documents 1694/VI/95 (Modelling Environmental Fate of Plant Protection Products in the Context of their Authorization within the European Union) and 4952/VI/95 (Leaching Models and EU Registration). Each model gives the estimation of the PEC in the ground water based on a standardised scenario taking into account meteorological, hydrological and application data. The main parameters are the soil degradation DT_{50} and the soil sorption characteristic K_{oc}.
The PNEC is derived from the acute toxicty test in *Daphnia magna* (see Annex I and Annex III Tier A, 2.3. and 2.4.).

5. PRODUCTS WITH DIRECT ENTRY TO THE AQUATIC ENVIRONMENT

This refers specifically to the fish medicines, used either in marine or freshwater situations. These products have the most direct entry into the environment, particularly in the case of caged fish placed in natural waters. Aquatic life in waters surrounding cages, or in rivers receiving effluent from fish farms are at risk from exposure.

5.1. PEC estimate for fish medicines used in freshwater

PEC estimates should be based on the concentration of drug in the effluent from the fish farm and the subsequent dilution of this on entry into receiving waters. There are a number of reference books on fish husbandry which could provide suitable values for input into a PEC calculation.

An example is given below of the parameters that may be used in such a calculation, with some typical values for a trout farm in the south of England:

- annual production 50 tonnes of trout for the table each year;
- water requirement 4,550,000 litres/day/10 tonnes annual production;
- information on dose, time period over which this is given, metabolism and excretion and any wastage by fish, can be used with the above values to estimate the concentration in effluent leaving the farm fish;
- dilution of effluent on entry to the receiving water, this will usually be in the ratio of effluent:receiving water of 1:2, 1:1 or 2:1.

The assumptions made may need to be varied according to local practices governed by national regulations in the Member States.

5.2. PEC estimates for fish medicines used in marine situations

See specific guidance in the detailed explanation in annex IV.

ANNEX III

PHASE II - TIER A AND TIER B

TIER A

1. TERRESTRIAL ENVIRONMENT

These notes provide information to users on appropriate types of studies to use in the environmental risk assessment, and an explanation of how such data are used.

1.1. Degradation in soil (DT 50)

This study will indicate whether data are needed on effects on microbial function and an appropriate extrapolation factor to use in conjunction with the earthworm data. There is no OECD guideline for this type of study, but as such information is collected for pesticides suitable protocols are available.
Studies on fate and behaviour of the test substance in soil should be conducted in the laboratory rather than in the field. Soils used for these studies should be freshly sampled from agricultural land and from sites where there have been no treatments during the previous 5 years that may have affected microbial populations, e.g. soil sterilants. Soil should be properly handled and the type used should be appropriate for the particular study. Soil properties such as pH, percentage organic matter, particle size distribution and water holding capacity should be reported.
The transformation rate (disappearance time, DT_{50}) is dependent on many factors, such as temperature, soil moisture content, as well as soil type. This is measured for 3 soils in total as part of Tier A. The soils should cover a range of pH, organic matter and clay contents representative of soils where residues may occur, including one with low microbial activity. If degradation is expected to be pH dependent then soils should have a wider range of pH values, e.g. 4.5 - 5.5, 6 - 7 and approximately 8.

1.2. Toxicity tests

Toxicity to earthworm
The artificial-soil test according to EU Testing Method C8: Toxicity to Earthworms: Artificial Soil Test or the OECD guideline 207 are suitable when either of the trigger values in soil or in dung have been reached. When residues of drug and/or metabolites present in dung excreted in pasture exceed the trigger for PEC in dung of ≥ 0.1 µg/kg, the trigger for further testing of earthworm toxicity in dung is : PEC dung/LC_{50} earthworm > 0.1.

Phytotoxicity to terrestrial plants
OECD Guideline 208 provides suitable guidance on testing. Initially tests should be conducted in a minimum of three species, as suggested by OECD, the endpoint of testing is the EC_{50}. Such testing is only required where residues will be disposed of to land that is to grow crops, not where disposal is to grassland or to non-crop land, nor for residues in dung.

Effects on microorganisms
The microbial growth inhibition study outlined in the Environmental Assessment Technical Handbook produced by the FDA is suitable (7). Such testing is only required where the DT_{50} in soil is more than 60 days.

Insecticidal activity
This is only of concern for products where there may be excreted residues in dung, for high volume topical products where disposal to land is permitted and/or where residues in fleece may affect vertebrate wildlife.

The following may be used as evidence of insecticidal activity:

- product indications may include activity against arthropod species;
- other compounds in the same chemical group may have been shown to have activity against arthropod species;
- drug screening data show activity against arthropod species;
- other evidence, e.g. in the literature, indicating insecticidal activity.

The following information may be used as evidence of lack of insecticidal activity:

- related compounds may have been shown to have a lack of activity against arthropod species;

Where there is no information a test should be conducted if any of the following apply:

- where residues of drug and/or metabolites are likely to be present in dung excreted in pasture (see Tier B Test on Dung Fly);
- where residues of used high volume topical application are likely to be spread onto land (see Tier B Grassland Invertebrates);
- where residues of high volume topical application are likely to be present in fleece (see Tier B Vertebrate Wildlife).

2. AQUATIC ENVIRONMENT

2.1. Estimation of PEC in surface water

This section applies to:

- Drug substances for which the K_{oc} is less than 500 and the PEC in soil is greater than 10 µg/kg.
- Treatments of animals in grazing pastures with veterinary medicines that are subsequently excreted in dung, and where residues have insecticidal activity. In the case of these products the aquatic effects assessment should be concerned with aquatic invertebrates and it is not necessary to test other taxonomic groups.

For dung excreted into surface waters containing residues with insecticidal activity, it is assumed that 1% of the dose given/ha is excreted into a stream of volume $100m^3$. This is based on the following assumptions:

- that livestock roam freely over pasture and do not spend a greater proportion of time in any one area, including any stream passing through the field;
- that excretion is as likely to occur into the stream as into the pasture;
- that a hectare of pasture contains a slow-flowing stream 100m long, 1m wide and 1m deep.

2.2. Other exposure scenarios

For run-off from land into surface waters consult exposure scenario 4.1. in Annex II.

As indicated in the Phase I desicion tree and in the exposure scenario 4.2. in Annex II, the trigger limit of 0.1 µg/L for ground water apply in principle only to substances which are readily dissolved in water (solubility >30 mg/L) and which do not bind to soil (K_{oc}<500).

Fish medicines should be considered in Tier B (Annex IV Fish Medicines).

2.3. Acute toxicity tests

The requirements are for a 96-hour LC_{50} study for a species of fish; a 48-hour EC_{50} study for *Daphnia magna* (the only study required for assessment of excreta deposited into surface waters or where the PEC in ground water is above 0.1 µg/L); and an EC_{50} study for a species of alga. Suitable methods are listed in Annex I (e.g. EU testing methods C1-C3 or OECD guidelines 201-203).

2.4 PEC/PNEC ratio for surface water and ground water

Laboratory data for individual species are used to predict no-effect concentrations in the environment (PNEC), i.e. for ecosystems and communities. Therefore, extrapolation is required to account for the many variables which may influence the predicted no-observed effect concentration in the environment. When using single species laboratory acute data to predict no effect levels in the field, the extrapolation factor must take into account intraspecies variation, interspecies variation, potential duration of exposure, and artificial test conditions.

The PEC/PNEC is the ratio between the Predicted Environmental Concentration (PEC) and the Predicted No-Effect Concentration (PNEC) for the environmental compartment under consideration. Clearly the PEC is not a single value but will alter both temporally and spatially as the substance is degraded and dispersed. On initial entry into the environment the PEC will be relatively high, whether it remains high will depend on whether it becomes more widely dispersed in the receiving environment and on how stable it is. At Tier A there will generally be insufficient information to determine this as data will not have been obtained on fate of the substance in surface waters.

The PNEC is designed to protect ecological communities within the environmental compartment, not just individual species, and from long-term as well as short-term effects. Where only limited laboratory data on effects are available extrapolation factors are applied to the data to derive the PNEC. When using single species laboratory acute data to predict no effect levels in the field, extrapolation must take into account intraspecies variation, interspecies variation, potential duration of exposure, and artificial test conditions. Arbitrary safety factors are used to quantify this variation. In these guidelines arbitrary safety factors for extrapolation from acute effects to potential chronic and field effects have been adopted. The factors are applied to the lowest LC_{50} value, i.e. based on the most sensitive species, in the acute toxicity dataset.

A factor of 100 is used on the basic acute toxicity data set. This is based on the recommendations of an OECD workshop on extrapolation of laboratory toxicity data to the environment (22). For instance, if the LC_{50} in the most sensitive species was 100 mg/l, the PNEC would be 100/100, i.e. 1 mg/l.

3. AIR EXPOSURE

Based on the volatility and Henry's Law constant an indication can be provided about the potential of a substance to be released into air. A vapour pressure in Pascal (Pa) lower than 0.0001 Pa generally can be classified as very slightly volatile and is therefore not considered as relevant for the air compartment. Henry's Law constant (H) may be expressed in a dimensionless form (also called air-water partition coefficient) as follows: H=(PxM)/(SxRxT)

where: H = Henry's Law constant (-) S = Solubility (g/m^3)
P = Vapour pressure (Pa) R = Gas constant (= 8.3) (Pa. m^3/mol.K)
M = Molecular weight (g/mol) T = Absolute temperature (K)

TIER B

If the PEC/PNEC ratio is greater than one or any other of the trigger values are exceeded further assessment at Tier B is required. It is only those products for which there is an indication of adverse ecotoxicological effects that should progress to Tier B. An assessment should only be conducted in those sections of Tier B for which Tier A indicates that this is necessary.

The Tier B guidelines are intended to provide information to users on issues that are likely to be relevant to the environmental risk assessment of their product. The guidelines are also a source of additional references in the literature that may be of use in deciding the further data that may need to be generated. These guidelines should not be used by industry or regulators in a prescriptive way, and the reference section should not be treated as a checklist of data requirements.

It is recommended that the applicant discuss the data they propose to generate and their approach to the risk assessment with the appropriate regulatory authority at the planning stage. Prior to such discussions the applicant should be familiar with the approach used in the guidelines and should have a proposed plan for discussion.
Parts of Tier B deal with areas of ecotoxicology that are still under development, and in some cases there are no internationally recognised guidelines in place for studies that may need to be conducted. Information has been included on approaches that may be used but these are not exhaustive. It is accepted that other approaches may be equally valid, and that as the science develops new techniques and study guidelines may become available.

1. SOIL FAUNA

This scheme deals with risk assessment for soil fauna and is based to some extent on risk assessment for pesticides, using earthworms as the indicator species (14). Earthworms contribute to soil fertility and are an important part of terrestrial food webs. The soil mesofauna, made up of numerous species and taxa, is known to contribute to decomposition processes.

1.1. Degradation in soil (DT50) and soil transformation pathway

Further data on effects on earthworms are triggered by stability of residues in soil, where the DT_{50} (degradation half-life) is more than 60 days, which is equivalent to a DT_{90} (time to 90% degradation) of 180 days. If a drug substance is present in soil for this length of time it will have the potential to exert toxic effects during the lifecycle of earthworms, which could lead to population changes.

Data on chemical and physical properties, as well as specific studies in soil should be used to provide information on environmental fate (see methods listed in Annex I). Studies on fate and behaviour of the test substance in soil should be conducted in the laboratory rather than in the field.

The transformation pathway (identification of metabolites) is independent of soil type and therefore only needs to be studied in one soil. The soil used should not be an extreme type and should be relevant to the situations in which soil residues might be found.

1.2. Sublethal effects on earthworms

There are no internationally accepted guidelines for a test on sublethal effects, but several drafts exist and examples of studies can be found in the literature. If the need for a test on sub-lethal effects is indicated then a suitable protocol should first be discussed with the regulatory authority.

The use of juvenile worms should be considered for specific tests in which growth is an endpoint, and where it is intended to examine effects on bodyweight changes. If the environmental fate of the test substance indicates that there is likely to be exposure of earthworms during periods when they are reproducing, then specific data may need to be

obtained. In tests assessing effects on reproduction, both fecundity and fertility should be examined (19).

1.3 Field studies

Tests carried out under field conditions have the advantage of realism, but there may be difficulties in designing suitable experiments and in interpretation of results. Because field conditions are highly variable, a flexible approach to design of test protocols is appropriate where the need for such tests is indicated. Protocols should be discussed with the regulatory authority before any work is conducted.

Test sites can be chosen according to soil characteristics, earthworm density and whether grass or cultivated fields are more appropriate. Size of test plots is a compromise between the requirement for replication to enable statistical validity and the need to have large enough plots to mitigate the effects of immigration/emigration. They should also take account of the size of areas likely to contain residues. There are various sampling methods, each with their particular limitations, and therefore it may be preferable to use more than one method (17).

Interpretation of field effects data
Interpretation of effects should include measures of abundance and biomass. In addition adult:juvenile ratios may demonstrate changes in reproduction. Ideally each species should be evaluated separately, but for reasons of practicality it may be necessary to combine data from species. Interpretation of results should take account of 'natural' fluctuations in populations, the biological significance of effects and recovery from effects. The assessment should take acccount of the kind of effect; initial degree of effect; the time course of effect and subsequent recovery time; the ecological categories that are affected and the number of species affected. Generally effects should be judged as especially serious if the decline in the overall number and/or biomass is > 50% or if the recovery time exceeds the interval between two exposures.

2. SOIL MICROBIAL FUNCTION

Soil microflora have a critical role in the maintenance of soil fertility. It is the potential adverse effects on this function that are being considered, rather than any measured effects on abundance or diversity of micro-organisms. This part of the scheme has been adapted from the EPPO scheme for pesticides (15). Testing is based on the requirements for pesticides as set out in the Uniform Principles.

2.1. Degradation in soil (DT50)

See notes for soil fauna.

2.2. Tests for effects on soil microflora

Testing is based on the application of methods which will detect changes in both nitrogen mineralisation and carbon mineralisation in a sensitive agricultural soil. Suitable tests for agricultural soils can be found in (16). Deviations from these guidelines may be acceptable provided good reasons exist.
The soil used should be agricultural soil, usually sandy soil (<70% sand), pH 5.5-7, low organic matter content (0.5 - 1.5% organic C) and have a microbial biomass of not less than 1% of the total soil organic C content. This represents a 'worst case' scenario agricultural soil. The selected sampling site should not have been treated with plant protection products for at least one year, nor with organic fertiliser for at least 6 months, nor mineral fertiliser for 3 months.
Results should be reported in comparison to the untreated control. If deviation from the control treatment at the end of the test is more than 25% then further consideration of effects is necessary.

2.3. Natural fluctuations in soil microflora

Compare the scale of the observed effect with fluctuations known to occur as a result of natural events in soil. The magnitude of 'natural' fluctuations in soil microflora can be assessed from the published literature (e.g. 5).

A duration of effects of less than 30 days may be considered to be within normal fluctuations. A duration of 31 - 60 days may be considered to be similar to normal fluctuations, and a duration of more than 60 days greater than normal fluctuations. In general soil microbial function tests are conducted for 30 days therefore, if there is an adverse effect at the end of the 30-day tests, there may be a need to conduct additional tests for a period of up to 100 days to clarify these effects.

3. PHYTOTOXICITY

The main concern in this scheme is the potential effects of residues in slurry on crop plants, i.e. phytotoxicity. At Tier A - Soil, EC_{50} data will have been generated for 3 species.

3.1. Refinement of PEC

At this point there is an opportunity to determine the time period over which the PEC may occur, or how it may alter over time. However, even if soil residues occur over a relatively short period they may still exert a phytotoxic effect if crops come into contact with them soon after spreading of slurry onto crop land. If there is evidence of phytotoxicity, data on environmental fate will be useful in devising suitable risk management strategies to protect against crop damage, e.g. delaying sowing.

3.2. Phytotoxicity to terrestrial plants

OECD Guideline 208 provides suitable guidance on testing, one species from each of the three groups listed by OECD will have so far been tested. If phytoxicity has been indicated it will be necessary to test these again, together with additional species, to determine the range of species in which effects occur.
In Tier B studies should be conducted so that a total of 6 species have been tested, the additional 3 species tested should be related to the most sensitive species tested at Tier A.

3.3. Effects in the field

There are no internationally agreed guidelines, however studies may be based on similar requirements for pesticides. The drug should be applied during the study in the form in which it would be expected to be applied to crops, i.e. in slurry, so as to simulate bioavailability of residues. It is recommended that studies are conducted using small plots within a field rather than whole fields to enable replication, so as to produce more readily interpretable results. Use of a range of doses (e.g. 3 - 5) and an untreated control (slurry without residues) will enable determination of a dose-response, which will also aid interpretation of data.

4. DUNG FAUNA

This part of the assessment concerns the potential risks to dung fauna exposed to residues of product present in dung pats produced by treated livestock. Such an assessment is only relevant where dung of cattle, sheep, horses or deer are likely to contain significant residues, as identified by a Phase I and Tier A-Terrestrial environmental risk assessment.

4.1. Estimation of PEC

Information from pharmacokinetic studies will indicate the percentage of dose excreted in dung. If data are not available then 100% excretion of dose in the dung should be assumed. The PEC should be expressed as amount drug/weight of dung, not as amount of drug/ha. To estimate the PEC information is needed on the amount of dung produced daily by livestock. Such information is not generally available, only on total excreta production (see below). Use of total excreta amounts in the PEC calculation will underestimate residues in dung and this should be taken into account when comparing data on effects.

Amounts of excreta produced by livestock (20):

Dairy cow (450 - 650 kg)	57 l/day
Beef bullock (200 - 450 kg)	27 lday
Fattening lamb (45 kg)	2.2 l/day
Mature sheep (60 - 80 kg)	4 l/day

4.2. Laboratory studies

Earthworms
Earthworms are quantitatively the most important decomposers of dung organic matter under temperate conditions. The toxicity to earthworm is tested at Tier A using the artificial soil test, a standard test which provides some indication of whether residues are likely to be toxic in dung. Having exceeded the triggers for further testing at Tier A, it is important to test the bioavaibility of drug residues in dung. Although further testing is based on artificial soil test, the use of dung as a medium has not been standardised and therefore results may be more variable.

Two possible approaches to testing are suggested here, In all cases it is advisable to include both an untreated soil control and an untreated manure control, together with artificial soil treated at one concentration, e.g. the maximum concentration previously tested or a concentration similar to the LC_{50}. With respect to manure treatments, either a single maximum dose may be used, or a range of concentrations in order to obtain a dose:response.

Trigger values and further testing should be in accordance with Tier B: Soil Fauna-Earthworm. However, should testing in the field be necessary, proper consideration will need to be given in the protocol to the route of exposure, i.e. via dung pats.

Dung insects
Several aspects have to be taken into account when selecting which species of dung feeding insects to test. In practice, species which are easy to rear in the laboratory can be used but they must also be representative of species likely to be exposed in the field. The species to be tested should represent the two major insect orders occurring in dung, i.e. Coleoptera and Diptera, and relevant stages of the life-cycle should be tested. As regards beetles feeding on dung, there are certain difficulties to rear species common in Europe.

Examples of the types of studies that may be used are given in the list of references (30, 31 and 35)

4.3. Use of trigger values for field testing

The trigger values used are based on those of the IOBC (International Organisation for Biological Control), which are based on their experience of testing with species that are natural enemies of crop pests. The IOBC currently have trigger values for laboratory tests with natural substrates and field trials, these are listed below:

less than 25%	harmless
25 - 50%	slightly harmful
51 - 75%	moderately harmful
greater than 75%	harmful

If there is more than 50% effect on dung fauna in laboratory studies at relevant concentrations, data from field studies will be needed to assess the significance of these effects.

4.4. Field studies

These tests are the most similar to the 'real world' and give information on a range of species. The results are, however, usually the most difficult to interpret and to determine the mechanisms underlying the observed effects. There are examples of field studies having been conducted with these types of treatment, however such studies have been for regulatory purposes and are not reported in the literature.

Design of the study should enable establishment of baseline measurements, e.g. use of an untreated control or a negative control (treatment without activity against dung fauna). Sufficient replication must be included in the trial to enable effects, if they occur, to be detected by statistical methods. A toxic standard, relevant to the drug under consideration, may be a useful inclusion so that the ability of the trial design to detect effects can be confirmed. The rate of dung degradation should also be investigated.

Interpretation of results should take into account the usage pattern of the drug, the extent of contamination of dung, the season in which contamination may occur. An assessment should be made of the likely scale of effect and its biological significance. P. Skidmore has produced a classification of dung insects which may aid in interpretation of effects (34).

5. GRASSLAND INVERTEBRATES

This scheme is concerned with assessing the potential risks to grassland invertebrates, more specifically arthropods. It applies to surface-dwelling invertebrates exposed to drug residues through disposal of used high volume topical applications, e.g. sheep dips, that have insecticidal activity. It is derived from the Arthropod Natural Enemies scheme of the EPPO/Council of Europe Joint Panel on Environmental Risk Assessment (14).

5.1. Choice of test species

Several aspects have to be taken into account when selecting which grassland invertebrate species to use in laboratory studies. In practice, species which are easy to rear in the laboratory can be used but they must be representative of species likely to be exposed in the field. The species to be tested should be at least from different families or preferably different orders. Suitable species for testing and their groupings are given below:

Polyphagous predators	*Pterostichus cupreus*
	Bembidion lampros
	Tachyporus hypnorum
	Aleochara bilineata
Aphid parasitoids	*Aphidius* spp
Lepidopteran parasitoids	*Trichogramma cacoeciae*

5.2. Laboratory toxicity test: susceptible stage

Products applied to land in liquid form can be evaluated by determining toxicity in simple worst-case laboratory tests, which should be conducted for two species. The stage of development which would be exposed in the field and thought most susceptible to the product should be tested, to represent a standard 'worst case' exposure, using an inert substrate such as glass or sand. Any mortality would be recorded along with appropriate measures of sub-lethal effects. The use of a toxic standard, of known effect, would prove useful in the evaluation, e.g. dimethoate.

Guidelines for testing are produced by IOBC (International Organisation for Biological Control) and the BART Group (Beneficial Arthropods Testing Group). There are few recognised protocols for regulatory testing, although some aspects of testing procedures are widely agreed upon. Invertebrates should be exposed to fresh, dry residues, applied to an inert surface, to measure initial contact toxicity. Forced ventilation should be used in cages to reduce vapour effects. Test organisms should be laboratory-reared and of known sex, weight, and uniform age, they should be exposed to the maximum estimated dose or concentration of the drug. Sub-lethal effects, such as a decline in reproductive potential or reduced feeding, in addition to mortality, should where possible be used as a measure of the effect. Food should be offered in excess and environmental conditions should be optimal for the species.

Many of the products likely to be tested may also be used as pesticides, e.g. organophosphorus sheep dips. It is quite likely that these will have been tested for their effects on natural enemies of pests in arable crops, particularly natural enemy species found in cereals. Laboratory data generated for this purpose may also be extrapolated to grassland invertebrates.

The IOBC currently have a classification for products based on percentage effect (either mortality or predation/reproduction effects), which can be used as trigger values for further testing:

less than 30%	harmless
30 - 79%	slightly harmful
80 - 99%	moderately harmful
greater than 99%	harmful

If data from worst-case laboratory tests indicate a more than 79% effect, in any of the test species, then the next stage of testing will be required.

5.3. Dose-response laboratory tests using natural substrate

Further laboratory tests may be useful to clarify the toxicity of the test substance. Such tests should use a maximum of 4 species and include at least one of the species tested at in the single dose, worst-case study. The dose-response studies may involve testing other, more robust stages, should use a range of doses or concentrations to obtain dose-response curves, and more realistic surfaces such as natural soil or plant material. The use of an inert substrate for the original laboratory test probably represents a 'worst case' situation and the effects may be reduced if a more realistic substrate is used instead. The substrate, with or without test species, can be treated either in the laboratory or in the field and returned to the laboratory for testing. Material treated in the field can be returned to the laboratory at intervals after treatment to measure persistence of residue effects.

The concept of trigger values can be applied to laboratory studies with natural substrates, although the values are reduced because the conditions are no longer considered to be 'worst case'. A greater than 50% effect at this stage of testing triggers the need for further studies:

less than 25%	harmless
25 - 50%	slightly harmful
51 - 75%	moderately harmful
greater than 75%	harmful

5.4. Field studies

These tests are the most similar to the 'real world' and give information on a range of species. The results are, however, usually the most difficult to interpret and to determine the mechanisms underlying the observed effects.

Numbers of grassland invertebrates will vary according to the time of year and therefore any field study should be conducted when numbers are high (summer months), ensuring that this is also relevant to the time of year when the product will be used and disposed of.

A replicated field study should be conducted with a minimum of 4 replicates for sufficient statistical validity, and preferably in a randomised block design. Small barriered plots at least 10m x 10m are suitable and can be sited within a 1 ha area. The use of barriers limits the re-invasion of plots from surrounding areas by epigeal fauna. Sites treated with insecticides or molluscicides in the previous year should be avoided.

Treatment of plots should be at the maximum rate of disposal to land. A toxic standard, e.g. propetamphos, should be included so that the ability of the trial design to detect effects can be confirmed. An untreated control is also needed to measure natural fluctuations in populations during the trial.

Sampling must be done before as well as after treatment to establish baseline measurements. The species groups referred to above should be given particular attention and identification should be to species level where possible. Sampling can be by pitfall traps for polyphagous predators, sweep netting or suction sampling can be used to collect other species. Parasitoid emergence can be assessed in the laboratory from mummies collected in the field.

Interpretation of data should take into account the range of species affected, the magnitude and duration of effect. Categories for persistence of effects have been devised by IOBC, which may also be helpful in interpreting data from the grassland situation:

less than 5 days	short-lived
5 - 15 days	slightly persistent
16 - 30 days	moderately persistent
more than 30 days	persistent

6. TERRESTRIAL VERTEBRATE WILDLIFE

There have been reported incidences of poisoning of terrestrial vertebrate wildlife following use or disposal of veterinary medicinal products. These have generally resulted from short-term exposure and toxicity, and have involved substances of high acute toxicity. Therefore it is important to consider whether effects on terrestrial vertebrate wildlife may occur through these known routes of exposure, which have been identified at Tier A. In some cases exposure through bioaccumulation in aquatic life may have been identified.

6.1. Use of available short-term toxicity data

Some arbitrary classifications of toxicity to wildlife are given in the table below (8):

LD_{50} mg/kg	LC_{50} ppm diet	Classification
<10	<50	very highly toxic
10 - 50	51 - 500	highly toxic
51 - 500	501 - 1000	moderately toxic
501 - 2000	10001 - 5000	slightly toxic
>2000	>5000	practically non-toxic

The dossier on human safety or target species safety, should be consulted for an estimate of likely toxicity to wildlife. Using the acute oral and short-term dietary toxicity data it should be possible to place the product into one of the categories in the table above. If the data indicate that the veterinary drug is 'practically non-toxic' then there are unlikely to be concerns, even if exposure does occur, therefore there is no need to assess the product

further. If the data indicate that the product is 'highly toxic' or 'very highly toxic' it is likely that exposure will confirm a need for further assessment. For those products that are 'slightly toxic' or 'moderately toxic' the need for assessment will depend on whether the level of exposure is sufficiently high.

6.2. Potential routes of exposure

A number of potential routes of exposure are discussed below, not all will be relevant to the product under consideration. For some routes of exposure any indication of adverse effects could lead to risk management, rather than assessment, as discussed below.

Use and disposal of dips
Poisoning incidents have occurred when wildfowl have had access to dips, which they have drunk and subsequently died. If a product is used as a dip its potential toxicity to vertebrate wildlife should be considered. If toxic effects from drinking dip are likely then suitable warnings should be included on the product literature, warning of the toxicity to wildlife and the need to keep these animals away from the dip.

Exposure through feeding on invertebrates
Residues in invertebrates may lead to exposure of vertebrate wildlife feeding on them. These may be invertebrates present on the fleece of treated animals, or in areas where the product is disposed of. A relatively simple estimate can be made of exposure via these routes (18):

- for disposal of product to land estimate the application rate as kg/ha
- for application to animal hide/fleece estimate the application rate as mg/dm^2 hide/fleece
- residues in insects (mg/kg insect) will be in the range 2.7 - 29 * application rate

Food consumption estimates are based on Kenaga (18) and should be expressed as mg active ingredient consumed/kg bw/day. Consumption figures should be estimated assuming 10, 20 and 35% of bodyweight is consumed as food/day, to represent waders/small crows, thrushes and small songbirds respectively:

Consumption of residues through aquatic food chain
This should be considered if in a Tier B - Aquatic assessment a bioaccumulation study has confirmed that residues are likely to occur in fish or other aquatic life. As a 'worst case' exposure assume that a 2 kg heron eats 500 g of fish/day, all of which contain maximum residues achieved during the bioaccumulation study in fish. Express consumption as mg active ingredient/kg bw/day.

6.3. Toxicity data on vertebrate wildlife

Initial sources for such data should be from literature searches or from submissions made to other regulatory authorities. For instance, where the veterinary medicinal product is an organophosphate or a synthetic pyrethroid, there will often be a wide range of acute toxicity data in the published literature on both mammals and birds. Where the substance has other uses, e.g. as a pesticide, there are likely to be data on both birds and mammals that would have been generated to support registration. These data will show whether there is a large difference in sensitivity between mammals and birds, and also whether there is much interspecies variation in toxicity. If there is a large interspecies variation then data on the most sensitive species should be used in the assessment, even if it is not the most relevant. This is because there may be species of similar sensitivity amongst those exposed. If the toxicity data indicate that birds are much more sensitive than mammals then sufficient data must be available to properly assess the risk to birds.

If there are specific concerns over effects on birds there may be a need to generate data that may not otherwise be available. If there is a need for a specific study it may be possible to generate an approximation to the LD_{50}. This will be acceptable and account will be taken of the need to reduce the number of animals used in testing. If assessment progresses to the stage of considering consumption of contaminated food by birds over several days then an avian dietary toxicity test to OECD guideline 205 may be needed in order to assess the risk.

Having obtained a wider range of toxicity data, or data that are more relevant to the species likely to be exposed, the extrapolation factor is reduced to 10.

6.4. Additional studies to refine the assessment

The assessment might be refined by considering exposure in more detail. So far consumption of 100% contaminated food has been assumed. There may be factors which operate in the field that would make this unlikely. These factors can be investigated at this stage, using available information on consumption patterns, metabolism of residues in relation to rate of uptake, possible repellancy/palatibility of contaminated food and persistence of residues in food. The dietary LC_{50} data can be useful in indicating whether food is less palatable when contaminated with residues of the drug, and whether there is a threshold for this repellancy. The ratio between intake and toxicity can be reassessed taking account of these factors. If the trigger is still exceeded specific studies may be necessary. If this is the case it is recommended that these are first discussed with the appropriate regulatory authority.

7. AQUATIC ENVIRONMENT

This scheme is based on the recommendations of an OECD Workshop on Ecological Effects Assessment (22).

The need for an assessment of effects on freshwater aquatic life would be required if the PEC/PNEC ratio is greater than one, as derived from the basic acute toxicity data on aquatic organisms and the initial estimates of the PEC.

7.1. Refinement of the PEC and identification of relevant routes of exposure

It is important at this stage to refine the PEC (see methods listed in Annex I) as this will identify which areas of aquatic risk assessment are relevant to the product under consideration. The purpose is to estimate the dispersion and degradation of the substance in the aquatic environment so that a judgement can be made as to whether short and long term effects, and exposure in either sediment or water, should be assessed. This will also affect the extrapolation factors used and thus future estimates of the PEC/PNEC ratio.

Hydrolysis and photolysis
Hydrolysis and, to a lesser extent, photolysis studies can be used to estimate stability in water. However, it should be noted that photolysis may only be important near the water surface, particularly in areas where natural waters tend to be turbid. These are worst-case studies which do not take account of microbial degradation. A half-life of 14 days or more is taken to indicate that the test substance has potential for chronic effects in the aquatic ecosystem. However, before proceeding with chronic toxicity studies it may be more appropriate to obtain further data on fate in the aquatic environment.

Adsorption and bioaccumulation
The sediment/water adsorption coefficient can be used to estimate the PEC for sediment. If the DT_{50} in water is equal to or greater than 14 days and the substance is adsorbed to sediment then there is potential for the substance to affect sediment-dwelling aquatic species. If data are available on adsorption and degradation in soil from relevant parts of Tier B these may be useful in indicating fate in aquatic sediments. However, there may be a need to generate data for natural sediment/water systems (see below).
Adsorption may be expressed as the adsorption distribution coefficient (Kd), which is the ratio of chemical adsorbed by sediment to that remaining in water.

$$Kd = \frac{S}{Ceq} = \frac{As}{m} \times \frac{V}{Aliq}$$

where
- S = concentration sorbed (µg/g sediment)
- Ceq = concentration in water (µg/ml) at equilibrium
- V = volume (ml) water
- m = mass (g) sediment
- As = amount adsorbed (mg)
- Aliq = amount remaining in water (mg)

An example of how to estimate concentrations in sediment and water, using these calculations, is provided at the end of this section of notes.

Behaviour in natural sediment and water systems
This is most readily investigated in a natural sediment-water degradation study, which will provide information both on partitioning between sediment and water and on degradation. However, an initial estimate of partitioning between sediment and water can be made based on physical/chemical properties of the test substance as described in the previous section.
If a natural sediment-water study is to be conducted, data should be generated, using suitable protocols, that are relevant to the predicted level and type of exposure, on the biodegradation mechanism and half-life in natural sediment-water systems. There are no international guidelines, but it should be possible to devise suitable protocols to answer the particular concern with respect to persistence of the test substance. Such studies are now conducted regularly for plant protection products. Various Member States plant protection registration authorities (6 and 21) have produced national guidelines for studies on half-life in natural sediment-water systems which may be suitable. ISO are also producing guidelines on this subject. In addition, suitable data may have been generated to meet FDA requirements. Characteristics of sediments used in these type of test should be defined in the study report.

Bioaccumulation
Where the medicine is proposed for use, or is already licenced, in livestock such as cattle, sheep or pigs, the data on absorption, distribution, metabolism and excretion will have provided an indication of this potential. Pharmacokinetic studies in fish, carried out as part of the residues package, can also indicate whether bioaccumulation is likely to occur. If these studies indicate low potential for bioaccumulation then no further consideration is necessary.

For some substances, i.e. those showing potential for bioaccumulation in the above target species data, it will be necessary to study bioaccumulation in an aquatic species. OECD Test Guideline 305 provides further information on the properties that may trigger the need for a fish bioaccumulation study and further guidance (see methods listed in Annex I).

7.2. Assessment of relevant routes of exposure

Assessment of acute effects
Acute toxicity data have already been generated at Tier A, where an extrapolation factor of 100 was used to derive the PNEC. Where data on fate in the aquatic environment have been used to derive a short-term PEC in Tier B, the PNEC needs only to take account of acute effects. Therefore, an extrapolation factor of 10 should be used on the most sensitive species in acute toxicity studies to derive the PNEC.

Assessment of chronic effects in the water column
Refinement of the PEC in the previous section may have produced a long-term PEC in water, indicating potential for chronic effects on aquatic life. The long-term PEC will be the mean exposure over a period of *x* days, not the PEC at day 0 or day *x*. As a first step the PNEC may be derived using the acute toxicity data generated at Tier A with an extrapolation factor of 100. As the PEC has been revised downwards, the PEC/PNEC ratio may be less than one. However, if this is not the case then chronic toxicity studies are likely to be necessary.

Chronic toxicity testing should be conducted with the most sensitive species group in acute toxicity tests from fish, invertebrates or algae. Some suitable guidelines are listed below:

- OECD Guideline 210 *Fish Early-Life Stage Toxicity Test*
- OECD Guideline 202 *Daphnia sp.* Reproduction Test
- the 72-hour algae tests can be considered to be chronic tests as this time period accounts for 16 life cycles.

In deriving the PNEC from chronic toxicity studies an extrapolation factor of 10 should be used. If chronic toxicity data are available from a less sensitive species, then the chronic NOEC for the most sensitive species should be estimated in order to derive the PNEC. This involves calculating the acute/chronic ratio for the less sensitive species and applying that to the acute toxicity data for the most sensitive species to derive the chronic NOEC.

Assessment of effects on sediment fauna
The section on *Behaviour in natural sediment and water systems* provides a means of estimating the PEC for sediment. If exposure in sediment is confirmed there will be a need to assess both the timecourse and level of exposure and the likely effects on benthic fauna.

An estimate of a sediment PEC does not necessarily indicate the bioavailability of the test substance to benthic fauna. For non-ionic hydrophobic organic chemicals the equilibrium partitioning method has been found to be useful in assessing toxicity of sediments. For these substances it has been observed that the interstitial water concentration correlates more closely than the bulk sediment concentration with toxicity and/or bioaccumulation in benthic organisms. However, this method makes a number of assumptions and does have limitations which are discussed in OECD Monograph 60 (24). The interstitial water concentration should be compared with the PNEC derived for water column species. If the PEC/PNEC ratio is of concern then toxicity data for sediment species may be required.

For other compounds this method is not applicable and testing for effects on sediment species may be necessary using spiked sediments (24). The extrapolation factors used to derive the PNEC should be the same as for acute and chronic toxicity to water column species, but based on the sediment toxicity data. If no sediment toxicity data are available, then an additional factor of 10 on toxicity data from clean water should be used in deriving the PNEC for sediment fauna.

Further information on testing of effects on sediment fauna can be found in the list of references (24, 33, 3 and 4).

7.3. Further data on toxicity to aquatic species

The purpose of additional aquatic toxicity data at this stage is to refine the PNEC. With a greater number of studies an assessment of interspecies variation can be made and the extrapolation factor modified accordingly. Also if more relevant data are obtained, e.g. on toxicity to sediment species or from field exposure, a more reliable NOEC can be obtained which will require a lower extrapolation factor.

Before proceeding with further studies it would be worthwhile to consider risk management measures which may bring exposure down to an acceptable level. Also it would be advisable to discuss any additional data generation with regulatory authorities at the planning stage.

Further data that may be generated could include single or multi-species studies in the laboratory or field. Guidance on fish medicines may be consulted for additional information on suitable study guidelines (32).

Example of calculated sediment and water concentrations

Assumptions used: Stream depths of 25cm and 100cm used in PEC estimate
initial PEC for whole stream system 25cm depth = 1 mg/l
initial PEC for whole stream system 100cm depth = 0.25 mg/l
calculation based on section of stream 1m long x 1m wide
depth of sediment adsorbing = 5cm, density = 1.5 g/cm^3

$\log_{10}$Kd = 2.93
Kd = 851

1. PEC estimate for a 25cm deep stream:

$$Kd = \frac{As}{m} \times \frac{V}{Aliq}$$

$$Kd = \frac{As \times V}{m \times (250 - As)}$$

$$851 = \frac{500As}{3750 - 15As}$$

$$3191250 - 12765As = 500As$$

$$3191250 = 13265As$$

$$As = 240.58 \text{ mg}$$

From this: PECsediment = 32 mg/kg, PECwater = 0.038 mg/l

2. PEC estimate for a 100cm deep stream:

$$851 = \frac{2000As}{3750 - 15As}$$

$$3191250 - 12765As = 2000As$$

$$3191250 = 14765As$$

$$As = 216.14 \text{mg}$$

From this: PECsediment = 28.8 mg/kg, PECwater = 0.034 mg/l

ANNEX IV
FISH MEDICINES

1. STUDIES TO DETERMINE PEC AND PNEC

This scheme is based on the assessment of fish medicines used in the marine environment in the UK (37). Users are referred to that guidance in addition to the explanatory notes produced here.

1.1. Properties of the active ingredient(s) and relevant major metabolites

The following information is likely to be relevant to the assessment:

- UV/visible absorption spectrum - may indicate wavelengths at which a substance can be subject to photodegradation
- melting point and/or boiling point - combined with information such as water solubility or vapour pressure can be useful in predicting inherent potential for movement of the substance in the environment;
- solubility in water - influences movement and distribution of a substance between environmental compartments, governs the extent to which the substance may sorb to particulate matter, many degradation processes dependent on solubility;
- octanol/water partition coefficient - estimate tendency to accumulate in lipoid tissue and sorb onto organic matter;
- dissociation constants in water - affect water solubility, potential to bind to certain sediments, and potential to partition between lipid or octanol (bioavailability);
- vapour pressure - useful in predicting distribution of a substance in environmental compartments;
- molecular weight

In addition, obtain the following information:

- aquatic hydrolysis data under relevant conditions
- photolysis (will not be relevant in some situations due to poor light penetration of water bodies).

1.2. Predicted environmental concentration (PEC)

Data on physical and chemical properties of the drug, hydrolysis and photolysis should be used together with information on the use, release into the environment and excretion of the drug to estimate the PEC. This information can also be used to determine whether drug residues are most likely to be present in the water column or on sediment. The PEC at this stage will be a worst-case assessment, as dispersion and degradation are not fully taken into account.

1.3. Acute toxicity test methods

Use expert judgement to decide whether it is most appropriate to generate data on the active ingredient, or on its toxicologically significant metabolites or on the product. It may not be necessary to test the product if the excipients are unlikely to contribute to its toxicity or if there is reason to believe that environmental exposure will not occur. Suitable tests are listed below. One species should be tested from each group, i.e. fish, invertebrate and alga. Testing should either be with marine or freshwater species depending on use of the product.

Freshwater species

- a 96-hour LC_{50} study for a species of fish
- a 48-hour EC_{50} study for *Daphnia magna*, the only study required for assessment of excreta deposited into surface waters
- an EC_{50} study for a species of alga.

Suitable guidelines are listed in Annex I (EU testing methods C1-C3 and OECD guidelines 201-203).

Marine species

- acute toxicity to one species of fish, such as juvenile plaice or turbot;
- acute toxicity to one species of larval crustacean, such as *Homarus gammarus*, *Crangon crangon*, *Mysidopsis bahia*, *Acartia tonsa* and *Tisbe battagliai*;
- toxicity to one species of marine microalga;

Suitable protocols have been devised by the Paris Commission (PARCOM), by the International Standards Organisation (ISO) and by the US Environment Protection Agency (9 and 10).

1.4. Routes of environmental exposure

More than one of the routes of exposure may be applicable to the veterinary product under consideration.

Sediment

The sediment/water adsorption coefficient (Kd) can be used to estimate the PEC for sediment. Using the Kd it should be possible to work out the partitioning between sediment and water using the formula below:

$$Kd = \frac{S}{Ceq} = \frac{As}{m} \times \frac{V}{Aliq}$$

where

S = concentration sorbed (µg/g sediment)
Ceq = concentration in water (µg/ml) at equilibrium
V = volume (ml) water
m = mass (g) sediment
As = amount adsorbed (mg)
Aliq = amount remaining in water (mg)

Adsorption/desorption data from aquatic sediments should be obtained for substances which are likely to reach sediment.

Bioaccumulation

Where the medicine is proposed for use, or is already licenced, in livestock such as cattle, sheep or pigs, the data on absorption, distribution, metabolism and excretion will have provided an indication of this potential. Pharmacokinetic studies in fish, carried out as part of the residues package, can also indicate whether bioaccumulation is likely to occur. If these studies indicate low potential for bioaccumulation then no further consideration is necessary.

For some substances, i.e. those showing potential for bioaccumulation in the above target species data, it may be necessary to study bioaccumulation in an aquatic species. OECD Test Guideline 305 provides further information on the properties that may trigger the need for a fish bioaccumulation study and further guidance (see methods listed in Annex I).

Before conducting a study on bioaccumulation further data on degradation in the aquatic environment should be obtained to determine whether the time period of exposure is likely to be sufficient to lead to bioaccumulation.

Acute exposure in the water column

If exposure is for a short period of a few hours or for a similar duration to acute toxicity studies, then an assessment may be made on the basis of the data already generated. An

extrapolation factor of 10 may be used, on the EC/LC_{50} value for the most sensitive species in acute toxicity tests, to derive the PNEC where only a short period of exposure in the water column is expected to occur. This PNEC does not allow for chronic effects, bioaccumulation or exposure in sediment.

Chronic exposure in the water column
Inital data on degradation may indicate stability in sterile water, leading to exposure during the life-cycles of aquatic life. Before generating data on chronic toxicity, further data should be obtained on stability in water, taking into account microbial degradation. A natural water or natural sediment/water degradation study would provide such information.

2. PEC/PNEC RATIOS

See Tier B - Aquatic for explanation and derivation of PEC/PNEC ratios.

3. BEHAVIOUR OF SUBSTANCES IN NATURAL SEDIMENT AND WATER SYSTEMS

This is most readily investigated in a natural sediment-water degradation study, which will provide information both on partitioning between sediment and water and on degradation. However, an initial estimate of partitioning between sediment and water can be made based on physical/chemical properties of the test substance as described in the previous section.
If a natural sediment-water study is to be conducted, data should be generated, using suitable protocols, that are relevant to the predicted level and type of exposure, on the biodegradation mechanism and half-life in natural sediment-water systems. There are no international guidelines, but it should be possible to devise suitable protocols to answer the particular concern with respect to persistence of the test substance.

3.1 Additional toxicity studies

Acute toxicity studies
The additional tests are intended to provide further information on short term effects:

Freshwater
- acute toxicity to a warm water species of fish, if relevant, e.g. bluegill sunfish (*Lepomis macrochirus*) or a carp species (23);
- acute toxicity to *Gammarus pulex* (13);
- additional alga species.

Marine
- additional species from the list above (acute exposure in the water column) may be tested;
- acute toxicity to juvenile or larval molluscs of economic importance (11 and 12);
- growth test with additional species of microalgae or diatoms;
- growth inhibition test for a marine macrophyte, if exposure is expected

Chronic toxicity to pelagic species
Initially the most sensitive taxa in the acute toxicity studies should be used in a chronic toxicity test. Additional chronic toxicity studies may be useful if long-term exposure is shown to be important. Examples of tests are:

- OECD Guideline 210 *Fish Early Life Stage Toxicity Test*, marine or freshwater species
- OECD Test Guideline 202 *Daphnia* reproduction study
- chronic toxicity to marine crustacean, e.g. *Mysidopsis bahia* (1)
- the 72-hour algae tests can be considered to be chronic tests as this time period accounts for 16 life cycles.

Toxicity to obligate sediment feeders
An estimate of a sediment PEC does not necessarily indicate the bioavailability of the test substance to benthic fauna. For non-ionic hydrophobic organic chemicals the equilibrium partitioning method has been found to be useful in assessing toxicity of sediments. For these

substances it has been observed that the interstitial water concentration correlates more closely than the bulk sediment concentration with toxicity and/or bioaccumulation in benthic organisms. However, this method makes a number of assumptions and does have limitations which are discussed in OECD Monograph 60 (24). The interstitial water concentration should be compared with the PNEC derived for water column species. If the PEC/PNEC ratio is of concern then toxicity data for sediment species may be required.

For other compounds this method is not applicable and testing for effects on sediment species may be necessary using spiked sediments (24). The extrapolation factors used to derive the PNEC should be the same as for acute and chronic toxicity to water column species, but based on the sediment toxicity data. If no sediment toxicity data are available, then an additional factor of 10 on toxicity data from clean water should be used in deriving the PNEC for sediment fauna.

Further information on testing of effects on sediment fauna can be found in OECD (24), SETAC (33) and ASTM guidelines (2, 3 and 4).

Bioaccumulation
Pharmacokinetic studies in fish, carried out as part of the residues package, can indicate whether bioaccumulation is likely to occur. If these studies indicate low potential for bioaccumulation then no further consideration is necessary.

In some cases, however, it will be necessary to conduct a bioaccumulation study in an aquatic species, preferrably fish. There are suitable OECD guidelines for studies with fish. Guidelines are also available from the US Environment Protection Agency for studying bioaccumulation in other species such as mussels and oysters.

3.2 Fate in the natural environment

Dispersion
Data on dispersion are not normally required for substances which are extremely rapidly degraded and may not be needed for substances intended for administration in the feed. However potential leaching of the substances from uneaten feed into the water body and also possible excretion of the substance and/or its metabolites should be considered.

The need for dispersion studies will be indicated by the physico-chemical and toxicological properties of the substance and the likely pattern of usage. Dispersion studies are most likely to be needed for those medicines remaining in water. The advice of the Licensing Authority should be sought on the draft protocol.

Computer modelling may be used to predict dispersion of marine fish medicines remaining in and behaving similarly to sea water. It is recommended that study protocols or proposals for use of validated models be discussed with the regulatory authority.

Fate of residue in sediments
If the biodegradation studies in Tier 2 indicate that biodegradation in sediments will be slow, then further information on what will happen in the field will need to be obtained. A number of approaches to data generation are available, which may also be linked to data needed on biological effects. It may be possible to obtain sufficient data on environmental fate in sediments through use of laboratory microcosms, or by field mesocosms. Both types of study will provide much more controlled conditions than a field study. However, in some cases a field study may be the most suitable option. It is recommended that proposals or protocols for such studies be first discussed with the regulatory authority.

Bioaccumulation measurements
Bioaccumulation measurements under field conditions may be necessary if the bioconcentration factor is high in studies with fish or molluscs (for example, in the range 100 - 1000). The regulatory authority should be consulted if such tests are indicated.

3.3 Biological effects

Field studies
Before embarking on field studies to study biological effects, consideration should be given to whether the available data provide sufficient information to enable an assessment of risk. However, if the PEC/PNEC ratio is still less than one, consideration should be given to conducting additional studies representative of the situation in the field. Examples of suitable types of study are mesocosms for effects on benthic fauna, bioassays where a particular taxa is sensitive, or studies on the impact of treatments during experimental field trials at worst-case sites. The latter will be the most difficult to obtain interpretable results from because of the variety of impacts caused by fish farming. Protocols for these studies should be devised on a case by case basis, and in discussion with the regulatory authority.

If antimicrobial drugs have persistent residues in sediment it may be necessary to study their effects on microbial communities in sediment. Changes in these communities leading to adverse effects on microbial function would be of particular concern, e.g. a reduction in the normal degradation processes occurring in sediment. The MIC data already available may indicate the possibility of such effects. These may then be investigated in laboratory microcosms.

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