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Effects of ionising radiation on soil fauna

Science Technical Report P3-101/SP7

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This report outlines the results of research into effects of ionising radiation on soil fauna in order to better develop understanding and policy in the field of radiation protection to wildlife.

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Executive summary

There is a statutory need to confirm that the environment is protected from the harmful effects of contaminants released into it. The Environment Agency has responsibilities under the Habitats Regulations for England and Wales for assessing the environmental impact of authorised discharges of contaminants, including radioactive contaminants. All EU Member States are required to assess the impact of ionising radiation and, to facilitate this, the EC-funded Framework for ASSessment of Environmental impacT (FASSET) project has collated published data on the effects of radiation on plants and animals (Woodhead and Zinger, 2003).

In October 2003, the International Atomic Energy Agency (IAEA) held a conference in Stockholm on the protection of the environment from the effects of ionising radiation. Following this conference, it was stated that the FASSET project had highlighted major gaps in scientific knowledge concerning dose-response relationships for particular endpoints (e.g. morbidity, mortality, reproductive capacity and mutation) for a number of wildlife groups and that the data which do exist are old and relate to relatively high dose rates and acute exposures (Williams, 2004).

This project was commissioned to start addressing the gaps highlighted by the FASSET project. It utilised new documentation produced by the Agency – the SP2 handbook, *Developing experimental protocols for chronic irradiation studies on wildlife* (Wood *et al.*, 2003).

In order to study the effects of chronic radiation exposure on soil fauna, the following objectives were followed.

- To begin deriving empirical data for knowledge gaps identified by FASSET concerning soil fauna and the effects of environmentally relevant doses on morbidity, mortality and reproductive capacity.
- To prepare experimental protocols in line with the guidance provided in the SP2 handbook for the earthworm (*Eisenia fetida*) and the woodlouse (*Porcellio scaber*).
- To review the SP2 guidance following the completion of the experiments.

Groups of earthworms *E. fetida* and woodlice *P. scaber* were exposed constantly to one of six radiation dose rates (background, 0.2, 0.4, 1.4, 2.7 and 8.5 mGy/hour and background, 0.2, 0.4, 1.5, 2.8 and 8.9 mGy/hour, respectively). The periods of exposure were a total of 16 weeks for *E. fetida* and 14 weeks for *P. scaber*. The endpoints of mortality, number of viable offspring and average weight were recorded. A selection of individual earthworms and woodlice were also assessed using histopathological techniques to evaluate hyperplasia, necrosis and multinucleated cells.

The results for both species revealed that even individuals in the highest dose rate group of >8.5 mGy/hour showed no significant decrease in weight and reproductive capacity compared with individuals in the background and lower dose rate groups. There was also no significant increase in mortality or histopathology anomalies in the individuals from the >8.5 mGy/hour dose rate group during the course of the study compared with individuals in the background and lower dose rate groups.

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Introduction

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All EU Member States are required to assess the impact of ionising radiation on the environment. To facilitate this, the EC-funded Framework for ASSessment of Environmental impacT (FASSET) project (FIGE-CT-2000-00102) has collated published data on the effects of radiation on plants and animals (Woodhead and Zinger, 2003).

In October 2003, the International Atomic Energy Agency (IAEA) held a conference in Stockholm on the protection of the environment from the effects of ionising radiation. Following this conference, it was stated that the FASSET project had highlighted major gaps in scientific knowledge concerning dose response relationships for particular endpoints (e.g. morbidity, mortality, reproductive capacity and mutation) for a number of wildlife groups and that the data which do exist are old and relate to relatively high dose rates and acute exposures (Williams, 2004).

Part of the remit for the FASSET project was to collate all known published data on the effects of radiation on plants and animals and to group them under four umbrella endpoints. These umbrella endpoints are morbidity, mortality, reproductive capacity and mutation. FASSET Deliverable 4 (Radiation effects on plants and animals) shows that there are conspicuous data gaps for certain combinations of umbrella endpoint and wildlife group (Woodhead and Zinger, 2003). For example, data are lacking on morbidity for amphibians and reptiles, and little data exist on morbidity, mortality, reproductive capacity and mutation for soil fauna (Table 1.1).

As noted by Williams (2004), the majority of data collated through FASSET are for wildlife subjected to acute exposures. However, chronic radiation exposure is the most relevant form of radiation exposure in terms of environmental protection and regulation. There are natural as well as anthropogenic sources (in the form of authorised waste discharges) that give rise to this type of exposure affecting biota.

In response to this lack of information on the effects of low-level chronic exposure on biota, the Agency commissioned a handbook, *Developing experimental protocols for chronic irradiation studies on wildlife*, R&D Technical Report P3-101/SP2 (Wood *et al.*, 2003). This handbook provides guidance on how to conduct experiments (including the determination of environmentally relevant dose-response relationships) using non-human species. It aims specifically to facilitate and direct studies on the effects of ionising radiation on wildlife and hence the environment.

1.1 Objectives of this study

To start addressing the gaps highlighted by FASSET, the following objectives were followed in order to study the effects of chronic radiation exposure on soil fauna.

- To begin deriving empirical data for knowledge gaps identified by FASSET concerning soil fauna and the effects of environmentally relevant doses on morbidity, mortality and reproductive capacity.
- To prepare experimental protocols, in line with the guidance provided in the SP2 handbook for the

earthworm (*Eisenia fetida*) and the woodlouse (*Porcellio scaber*).

- To review the SP2 guidance following the completion of the experiments.

1.2 The approach used

The SP2 handbook (Wood *et al.*, 2003) was used in the planning of this study. The handbook promotes harmonisation of experimental approaches when researching the effects of radiation on wildlife and offers guidance on which species should be considered for a particular wildlife group and how laboratory experiments should be conducted. The handbook aims to:

- inform the development of experimental protocols, for a range of wildlife groups, that will enable the derivation of dose-effect relationships resulting from chronic exposure to ionising radiation;
- consider and advise on good practice for each stage of the design of experiments;
- provide examples of good experimental design;
- provide information on financial and staffing considerations to be taken into account when planning research.

In the handbook, researchers are guided via a key through four Good Practice Guides (GPGs). These are:

- Test species selection (GPG 1)
- Endpoint selection (GPG 2)
- Exposure guideline (GPG 3)
- Experimental design (GPG 4).

After working through these guides in a systematic manner, users are encouraged to record their decisions and justifications on the pro-forma provided. This information forms the basis of the experimental design.

The information provided in the handbook is by no means exhaustive. This is emphasised at the beginning of the document by the statement: *'Assessors are strongly advised to carry out a literature search at the time of planning any experiments in order to take into account the latest developments in this field.'* The need to identify the latest information is highlighted throughout the handbook and space is provided on the pro-forma to record the results of literature searches.

1.3 Outline of the study

Following the guidance given in the SP2 handbook (Wood *et al.*, 2003), numbers of earthworms (*E. fetida)* and woodlice (*P. scaber)* were segregated into groups and exposed constantly to one of six target radiation dose rates (background, 0.2, 0.4, 1.5, 4.0 and 8.0 mGy/hour. The periods of exposure were a total of 16 weeks for *E. fetida* and 14 weeks for *P. scaber.* The experimental set-up was staggered for the two species to allow sufficient preparation time for each species. The endpoints of mortality, number of viable offspring and weight of adult individuals were recorded.

The study methodology is given together with the results in Sections 2 and 3, and discussed further in Section 4. Section 4 also contains an evaluation of the guidance provided in SP2.

Table 1.1 Numbers of publications detailing experiments regarding ionising radiation and for FASSET selected wildlife groups (adapted from Woodhead and Zinger, 2003)

Wildlife group	Radiation effects (all types)	Morbidity (from July 1981)	Mortality (from December 1974)	Reproduction (from October 1991)	Mutation
Amphibians	357	0	62	62	7
Bacteria	6203	26	304	409	3364
Birds	1089	4	465	424	119
Crustaceans	217	1	390	364	50
Fish	1531	18	1881	1136	931
Insects	3415	4	643	1377	2057
Invertebrates	37	1	47	58	15
Mammals	26144	279	2529	1437	6990
Molluscs	194	1	239	157	34
Plants	16965	127	2533	3082	8053
Reptiles	100	0	22	62	7
Soil fauna	**12**	**0**	**3**	**11**	**3**

2

Chronic radiation experiments with earthworms

The Good Practice Guides in the SP2 handbook (Wood *et al.*, 2003) were used to design an experimental study of the effects of chronic ionising radiation exposure on the earthworm (*Eisenia fetida*).

2.1 Test species selection (GPG 1)

The earthworm has been widely used in toxicity testing (Wood *et al.*, 2003). SP2 recommends *Eisenia fetida* as a test species due to the ease of acquisition, short life cycle, easy handling and the lack of a need for a Home Office licence (as required for vertebrate studies). This species is used in:

- American Society for Testing and Materials (ASTM) standards
- US Environmental Protection Agency (US EPA) toxicity testing of hazardous waste sites
- the standard earthworm protocols for ecotoxicology generated by the International Standards Organisation (ISO) and the Organisation for Economic Co-operation and Development (OECD).

Further reading provided further examples of the worm's adaptability and suitability for toxicity testing. In a study by Bustos-Obregón and Goicochea (2002), reproductive, survival, body weight and histological parameters were used to assess the effects of a highly toxic organophosphoric agropesticide. Decreased numbers of sperm, cocoons and viable offspring were observed, in addition to decreased rates of survival and a reduction in adult body weight. They concluded that *E. fetida* was a suitable bioindicator of chemical contamination in the soil. Since the publication of SP2 in 2003, further use of *E. fetida* in ecotoxicology has been reported. For example, the cocoon production test is being used by the US EPA in its development of ecological soil screening level (Eco-SSL) benchmarks (Kuperman *et al.*, 2004).

2.2 Endpoint selection (GPG 2)

SP2 advocates the use of reproductive endpoints for assessment, as successful environmental protection requires the maintenance of ecosystem function. This function is inherently linked to the success of populations rather than individuals; therefore, any reduction in reproductive success or reproductive fitness could impact on the ecosystem.

However, the need to observe additional endpoints is also stressed in SP2. These include:

- mortality
- differences in physical appearance
- the weight of individuals.

For field studies, the use of complementary biomarker techniques, combined with methods that relate to organism fitness and site chemistry, provide the most useful data (Anderson *et al.*, 1998). Applying the same multifaceted assessment to laboratory studies, where test chemical concentrations substitute site chemistry, makes the results more comparable.

2.3 Exposure guideline (GPG 3)

SP2 provides information, where available, on the dose rate thresholds for specific endpoints for each wildlife group. The information is a composite of the information provided by the FASSET Radiation Effects Database (Woodhead and Zinger, 2003) – known as FRED – and Agency R&D Publication 128 (Copplestone *et al.*, 2001).

Earthworms have been reported to show reduced population size after exposure to <5 mGy/hour of

gamma radiation and after 100 mGy/hour of alpha radiation. However, most of the data for soil fauna are derived from field experiments undertaken after a nuclear accident or where soil activity concentrations have been artificially increased. This is very limited information and SP2 recommends that at least three or four dose rates should be used. The dose range proposed for further investigation for soil fauna is 1,000–5,000 mGy/hour (1–5 mGy/hour). SP2 also emphasises the need to ensure that the response threshold is spanned by the doses selected so that a dose response relationship can be constructed.

The pro-forma generated using SP2 (see Appendix A1), together with the handbook itself, were used to establish the design of this study. Appendix A1 also contains the completed checklist for data reporting from SP2. The methodology and the results are presented below. Tables of raw data for this study

2.4 Experimental design (GPG 4)

2.4.1 Setting up tanks

Consistent with draft ISO and OECD earthworm reproduction tests (Spurgeon *et al.*, 2002), commercially supplied topsoil was dried and its water holding capacity was derived (OECD, 2000). The soil was then homogenised and 1 kg of dried soil was rehydrated with 500 ml of distilled water to gain 50 per cent moisture content (1.5 kg final wet weight). The hydrated soil was placed in a 3.5 litre tank. Dried horse manure (10 g) was layered over the surface of each tank and rehydrated using a water spray to form a thin layer of slurry for the worms to feed on. A total of 84 tanks were prepared.

2.4.2 Dosimetry

The tanks were divided into six dose rate groups (14

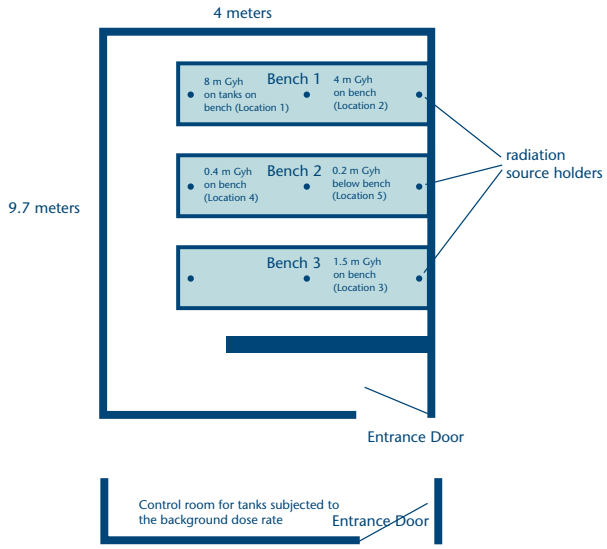

Figure 2.1 Layout of the irradiation facility

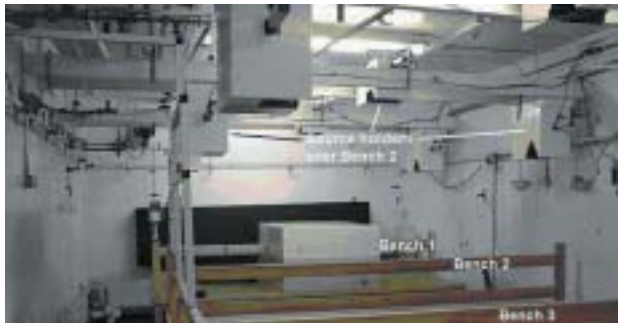

Figure 2.2 Interior of the radiation room. Empty tanks have been placed on bench 1 to give a raised surface and thus a higher dose rate to any organism placed on them.

tanks in each group). The six target dose rates chosen for this experiment were background, 0.2, 0.4, 1.5, 4 and 8 mGy/hour. Figures 2.1 and 2.2 show the irradiation room and the position of the tanks, respectively.

Dosimetry measurements were undertaken in three of the tanks along each bench (one at each end of the bench and one in the middle) using thermoluminescebt dosimeters (TLDs). This was to verify the dose rates the tanks actually received. Shielding effects from the soil were taken into consideration by measuring the dose rate from several widths and depths within the tank. The results are given in Table 2.1.

Table 2.1 TLD readings taken to verify dose rates actually received by Eisenia fetida

Location number	Aisle tank (mGy/hour)	Middle tank (mGy/hour)	Wall tank (mGy/hour)	Mean dose rate (mGy/hour)
1	9.1	6.9	9.5	**8.5**
2	2.5	2.5	3.2	**2.7**
3	1.5	1.4	1.3	**1.4**
4	0.4	0.4	0.4	**0.4**
5	0.2	0.2	0.2	**0.2**

2.4.3 Test animals

Adult *E. fetida* were supplied by Blades Biological Ltd, UK. Only mature worms (i.e. those having a clitellium) were used. Sexual differentiation was unnecessary, as worms are known to be hermaphrodites. Eight worms were weighed and introduced into each of the 84 tanks.

2.4.4 Duration of irradiation

The tanks were irradiated continuously for 110 days – longer than the usual 56 days exposure used in standard ecotoxicological protocols for non-radioactive chemicals (Spurgeon, *et al.*, 2002). This

ensured that any long-term effects would be more likely to be seen. The only interruption to the radiation exposure was when the tanks were brought out of the facility for assessment (see Section 2.4.5). The final doses received by each group of tanks are given in Table 2.2.

2.4.5 *Assessment of reproduction, growth and mortality parameters*

At two-week intervals, each of the tanks was removed from the irradiation facility for assessment. The soil of each tank was hand-sifted to search for the original worms and any progeny/cocoons that might be present.

Each worm was removed from the tank and the weight of the adult worms, the number of cocoons and the number of progeny recorded. After assessment the soil, worms, cocoons and offspring were returned to their tank, a fresh layer of manure (10 g) was added and the tank replenished with water (200 ml). The tanks were then placed back in the irradiation facility.

During week 8, 42 of the 84 tanks were removed for an interim kill after the usual 56-day study duration and to provide some intermediate histopathology. After the weight measurements had been taken, a proportion of the worms were put into 10 per cent buffered formalin ready for histopathological study. This step was repeated in the final week (week 16) for the remaining tanks.

2.4.6 *Histopathology*

The worms subjected to 10 per cent buffered formalin were sectioned into three blocks:

- a transverse section 3–4 mm from the mouth (anterior)
- a transverse section 3–4 mm anterior to the clitellum (intermediate)
- a transverse section 3–4 mm from the posterior of the saddle (posterior).

The tissues were embedded in paraffin using a Bayer Tissue-Tek III. Sections of tissue, 5 mm in thickness, were stained with haematoxylin and eosin (H & E) stain using a Varistainer. Slides were examined, for the tissue types listed in Table 2.3, using a Leitz Laborlux 11 microscope. Tissues were assessed for multinucleated cells, aberrant cell turnover and necrosis.

2.4.7 *Contaminant analyses*

When the study was complete, chemical analyses were carried out on a proportion of the worms in order to observe any preferential uptake of the soil components by worms from different dose rate groups.

The soil from each tank was homogenised with soil taken from other tanks in the same dose rate group and chemical analyses were undertaken on a sub-sample of the homogenised soil from each dose rate group.

The contaminants analysed for are listed in Table 2.4. Heavy metal analyses were carried out on both worms and soil sub-samples for all groups. Analyses for persistent organics were only performed on worm samples from the background dose rate group and the 8.5 mGy/hour dose rate group. Analyses were performed elsewhere.

Table 2.2 | Calculation of total dose received by *Eisenia fetida* during the study

Mean dose rate (mGy/hour)	Duration of irradiation (hours)	Total dose given (Gy)
0.2	2,568.7	0.51
0.4	2,568.1	1.03
1.4	2,563.4	3.58
2.7	2,567.9	6.93
8.5	2,568.4	21.83

Table 2.3 | Tissue types assessed in the histopathological study of *Eisenia fetida*

Basic tissue type	Specific regions
Integumen	Dorsal, lateral and ventral regions with intersegmental constrictions and chaetae
Alimentary canal	Oesophagus crop and gizzard and intestine with typhlosole and chloragenous cells
Nephridia	
Pseudohearts and blood vessels	
Nerve cord	Double ventral cord, giant nerve fibres and ganglia
Gonads	Testes, seminal vesicles, and vasa deferentia; ovaries and spermathecae
Musculo-skeletal system	Circular and longitudinal layers.

Table 2.4 | Determinands analysed for in worms and soil

Metals	Polycyclic aromatic hydrocarbons (PAHs)
Arsenic	Acenapthylene
Boron*	Anthracene
Cadmium	Benzo-(a)-anthracene
Chromium	Benzo (b) fluoranthene
Copper	Benzo (k) fluoranthene
Lead	Benzo-[a]-pyrene
Mercury	Benzo-[e]-pyrene
Nickel	Benzo-[ghi]-perylene
Selenium	Chrysene
Zinc	Dibenzo (ah) anthracene
	Fluoranthene
	Fluorene
	Indeno-[1,2,3-cd]-pyrene
	Naphthalene
	Perylene
	Phenanthrene
	Pyrene

* Boron is included with metals for the purposes of this analysis.

2.5 Results

2.5.1 Morbidity

Growth measurements

The results showed an increase in average weight after two weeks for worms from all dose groups, with worms from the 8.5 mGy/hour dose group having the heaviest average weight (Figure 2.3). Beyond two weeks, a marked decrease in average weight was observed in worms from all mean dose rate groups, with a significant decrease in average weight observed in worms from the 0.2, 0.4 and 8.5 mGy/hour average dose rate groups. This lower average weight was similar to the original average weight. For the remaining weeks in this study, the average worm weight was then constant for all dose groups.

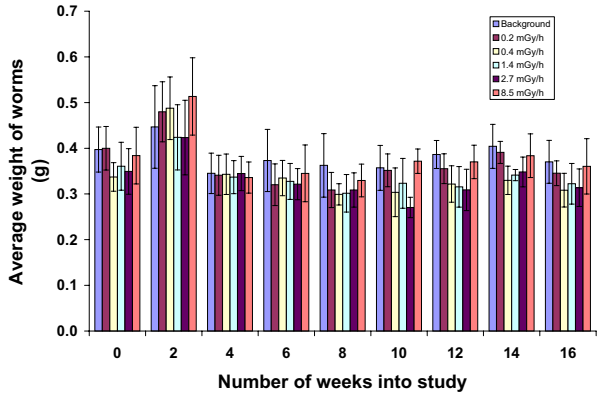

Figure 2.3 | Mean weight of worms in each dose group during the study (± 1 SD)

Histopathology

An intermediate cross-section of a specimen of *E. fetida* is depicted and annotated in Figure 2.4A. It is characterised by the bilateral lobes of the seminal vesicle and the bilateral seminal funnels where the dark blue regions consist of spermatogonia. The oesophagus, double ventral nerve cord and the circular and longitudinal muscle adjacent to the epidermis are also shown.

Table A2.2 (see Appendix A2) is a record of the observations made from the *E. fetida* sections taken from:

- eight individuals from the background, 1.4 mGy/hour and 8.5 mGy/hour dose rate groups at the interim stage of the study;
- eight individuals taken from each of the six dose rate groups at the end of the study.

None of the worms analysed showed sign of any pathological anomalies. Figure 2.4B shows a cross-section of an individual from the 8.5 mGy/hour dose rate group representative of the results from that dose group. However, monocystic infections (resulting from a protozoan infection endemic within terrestrial worms) were recorded in most of the worms analysed; this could indicate immunodepression due to exposure to contaminant levels (see Section 4.1). Figure 2.5 shows an example of a monocystic infection.

Table 2.5 lists the diagnostic criteria employed in the examination of the worm sections. These were based on the categories of histopathological lesions used for the assessment of hepatic pathology in dab by

the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) in their current fish monitoring programmes (CEFAS, 2001). The categories are given weighting scores from 0–9 in order of increasing severity of observed condition. After the observations were recorded for each individual, they were assessed and graded using the diagnostic criteria given in Table 2.5.

After grading, the number of recorded anomalies were grouped by dose groups (Table 2.6). A score was derived from each dose group by multiplying each entry in a column by the number of the category it was graded in. For example, one worm in the background dose rate group from the end of the study had an infection classification of 'no abnormalities detected (NAD)'. The number for the NAD category is 0, so the weighted score for this worm is 0 (0 x 1 = 0). The next category, which has a score weighting of 1, represented a trace level of monocystis. There are two entries in this category; therefore, the weighted score is 2 (1 x 2 = 2). Each of the categories was assessed in this manner. The totals for each category were then added together to obtain a total histopathology score for each dose rate group.

Table 2.5 Diagnostic criteria for histopathology of invertebrate tissue (CEFAS, 2001; Hingston, 2003)

	Score	Observation
	0	NAD (no abnormalities detected) and no infection
Infection specific	**1**	Trace
	2	Minimal
	3	Moderate
	4	Marked
Anomalies	**5**	Eosinophilic
	6	Basophilic
	7	Hyperplasia
	8	Necrosis
	9	Neoplasia

The histopathology of *E. fetida* relied upon comparative data from the histology of the earthworm *Lumbricus terrestris*. However, in *E. fetida*, there are no giant nerve fibres above the double ventral nerve cord as found in *L. terrestris*. Apart from this difference, the remaining histology of *E. fetida* is comparable to the histology of *L. terrestris (*Freeman and Bracegirdle, 1979). Furthermore, all worms were compared to *L. terrestris* data in the same manner; any differences observed between dose rate groups are therefore valid.

Figure 2.4 Cross-section of an earthworm representative of (A) the background dose rate group and (B) the 8.5 mGy/hour dose group (25× magnification)

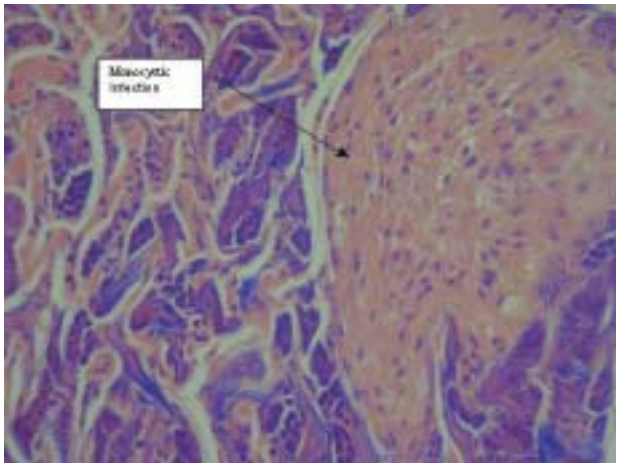

Figure 2.5 | Monocystic infection in a seminal vesicle (400× magnification)

The high standard deviation (SD) throughout all dose rate groups suggests that the observed differences between dose rate groups are not significant. One contributing factor to this variation could be the size and the colour of the cocoon. Figure 2.7 illustrates the average length of a cocoon in this study (i.e. 5 mm) and shows that the colour of the cocoon provides a level of camouflage. Therefore, while every effort was made to conduct thorough searches of the tanks for cocoons, it is possible that some cocoons may have been missed by those assessing the tanks (even though they had all been specifically trained).

Table 2.6 | Histopathological scores for both interim and final worms based on the diagnostic criteria in Table 2.5

	Dose rate (mGy/hour)	Level of infection					Anomalies						Total score
		0	1	2	3	4	0	5	6	7	8	9	
Interim	Background		3	3	1	1	8						16
	1.4		2	1	5		8						19
	8.5		3	4	1		8						14
Final	Background	1	2	3	2		8						14
	0.2		1	4	3		8						18
	0.4		6	1	1		8						11
	1.4		1	6	1		8						16
	2.7		2	4			8						10
	8.5		3	1	2		8						11

2.5.2 Mortality

Only one mortality was recorded during the study. This occurred within the first two weeks in a tank subjected to 8.5 mGy/hour, but the cause of death is unknown. However, because it occurred at the start of the experiment, it may be due to the age of the worm or poor health prior to the experiment. All worms were checked for obvious signs of low fitness prior to introduction, but it is possible that an unhealthy worm would not show external signs of ill health at this stage.

2.5.3 Reproduction

Number of cocoons

For all dose rate groups, the number of cocoons per tank fell from week 8 to week 14 (Figure 2.6) due to offspring starting to hatch. After week 14, the average number of cocoons per tank started to rise again.

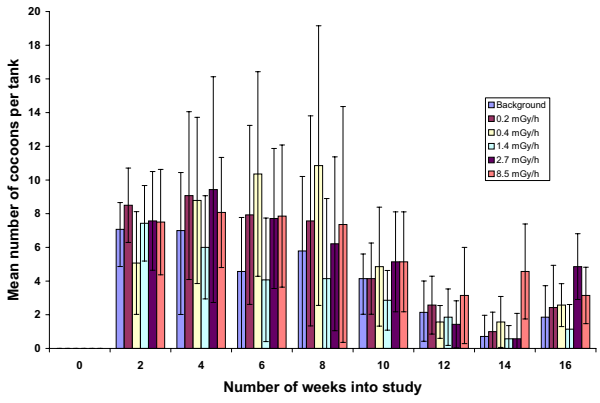

Figure 2.6 | Mean number of cocoons per tank (± 1 SD)

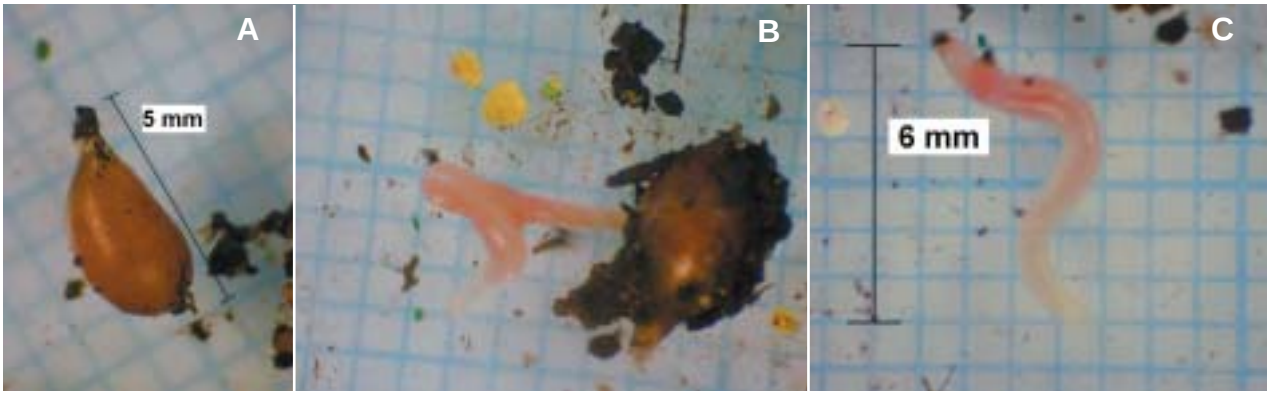

Figure 2.7 | Photographs showing the small size of (A) a worm cocoon and (B, C) newly hatched offspring

Number of offspring

Figure 2.8 shows a marked increase in the number of offspring hatched per tank during the course of the study. By the end of the study, the 8.5 mGy/hour group had the lowest mean number of offspring per tank, with the exception of the background group.

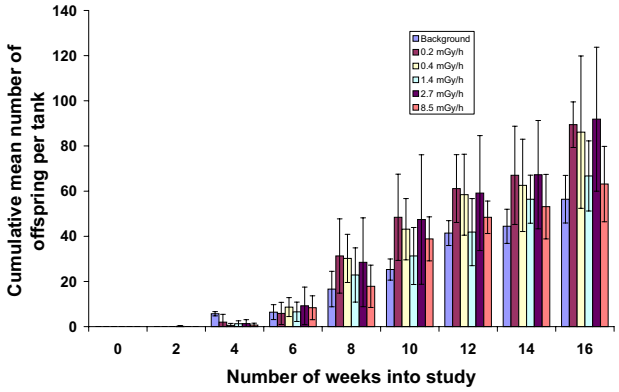

Figure 2.8 | Cumulative mean number of offspring per tank per dose group for all tanks for the duration of the experiment (± 1 SD)

Once the data for the number of offspring and the number of cocoons per dose rate group had been obtained and analysed, data for the number of cocoons were plotted alongside the data for the number of offspring for the same dose rate group (Figure 2.9). The aim was to see whether a delay in hatching could be detected in tanks containing worms exposed to higher dose rates. However, it is not apparent from the graphs that such a trend exists.

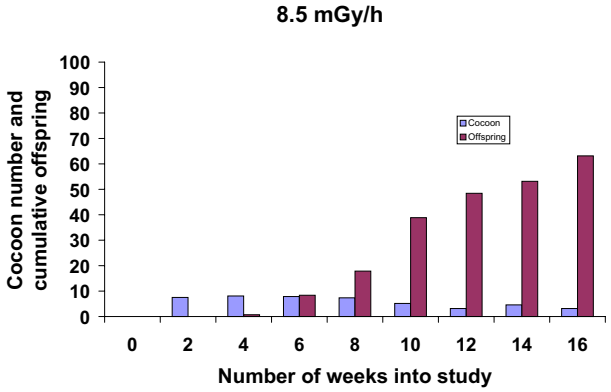

Figure 2.9 | Mean number of cocoons and cumulative offspring for each dose group for each week of assessment

The tanks subjected to background levels of radiation were kept in a separate control room. Temperature differences between the two rooms may therefore have influenced productivity in these tanks compared with those in the irradiation room. Figure 2.10 shows the temperature log data for the immediate vicinity of each dose group. However, it is clear from the graphs that there was no systematic difference between the two worms.

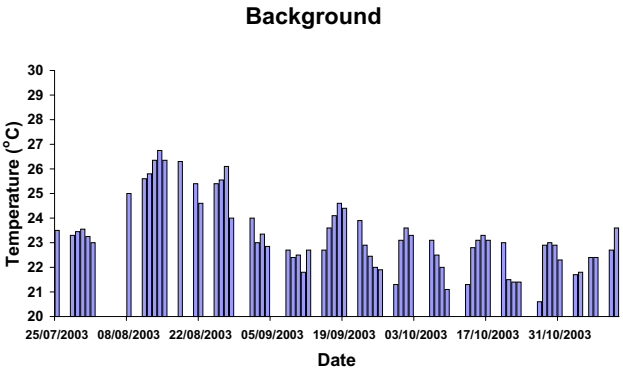

Figure 2.10 | Temperatures recorded at the location of each dose group within the irradiation and control rooms

It was subsequently observed that differing numbers of worms were being counted for each tank at different points during the study (Figure 2.11). Each tank should contain eight adult worms during the course of the study. In the first eight weeks of the study, there was a fall in the mean number of worms per tank in all dose groups, with the tanks in the background group suffering the most loss. However, the mean number of worms in the tanks of the 1.4 mGy/hour dose rate group increased after week 8 until week 14.

Unlike the problems associated with counting cocoons, human error is a much less likely explanation for the variation seen in adult worm numbers. The reasons for this are two-fold. First, adult worms are much larger then cocoons (approximately 70 mm long) and are consequently easier to detect. Secondly, dummy tanks had been set up with the right amount of soil and known complements of worms before the start of the study for training purposes. The results of counts by different assessors revealed a very low influence of bias when counting adult worms. Therefore, it was concluded that the worms were migrating out of their tanks. Figure 2.12 shows the probable route of migration. This was a completely unforeseen circumstance given the size of the worms, and the width and location of the slits in the lids of the tanks.

Numbers in neighbouring tanks increased, providing evidence that worms migrating from the tanks were not always lost from the experiment. This is highlighted in Figure 2.13, which represents each tank over the course of the experiment. A red square indicates when a tank was below its full complement of eight worms and a blue square indicates when a tank was above its complement of worms. A white square indicates that the tank held the correct number of worms.

Figure 2.13 suggests that, for each dose group, most of the worms that migrated did so in the first four weeks. At week 0, all of the tanks in the study (100%) had the correct complement of eight worms. By week 2, 70.2% of the tanks had the correct complement of eight worms. But, by week 4, only 23.8% of the total number of tanks in the study had its full complement.

Due to the lack of automated lighting in the irradiation room, it was deemed more suitable to have a continual light source (24 hours) for both the irradiation and control rooms. The worms, therefore, would not have been affected by a dark stage of a light cycle. Thus, the reason for migration is unclear.

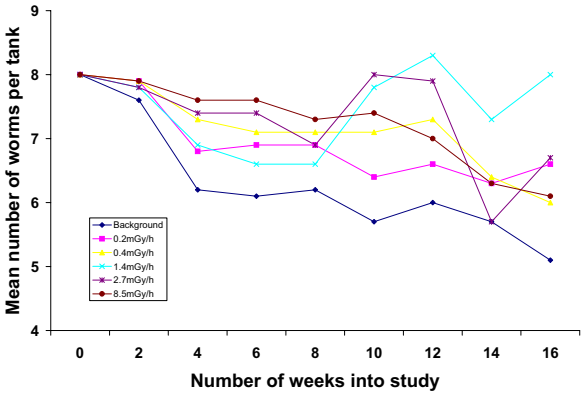

Figure 2.11 | Mean number of adult worms per tank during the study

Figure 2.12 | Possible migration route (the £1 coin on the lid illustrates the scale)

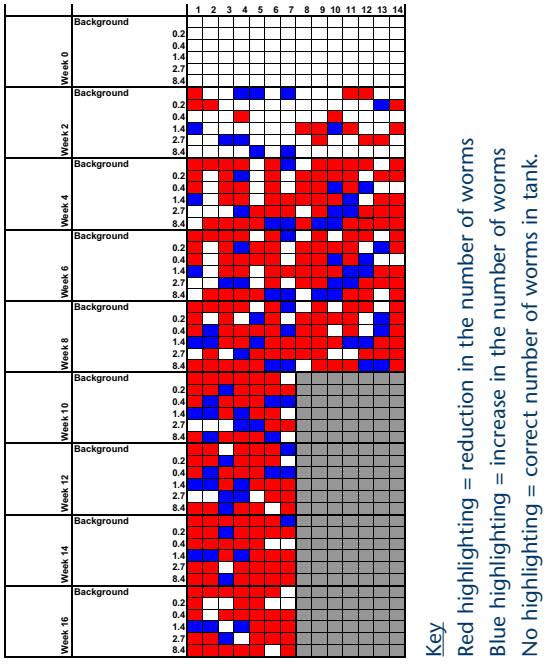

Figure 2.13 | Changes in the number of worms per tank during the course of the experiment

2.5.4 *Contaminant analyses*

Samples of soil and worms from each of the six dose rate groups were analysed for the presence of ten metals (see Table 2.4). To confirm that the soil metals burden was comparable with normal environmental concentrations, the metal concentrations in soil from the background dose rate group were compared with those in rural soil sampled for the UK soil and herbage survey (Wood *et al.*, in preparation) (Table 2.7).

Table 2.7 | Temperatures recorded at the location of each dose group within the irradiation and control rooms

Metal	Soil from background dose rate group (mg/kg)	UK soil and herbage rural soil (median) (mg/kg)	UK soil and herbage rural soil (range) (mg/kg)
Arsenic	29.9	7.09	0.5–143
Cadmium	0.386	0.29	0.1–1.80
Chromium	16.5	29.2	1.14–236
Copper	20.4	17.25	2.27–96.7
Lead	43.0	37.45	2.60–713
Mercury	0.151	0.10	0.07–1.22
Nickel	13.4	15.8	1.16–216

Metal concentrations in the soil taken from the background dose rate group tanks were consistent with those recorded in rural soil from the UK soil and herbage survey. Soil from each of the dose rate groups also had comparable metal concentrations (Table 2.8).

Concentration factors (CFs) were calculated using the worm and soil data of specific dose rate groups; the results are shown in Figure 2.13. One complication of using data sets containing 'less than' values is that they are actually reporting the limit of detection (LOD) for that sample, analyte and detection equipment. Inevitably, with samples of low mass or low concentration, the number of LOD values within a data set increases. There are four options available for handling these LODs (after Gilbert and Kinnison, 1981).

1. Ignore LOD values and calculate all the statistics using the remaining positive detected data.
2. Replace the LOD values with zero and then complete the statistical analysis.
3. Replace the LOD values by a value between zero and the reported LOD figure, and then complete the statistical analysis.
4. Use the LOD value for all statistical calculations.

Options 1 and 2 would produce values that which would be an overestimate and an underestimate, respectively, resulting in unrepresentative values for the data set. The third option assumes that all the values between zero and the LOD value are equally likely to occur and provides no easy mechanism for selecting a value to use: it simply increases the final reported uncertainty. The final option results in an overestimation of the mean and reduces the actual variation within the data set. However, although not ideal, this method has the advantage of placing an upper limit on the mean values. This is the method applied within the present study.

With one possible exception, the data show that increasing the dose to which the worms are subjected does not relate to an increase or decrease in the uptake of particular metals. For arsenic, there is an indication of a weak relationship between dose rate and concentration factor, which may suggest that arsenic uptake is increased as a result of radiation stress.

PAH analyses were also performed on worms from the background and 8.5 mGy/hour dose rate groups (Table 2.8). In general, the worms exposed to 8.5 mGy/hour had higher levels of PAHs, with only acenapthylene and benzo-[a]-pyrene having higher levels recorded for the background group. This may be linked to radiation stress, but further investigation is needed to determine the relevance of these values, i.e.

- whether the sets of values for the two dose rate groups are significantly different;
- how the PAH values for worms from different dose rate groups would compare;
- how the background levels relate to the values in soil.

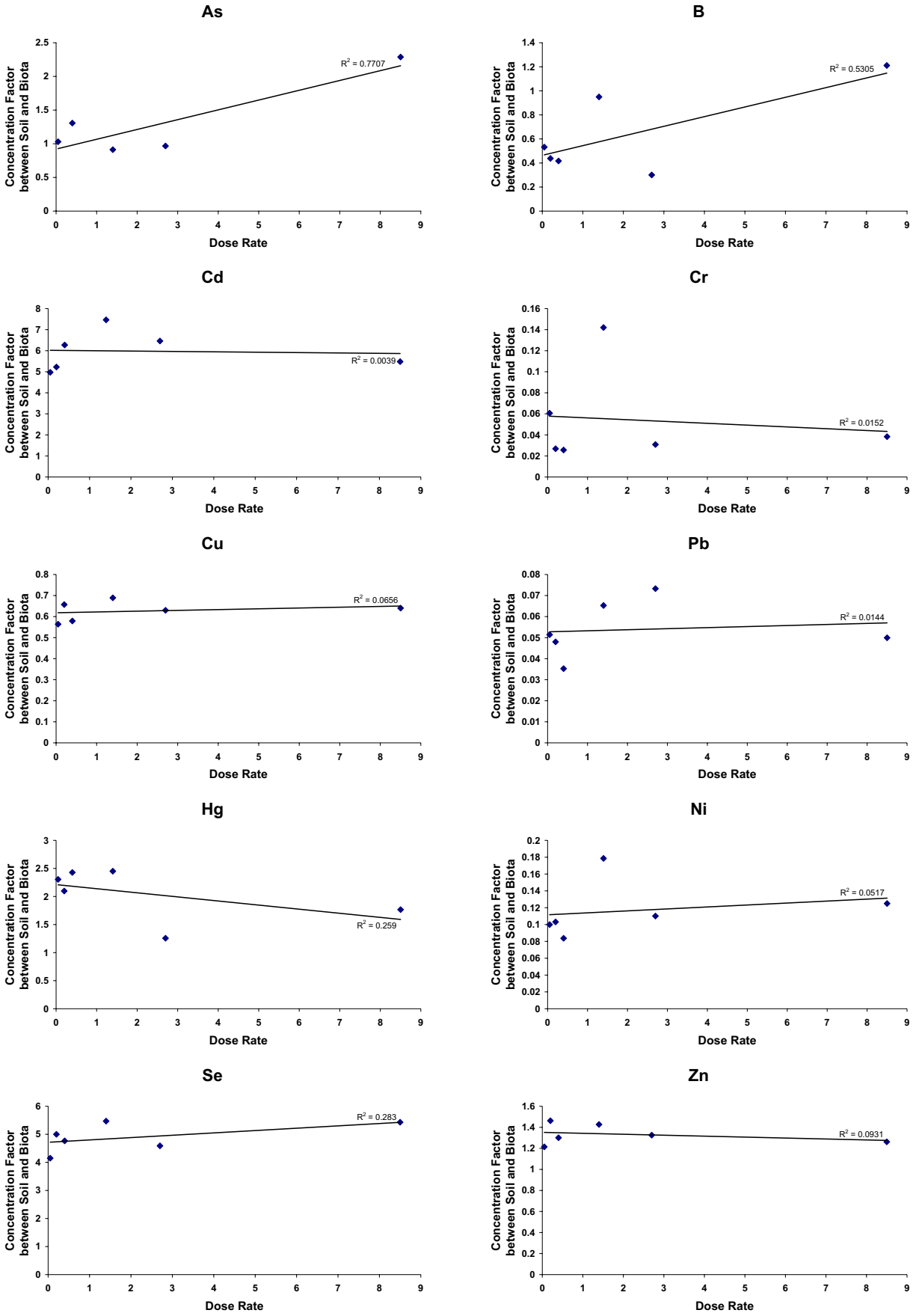

Figure 2.14 | Concentration factors derived from metal concentrations in soil and worms for each dose rate group (Note: Cr, Pb and Se contain LOD values)

Table 2.8 | Contaminant concentrations for soil and worms from all dose rate groups

Analyte	Background		0.2 mGy/hour		0.4 mGy/hour		1.4 mGy/hour		2.7 mGy/hour		8.5 mGy/hour	
	Soil	Worm	Soil	Worm	Soil	Worm	Soil	Worm	Soil	Worm	Soil	Worm
Arsenic	29.9	30.8	2.09	45.0	31.6	41.3	31.9	29.1	30.1	29.1	24.7	56.5
Boron	62.2	33.1	52.7	23.1	59.5	24.8	55.5	52.7	64.2	19.3	55.0	66.6
Cadmium	0.386	1.92	0.377	1.97	0.405	2.54	0.371	2.77	0.376	2.43	0.416	2.28
Chromium	16.5	<1.00	18.6	<0.500	19.5	<0.500	17.6	<2.50	16.2	<0.500	26.1	<1.00
Copper	20.4	11.5	17.5	11.5	20.2	11.7	19.3	13.3	18.1	11.4	21.4	13.7
Lead	43.0	2.21	37.5	1.80	51.9	1.83	38.3	<2.50	34.8	2.55	60.5	3.02
Mercury	0.151	0.348	0.0963	0.202	0.0873	0.212	0.102	0.250	0.113	0.142	0.111	0.196
Nickel	13.4	1.34	13.0	1.34	15.3	1.28	14.0	<2.50	12.9	1.42	12.8	1.60
Selenium	1.28	5.31	1.05	5.25	1.41	6.72	1.17	6.4	1.39	6.38	1.21	6.57
Zinc	90.6	110	80.0	117	93.0	121	86.9	124	84.5	112	95.1	120
Acenaphthene		1.23										7.71
Acenapthylene		23.4										10.6
Anthracene		2.11										11.0
Benz-[a]-anthracene		<19.7										<70.6
Benzo (b) fluoranthene		<5.59										<24.0
Benzo (k) fluoranthene		<1.21										<7.70
Benzo-[a]-pyrene		<76.2										<52.6
Benzo-[e]-pyrene		<16.0										<21.9
Benzo-[ghi]-perylene		<1.48										<56.0
Chrysene		<27.0										90.7
Dibenzo (ah) anthracene		<13.6										<14.7
Fluoranthene		8.12										33.5
Fluorene		0.720										1.68
Indeno-[1,2,3-cd]-pyrene		<13.3										<64.1
Naphthalene		7.95										16.4
Perylene		<36.2										<63.1
Phenanthrene		0.820										0.970
Pyrene		10.2										16.3

Chronic radiation experiments with woodlice

The Good Practice Guides in the SP2 handbook (Wood *et al.*, 2003) were used to design an experimental study of the effects of chronic radiation exposure on the woodlouse (*Porcellio scaber*).

3.1 Test species selection (GPG 1)

Woodlice have been used in standard toxicity testing as well as in ecotoxicology and chronic irradiation studies. For example, in studies of woodlice exposed to beta emitters (Kanao *et al.*, 2002), it was observed that, at very low levels of ionising radiation (4.5 mGy/hour), woodlice migrated towards the source of the radiation rather than retreating from it.

Woodlice are easy to maintain and require little space, and the laboratory conditions required are similar to those for the earthworm. Experiments can be carried out simultaneously on the two species (but using separate tanks).

3.2 Endpoint selection (GPG 2)

SP2 advocates the use of reproductive endpoints for assessment (Wood *et al.*, 2003), as successful environmental protection requires the maintenance of ecosystem function. This function is inherently linked to the success of organisms at a population level; therefore, any reduction in reproductive success or reproductive fitness could impact on the ecosystem.

However, the SP2 handbook also documents the need to observe additional endpoints, namely:

- mortality
- differences in physical appearance
- the weight of individuals.

For field studies, the use of complementary biomarker techniques, combined with methods that relate to organism fitness and site chemistry, provide the most useful data (Anderson *et al.*, 1998). Applying the same multifaceted assessment to laboratory studies, where test chemical concentrations substitute site chemistry, makes the results more comparable.

Studies on histopathological alterations in the hepatopancreas of woodlice as indicators of environmental stress have already been undertaken. Odendaal and Reinecke (2003) studied alterations in histological sections of hepatopancreatic tissue from *P. laevis* as a biomarker of cadmium exposure and found that exposure to cadmium sulphate could change the structure of the hepatopancreas. Znidarsic *et al.* (2003) used transmission electron microscopy to scan hepatopancreatic tissue from *P. scaber* exposed to sub-lethal concentrations of zinc and cadmium for cellular alterations. They found that hepatopancreatic tissue exposed to the metals had gained electrodense deposits compared with the control hepatopancreatic tissue. These studies lend support to the inclusion of histopathological techniques in this study alongside the techniques for measuring reproductive endpoints, mortality and growth.

3.3 Exposure guideline (GPG 3)

SP2 provides information, where it exists, on the dose rate thresholds for specific endpoints for each wildlife group. The information is a composite of the information provided by the FASSET Radiation Effects Database (Woodhead and Zinger, 2003) – known as FRED – and Agency R&D Publication 128 (Copplestone *et al.*, 2001).

No data were available for woodlice specifically, but

data do exist, for example, for earthworms (Section 2.3). However, most of the data for soil fauna are derived from field experiments undertaken after a nuclear accident or where soil activity concentrations have been artificially increased. This is very limited information and SP2 recommends that at least three or four dose rates should be used. The dose range proposed for further investigation for soil fauna is 1,000–5,000 mGy/hour (1–5 mGy/hour). SP2 also emphasises the need to ensure that the response threshold is spanned by the doses selected so that a dose-response relationship can be constructed.

The pro-forma generated using SP2 (see Appendix A3), together with the handbook itself, were used to establish the design of this study. Appendix A3 also contains completed checklist for data reporting from SP2. The methodology and the results are presented below. Tables of raw data for this study with *P. scaber* are given in Appendix A4.

3.4 Experimental design (GPG 4)

3.4.1 Setting up tanks

Commercially supplied bark compost was dried and its water holding capacity derived. The compost was then homogenised before 500 g of dried bark compost was rehydrated with 500 ml of distilled water to gain 50 per cent moisture content (0.5 kg final wet weight). The rehydrated bark compost was placed in a 3.5 litre tank. This was repeated for 72 tanks. Bran (10 g) was then layered over the surface of each tank. A length of plywood (15 cm x 10 cm) was added to each tank to provide cover for the woodlice.

3.4.2 Dosimetry

The tanks were divided into six dose rate groups (12 tanks in each group). The six dose rates chosen for this experiment were background, 0.2, 0.4, 1.5, 4 and 8 mGy/hour. Dosimetry was undertaken at three positions along each bench to verify the dose rates the tanks actually received. The results are given in Table 3.1.

The dose rates administered to the woodlice vary from those administered to the earthworms (see Section 2.4.2) even though the woodlice tanks were placed in random order amongst the worm tanks. This is due to the relative shielding effect of the soil in the worm tanks compared with that of the bark compost and plywood in the woodlice tanks.

Table 3.1 TLD readings taken to verify dose rates actually received by *Porcellio scaber*

Location number	Aisle tank (mGy/hour)	Middle tank (mGy/hour)	Wall tank (mGy/hour)	Mean dose rate (mGy/hour)
1	11.0	7.7	8.1	**8.9**
2	2.5	2.7	3.2	**2.8**
3	1.5	1.4	1.5	**1.5**
4	0.5	0.4	0.4	**0.4**
5	0.2	0.2	0.3	**0.2**

3.4.3 Test animals

Woodlice were supplied by Blades Biological Ltd, UK. Fifteen woodlice were introduced into each tank. In accordance with the sexual differentiation described by Oliver and Meechan (1993), the animals were sexed (Figure 3.1) and weight measurements taken before they were introduced into the tanks. Eight females and seven males were placed in each tank.

3.4.4 Duration of irradiation

The tanks were irradiated continuously for 96 days. The only interruption to radiation exposure was when the tanks were brought out for assessment. The final doses received by each group of tanks are given in Table 3.2.

Table 3.2 Calculation of total dose received by *Porcellio scaber* during the study

Mean dose rate (mGy/hour)	Duration of irradiation (hours)	Total dose given (Gy)
0.2	2,296.1	0.45
0.4	2,295.7	0.92
1.5	2,296.7	3.44
2.8	2,295.6	6.42
8.9	2,296.3	20.44

3.4.5 Assessment of reproductive, growth and mortality parameters

At two-week intervals, each tank was removed from the irradiation facility for assessment. The plywood was removed and the bark compost of the tank hand-sifted to search for the original woodlice and any progeny. Each woodlouse was removed from the tank and weighed, and the number of progeny recorded. Any mortalities were also recorded. After assessment, the bark compost, plywood and woodlice were returned to their tank, which was placed back in the irradiation facility.

During the experimental phase (week 8 out of 14 weeks in total), 36 of the 72 tanks were removed for the interim kill. After the weight measurements had been taken, a proportion of the woodlice were put into 10 per cent buffered formalin ready for histopathological study. This step was repeated in the final week for a proportion of the remaining woodlice.

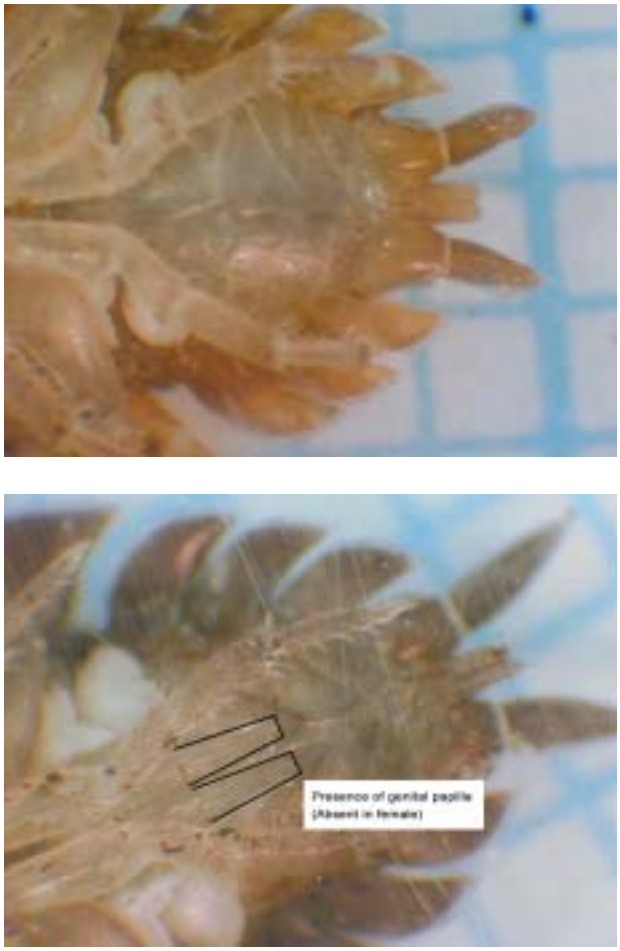

Figure 3.1 External differences between the male and female sexes of the woodlouse: (A) female Porcellio scaber and (B) male *Porcellio scaber* depicting outline of genital papilla in black

3.4.6 Histopathology

The woodlice subjected to 10 per cent buffered formalin were sectioned into three blocks:

- a transverse section at the 3rd/4th segment from the perion
- a transverse section anterior to the pleopods on the abdomen
- a transverse section to the posterior.

The tissues were embedded in paraffin using a Bayer Tissue-Tek III. Sections of tissue, 5 mm in thickness, were stained with H & E stain using a Varistainer. Slides were then examined, for the tissue types listed in Table 3.2, using a Leitz Laborlux 11 microscope.

Table 3.3 Tissue types assessed in the histopathological study of *Porcellio scaber*

Basic tissue type	Specific tissues
Cuticle	Lateral and ventral regions
Alimentary canal	Hind gut with and without typhlosole, rectum and hepatopancreas lobes
Nerve cord	Double ventral cord and ganglia
Gonads	Testes, androgenic glands, seminal vesicles, vasa deferentia and ovaries
Brood pouch	
Pleopods	
Musculo-skeletal system	

3.4.7 Contaminant analyses

At the end of the study, chemical analyses were undertaken on a proportion of the woodlice. The compost from each tank was bulked with compost taken from the other tanks in the same dose rate group and homogenised. Chemical analyses were also undertaken on a sub-sample of the homogenised compost from each dose rate group.

The contaminants analysed for are listed in Table 3.4. Heavy metal analyses were carried out on both woodlice and compost sub-samples for all groups. PAH analyses were only performed on woodlice samples from the background dose rate group and the 8.9 mGy/hour dose rate group.

Table 3.4 | Determinands analysed for in woodlice and bark compost

Metals	PAHs
Arsenic	Acenapthylene
Boron*	Anthracene
Cadmium	Benzo-(a)-anthracene
Chromium	Benzo (b) fluoranthene
Copper	Benzo (k) fluoranthene
Lead	Benzo-[a]-pyrene
Mercury	Benzo-[e]-pyrene
Nickel	Benzo-[ghi]-perylene
Selenium	Chrysene
Zinc	Dibenzo (ah) anthracene
	Fluoranthene
	Fluorene
	Indeno-[1,2,3-cd]-pyrene
	Naphthalene
	Perylene
	Phenanthrene
	Pyrene

* Boron is included with metals for the purposes of this analysis.

3.5 Results

3.5.1 Morbidity

Growth measurements

The general trend was of an increase in average weight, over time, for woodlice from all dose groups (Figure 3.2). The only apparent exceptions were the groups of woodlice exposed to a dose rate of 0.2 mGy/hour and the background level for week 0. This observation cast doubt on the accuracy of the balance used for weighing woodlice and it was replaced before week 2.

The woodlice from the background and 0.2 mGy/hour dose rate groups were not obviously larger than their counterparts in other dose rate groups and, as it was at the start of the study, none of the individuals had been exposed to any levels of radiation. In subsequent weeks, the mean weight values of the woodlice from the background and the lowest dose rate groups were comparable with the the data for the other dose rate groups.

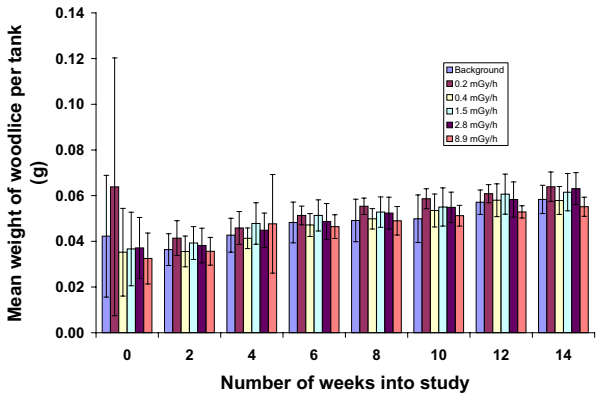

Figure 3.2 | Mean weight of woodlice per tank per dose group during the study (± 1 SD)

Histopathology

A cross-section of a specimen of *P. scaber* is depicted and annotated in Figure 3.3A. It is characterised by the bilateral lobes of the hepatopancreas, the hindgut, rectum and the heart.

Table A4.2 (see Appendix A4) is a record of the observations made from the *P. scaber* sections taken from:

- eight individuals from the background, 1.5 mGy/hour and 8.9 mGy/hour dose rate groups at the interim stage of the study;
- eight individuals taken from each of the six dose rate groups at the end of the study.

None of the woodlice analysed showed signs of any pathological anomalies. Figure 3.3B shows a cross-section of an individual from the 8.9 mGy/hour dose rate group representative of the results from that dose rate group). Figures 3.3C and D illustrate the presence of spermatogonia and oocytes, respectively.

After the observations had been recorded for each individual, they were assessed and graded using the diagnostic criteria in Table 2.5.

After grading, the number of recorded anomalies were grouped into dose groups (Table 3.5). A score was derived from each dose group by multiplying each entry in a column by the number of the category it was graded in. Unlike Table 2.6 (for earthworms), this table does not grade infection as monocystis is worm-specific and no other infections were observed in woodlice.

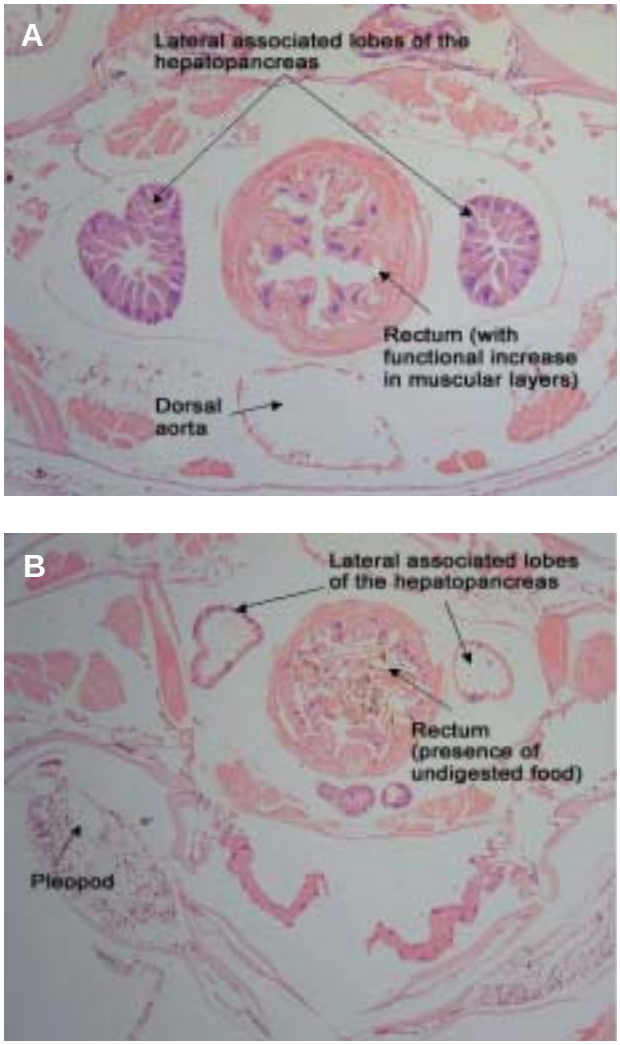

Figure 3.3 Cross-section of a woodlouse representative of (A) the background dose rate group and (B) the 8.9 mGy/hour dose rate group (63× magnification)

Table 3.5 Histopathological scores for both interim and final woodlice based on the diagnostic criteria in Table 2.5

		Anomalies						Total score
		0	5	6	7	8	9	
Interim	Background	8						0
	1.5	8						0
	8.9	8						0
Final	Background	8						0
	0.2	8						0
	0.4	7	1					5
	1.5	7	1					5
	2.8	8						0
	8.9	8						0

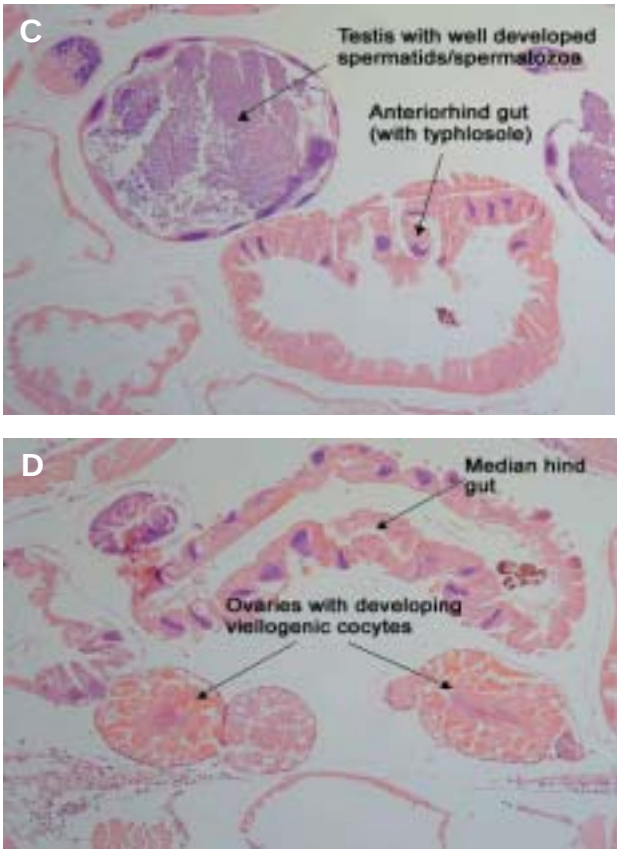

Figure 3.3 | Presence of (C) spermatogonia and (D) oocytes in woodlice

3.5.2 Mortality

Figure 3.4 shows the cumulative number of woodlice mortalities per dose rate group. No obvious relationship between dose rate and mortality can be observed. However, numbers of adult woodlice plus numbers of mortalities did not equate to 15 (the original number of woodlice in each tank) for all the tanks. This is discussed further in section 3.5.3.

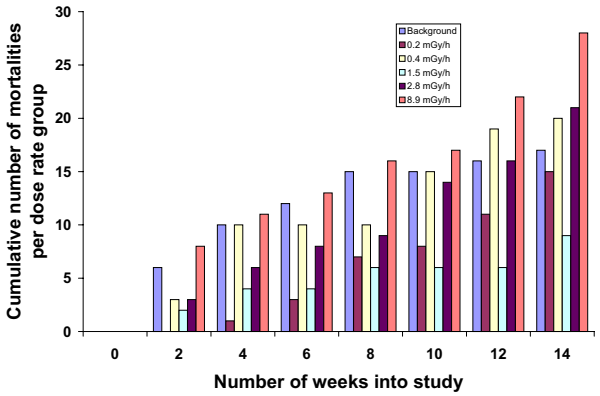

Figure 3.4 | Cumulative number of mortalities per dose group for all tanks during the study

3.5.3 Reproduction

Number of offspring

The numbers of offspring increased for all dose rate groups up to 4 weeks after the beginning of the study (Figure 3.5). After week 4, the general trend for all dose rate groups was a fall in the number of offspring –though no observations of offspring mortality were recorded. In week 14, however, the numbers of offspring for dose rate groups background, 0.2, 0.5, 1.5 and 2.6 mGy/hour were higher than those recorded in week 12.

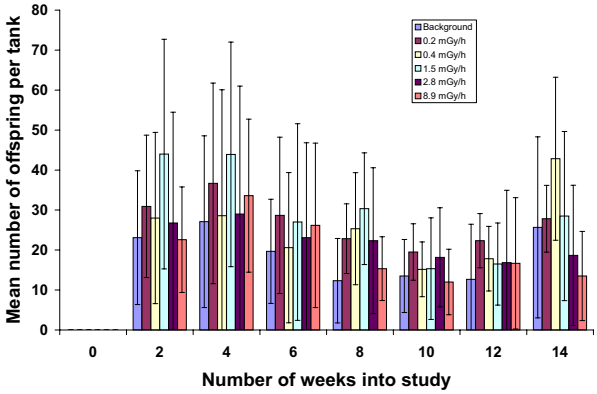

Figure 3.5 | Mean number of woodlice offspring per tank

It is possible that the variations in number of progeny recorded during the assessments were partly due to counting errors: the progeny are small (Figure 3.6) and move quickly.

Figure 3.6 | Photograph illustrating the small size of woodlice offspring

Figure 3.7 shows the average number of offspring per tank for each dose rate group for weeks 8, 10 and 12. The woodlice were counted solely by one assessor; however, there is no obvious indication whether the assessor accounted for the presence of each individual offspring. It was thought that a more suitable indication of the counting error might be to compare the number of adults counted each week with the number of mortalities that had occurred for that period. Figure 3.8 shows such a comparison for the background tanks during the first six weeks of the study.

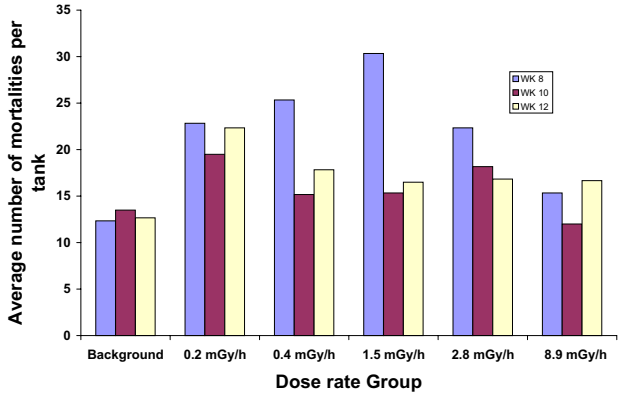

Figure 3.7 Mean number of offspring per tank for all dose rate groups at weeks 8, 10 and 12

The total number of adults and mortalities should all tally at 15 (as at assessment 1, week 0, section 3.5.3). During the assessment at week 2, however, it was clear that either the total number of adults or the total number of mortalities were not accounted for as only two out of 12 tanks had 15 of the original individuals. In week 4, only one tank out of 12 had all 15 of the original individuals accounted for and one tank (6b) had 16 individuals.

The reason for this is unclear. The woodlice could not match the worms' mobility to traverse the sides of the tank (see Section 2.5); therefore, migration of the woodlice out of the tanks is thought highly unlikely. The comparison was repeated for weeks 8, 10 and 12, with only a single assessor carrying out each assessment (four different assessors had carried out the previous comparison). As in weeks 2 and 4 (Figure 3.8), the full complement of 15 woodlice could not be accounted for in any of the tanks (Figure 3.9). Therefore, there is no indication that the number of assessors was a factor that biased the data.

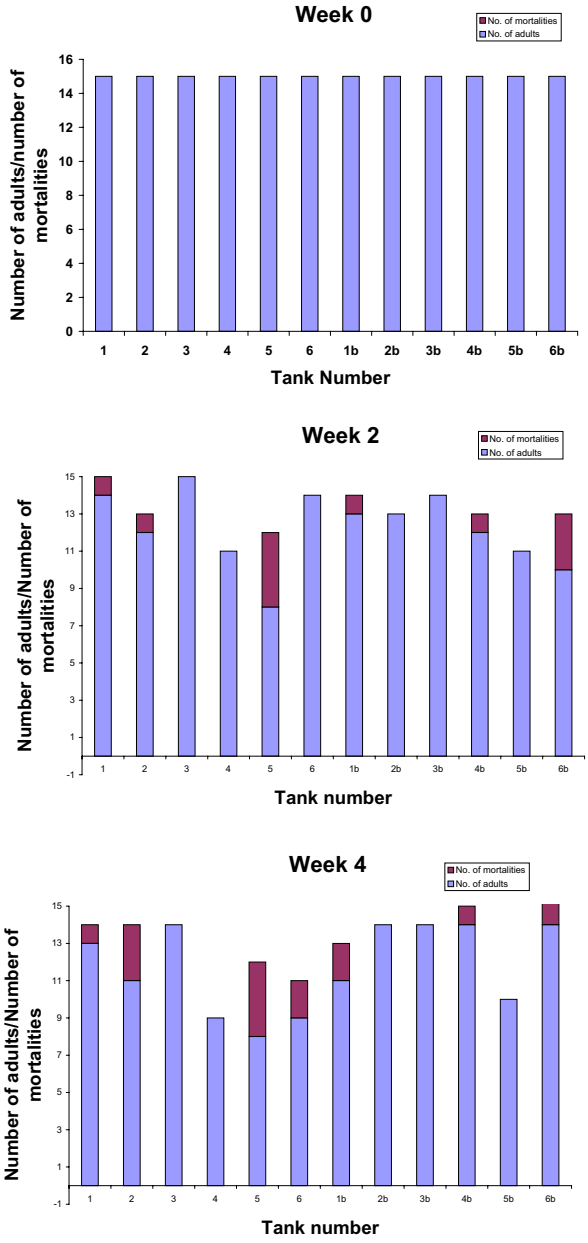

Figure 3.8 Comparison of the number of adults counted and the total number of mortalities in background tanks at weeks 2 and 4 (multiple assessors)

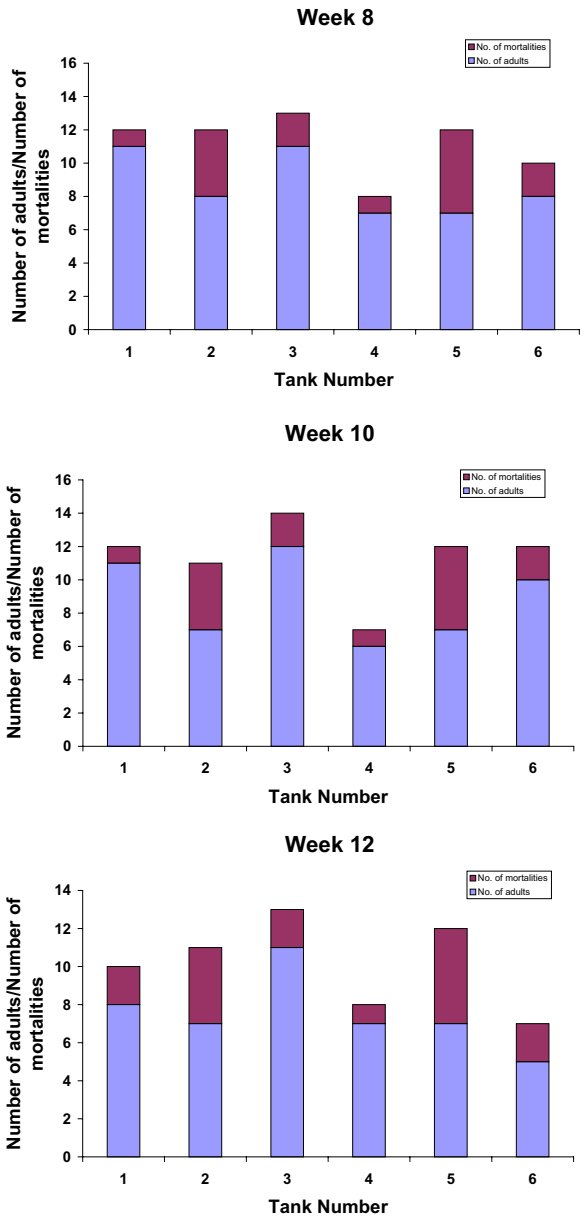

Figure 3.9 Comparison of the number of adults counted and the total number of mortalities in background tanks at weeks 8, 10 and 12 (single assessor)

3.5.4 Contaminant analyses

Samples of soil and woodlouse from each of the six dose rate groups were analysed for the presence of ten metals (Table 3.4). To confirm that the soil metal burden was comparable with normal environmental levels, the metal concentrations in the soil from the background dose rate group were compared with those in rural soil sampled for the UK soil and herbage survey (Wood *et al.*, in preparation) (Table 3.6).

Table 3.6 Comparison of heavy metal concentrations in the test bark compost and rural soils in the UK

Metal	Bark compost from background dose rate (mg/kg)	UK soil and herbage rural soil (median) (mg/kg)	UK soil and herbage rural soil (range) (mg/kg)
Arsenic	2.18	7.09	0.5–143
Cadmium	0.607	0.29	0.1–1.80
Chromium	13.5	29.2	1.14–236.
Copper	13.0	17.25	2.27–96.7
Lead	17.8	37.45	2.60–713
Mercury	0.322	0.10	0.07–1.22
Nickel	12.7	15.8	1.16–216

Metal concentrations in the soil taken from the background dose rate group tanks were consistent with those recorded in rural soil from the UK soil and herbage survey. Soil from each of the dose rate groups also had similar heavy metal concentrations to each other (Table 3.7). Concentration factors were calculated using the woodlice and soil data of specific dose rate groups and the results are shown in Figure 3.10. LOD values were treated as explained in Section 2.5.4.

With one possible exception, the data show that increasing the dose to the woodlice does not correlate with the uptake of particular metals. For chromium, there is an indication of a weak relationship between dose rate and concentration factor, which may suggest that uptake is increased as a result of radiation stress. However, the concentration values for chromium in woodlice are LOD values and not absolute values. Further investigation would be needed to confirm any such relationship.

PAH analyses were also performed on woodlice from the background and 8.9 mGy.hour dose rate groups (Table 3.7). In general, the woodlice exposed to 8.9 mGy/hour had higher levels of the PAHs analysed for (12 out of the 18 PAHs listed in Table 3.4) than those recorded for the background group. Further investigation is needed to determine the relevance of these values, i.e.

- whether the sets of values for the two dose rate groups are significantly different;
- how the PAH values for woodlice from different dose rate groups would compare
- how the background levels relate to the values in soil.

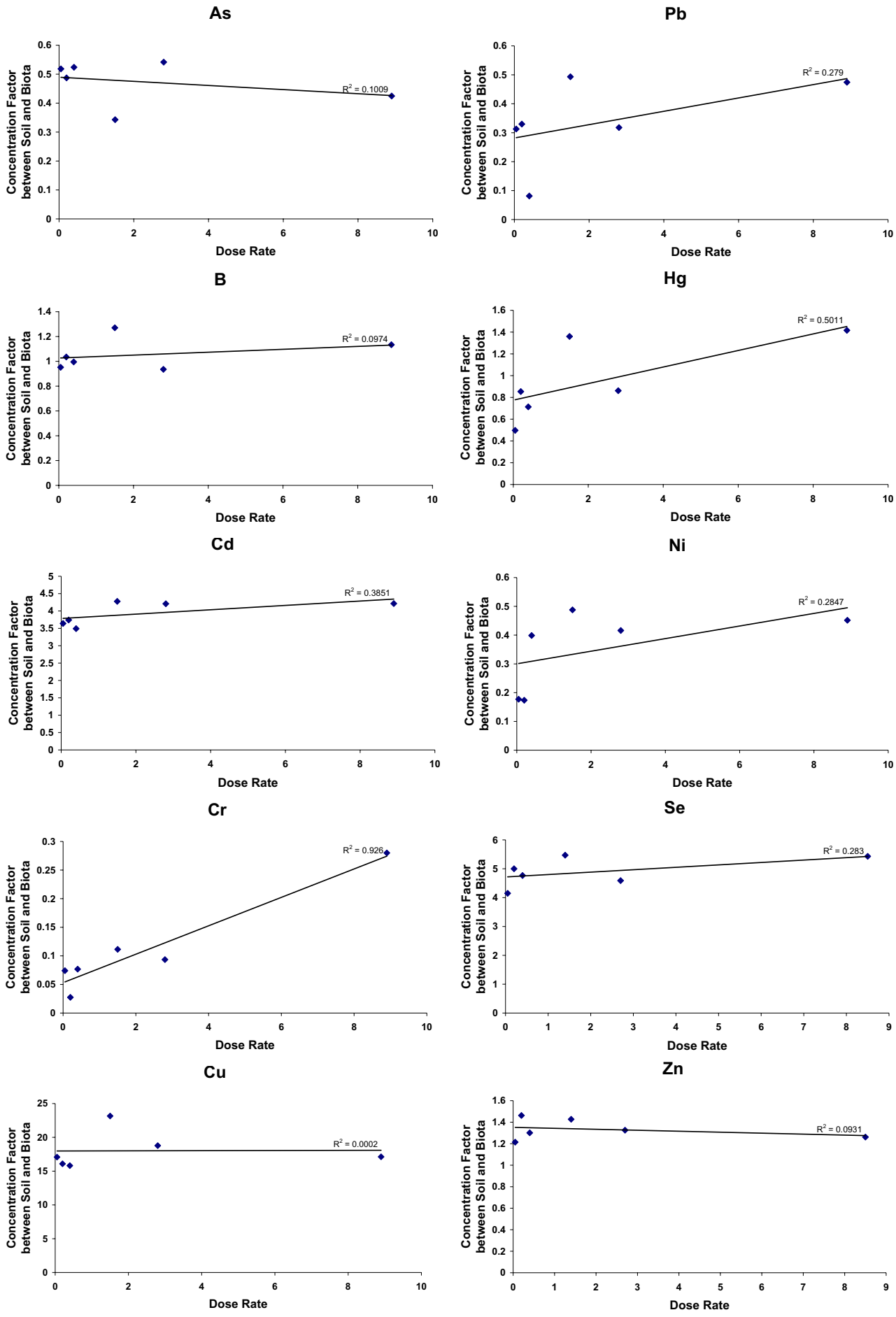

Figure 3.10 | Concentration factors derived from heavy metal concentrations in soil and woodlice for each dose rate group (Note Cr and Se include LOD values)

Table 3.7 | Contaminant concentrations for both soil and woodlice from all dose rate groups

Analyte	Background		0.2 mGy/hour		0.4 mGy/hour		1.5 mGy/hour		2.8 mGy/hour		8.9 mGy/hour	
	Litter	Woodlice	Litter	Woodlice	Litter	Woodlice	Litter	Woodlice	Litter	Woodlice	Litter	Woodlice
Arsenic	2.18	1.13	1.48	0.721	1.67	0.875	2.71	0.929	1.92	1.04	2.03	0.862
Boron	81.3	77.4	66.0	68.3	82.5	82.1	60.3	76.6	87.0	81.4	70.3	79.7
Cadmium	0.607	2.21	0.580	2.17	0.570	1.99	0.505	2.16	0.575	2.42	0.579	2.44
Chromium	13.5	<1.00	18.2	<0.500	8.73	<0.670	8.98	<1.00	10.7	<1.00	8.93	<2.50
Copper	13.0	222	13.8	222	14.1	223	11.4	264	13.9	261	14.6	250
Lead	17.8	5.57	21.7	7.16	54.2	4.41	16.5	8.14	19.0	6.04	25.3	12.0
Mercury	0.322	0.160	0.212	0.181	0.244	0.174	0.175	0.238	0.260	0.224	0.214	0.303
Nickel	12.7	2.25	12.1	2.10	5.62	2.24	5.29	2.58	6.73	2.80	5.58	2.52
Selenium	<0.400	0.422	<0.400	<0.400	<0.400	<0.400	<0.400	<0.400	<0.400	<0.400	<0.400	0.420
Zinc	130	323	125	334	126	332	105	359	121	360	123	350
Acenaphthene		<12.6										11.3
Acenapthylene		2.20										2.73
Anthracene		46.2										4.62
Benz-[a]-anthracene		<87.8										<91.4
Benzo (b) fluoranthene		<33.2										3.86
Benzo (k) fluoranthene		<27.4										16.2
Benzo-[a]-pyrene		<4.98										7.59
Benzo-[e]-pyrene		<1.27										14.9
Benzo-[ghi]-perylene		<26.0										4.62
Chrysene		19.7										32.4
Dibenzo (ah) anthracene		<8.91										<9.90
Fluoranthene		0.920										4.96
Fluorene		5.87										14.0
Indeno-[1,2,3-cd]-pyrene		<7.81										25.3
Naphthalene		15.5										36.6
Perylene		<14.1										<9.15
Phenanthrene		20.8										32.2
Pyrene		<9.58										<17.2

Discussion

In a previous study on the effects of different heavy metals on *Eisenia fetida*, increasing concentrations of cadmium resulted in severely affected growth (Reinecke and Reinecke, 1996). In the same study, it was observed that worms grew well in soil contaminated with lead, though reproductive performance was affected.

4.1 Discussion of results concerning *Eisenia fetida*

Although a drop in weight occurred after four weeks of the present study, the decreased weight is only just lower than the worms' original weight. The initial and unexpected increase in weight at the beginning of the study could be attributed to the worms being introduced into a new environment with an abundant food supply.

The histopathological study of *E. fetida* did not reveal any anomalies – merely a high prevalence of monocystis (an endemic protozoan infection in worms). The number of individuals with monocystis for each dose rate group was assessed and recorded, as prevalence of infection can also be a biomarker for increased levels of environmental contamination. When the oil tanker *Sea Empress* spilt over 70,000 tonnes of crude oil into the sea off the Milford Haven coastline, the mussels in the vicinity suffered severe immunodepression (Dyrynda *et al.,* 2000). This was measured by the levels of superoxide production and phagocytic activity in the haemocytes of the mussels from the contaminated shoreline. These levels were greatly reduced in affected individuals, which could have seriously undermined their ability to resist disease. Environmental contamination may lead to increased susceptibility to disease in other species. For example, in a study of mean concentrations of mercury, selenium and zinc in the liver of porpoises (*Phocoena phocoena*), levels were significantly higher in those that had died of infectious disease than those that had died of other causes such as physical trauma (CEFAS, 2001). However, no link could be made in the present study between levels of infection and dose rate as the majority of worms exhibited monocystis.

However, a variation in endpoints (e.g. offspring number) between tanks in the same dose group was increasingly apparent. Arnaud *et al.* (2000) showed that biomarkers such as oxidative stress could vary significantly in the short time period of two weeks and not be linked to changes resulting from alterations in season, weather, temperature or any other stress. The worms and woodlice in the present study were kept in a clean and constant environment (similar temperature readings between dose groups were verified) and these factors would not cause inter-tank differences. Of more importance in this instance are the other modifying factors that have been reported which relate to the organism itself and not its surroundings. These factors include health, sex, age and, of certain consequence in the earthworm experiment, migration behaviour. The extent and influence of these modifying factors and how natural or controlled environments affect them have rarely been quantified previously (Arnaud *et al.*, 2000).

4.2 Discussion of results concerning *Porcellio scaber*

The protective effects of small doses of ionising radiation have been promoted and discussed (Johansson, 2003). Indeed, studies on woodlice have proved that aversion to all ranges of radiation sources is not automatic. Kanao *et al.* (2002) observed that, at very low levels of ionising radiation (4.5 mGy/hour) produced by a beta emitter, woodlice migrated towards the source of the radiation as opposed to retreating from it. This phenomenon was also observed when the woodlice were near a gamma emitter (^{57}Co) where the exposure rate was 10 mGy/hour (Kanao *et al.*, 2002).

However, the woodlice moved away from the source when they were exposed to higher levels of ionising radiation irrespective of source type. In the present study, no benefit to the woodlice from being exposed to lower doses of radiation was perceived; likewise, no detrimental effects were observed in woodlice at higher dose rates. Similar growth and mortality rates and number of offspring were observed in woodlice exposed to different dose rates.

One concern relating to the experimental design of this study was that some woodlice generated their offspring very quickly. As woodlice only have 1–2 broods each year, it was suspected that some of the woodlice may have been pregnant before the start of the study and, as a result, the full effects of the differing doses of radiation would not have been represented.

4.3 General discussion and evaluation of SP2

The SP2 handbook (Wood *et al.*, 2003) states that the organisms under examination should be kept in optimum living conditions by regulating factors such as diet, temperature and light regime to ensure that any effects observed are contributable to the contaminant under assessment. This guidance is consistent with the internationally adopted approach for carrying out ecotoxicological assessments of chemical contaminants. This does pose a dilemma, however, as organisms kept in the laboratory have, in some instances, been more resistant to the effects of radiation due to their 'protected' environment compared with when kept in their indigenous, naturally stressful habitats (Mothersill, 2001). The worms and woodlice in this study appeared to be unaffected by the doses given. But the organisms were removed from predation, overcrowding, competition for food, and extreme conditions. The results, therefore, could be indicative of a 'protected' environment.

The SP2 handbook provided suitable guidance on a wide range of issues. However, it must be stressed that researchers should not use it as a prescriptive tool and must be aware that unforeseen problems can still arise (e.g. migration of worms from tanks). The handbook also states that researchers should consider costs and staffing levels. Time constraints were a major factor in this study (as in many) and it proved difficult for each assessor to complete the assessment of the number of tanks required.

4.4 Conclusion

It has previously been reported that the consensus for most studies on biota is that the threshold for statistically significant effects on individual organisms is about 100 mGy/hour, with responses increasing progressively with increased dose rate (Zinger et al., 2003). At sites contaminated by regulated release of radionuclides, existing assessments indicate that, in the UK, the absorbed dose rates are generally much less than 100 mGy/hour and always less than 1,000 mGy/hour (Woodhead, 2004).

In the present study, the maximum dose rate that individuals of *E. fetida* and *P. scaber* were exposed to was approximately 8 mGy/hour (8,000 $mGyh^{-1}$). The study revealed that neither species showed deleterious effects for the endpoints studied at this dose rate or below.

4.5 Recommendations for further research

This study has provided more data to fill gaps in the FASSET database (Woodhead and Zinger, 2003) concerning soil fauna. It has also provided an evaluation of the SP2 handbook. If time and resources had allowed, the following would have been undertaken. These should now be considered as recommendations to be incorporated into similar future studies.

- The F1 generation would have been retained and studied further to assess their reproductive success and survival rates.
- Quality control checks of tank assessment would have been undertaken. For example, three or four tanks containing a known number of woodlice and offspring should have been maintained to allow assessment of inter-assessor differences and to ascertain whether any variation in results was due to human error.
- The comet assay would have been undertaken on animals from each dose rate group to ascertain the number of DNA strand breaks occurring within each group. The inclusion of the comet assay in this study would have been a valuable tool, because it provides another endpoint for comparison and is a simple and low-cost technique to perform (Hingston, 2003). The comet assay was undertaken on *E. fetida* in a recent study on the effects of nickel (in the form of nickel chloride), which succeeded in highlighting the genotoxic potential of this heavy metal compound (Reinecke and Reinecke, 2004).
- Consideration of the reproduction specific biomarker annetocin would have been included. Annetocin is a novel biomarker that has been developed to evaluate reproductive fitness in the earthworm *E. fetida* (Ricketts *et al.*, 2004). Annetocin is involved in the induction of cocoon laying behaviour. By measuring the expression of this gene in earthworms subjected to soils with different contaminant levels, the effects such levels has on egg laying can be ascertained (Ricketts *et al.*, 2004).

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Appendix A1:

Table A1.1 | Pro-forma for earthworm experiment (following guidance key in Section 3.1 of SP2)

Main experiment (delete as appropriate)					
Key instruction			Page	Section	Table
a	**Umbrella endpoint of interest** (e.g. reproduction)	Reproduction	53 57	5.1.2 5.2.1	5.1+5.2
b	**Wildlife group and species**	Soil fauna *Eisenia fetida* (compost worm)	44	4.17	4.13a
c	**Maintenance conditions** (e.g. temperature, light regime, diet)	Room temperature, continual light 50% soil moisture, horse manure	44	4.17	4.13 a,b,c
d	• **Specific endpoint(s) to study** (e.g. No. of eggs produced)	No. of cocoons produced No. of juveniles	53	5.2	5.1+5.2
	• **Compulsory measurements to record**	Weight Length Growth rate No. of mortalities occurring			
e	**Irradiation type** (internal/external/mixed)	External	59	6.2	–
f	**Facilities required** (e.g. Cs-137 source)	Cs-137 source	68	6.7.1	–
g	**Dose rates to use** (e.g. Background, 10, 20, 40, 80, 160, 320, etc. Gy or µGy/hour). Where known also indicate, in the brackets, the total dose received at each dose rate.	Background = minimal Dose rate 1 = 0.2 mGy/hour (0.3 Gy) Dose rate 2 = 0.4 mGy/hour (1.0 Gy) Dose rate 3 = 1.5 mGy/hour (4.0 Gy) Dose rate 4 = 4.0 mGy/hour (10 Gy) Dose rate 5 = 8.0 mGy/hour (20 Gy)	66	6.5.6	6.9
h	**Need for a pilot experiment?**	No	67	6.6	–
	• No. of dose rates, including control				
	• No. of individuals per dose rates				
	• Dose/dose rates used				
	• Duration of irradiation				
	• Duration of experiment				
	• **Notes/other considerations**				
i	**Duration of irradiation** (e.g. list daily time period for irradiation – 20 hours)	Continual – irradiation only interrupted for assessing endpoints	58	6	–
	Duration of experiment	108 days	–	–	–
j	**No. of dose rates, including control**	6	–	–	–

Table A1.1 | Pro-forma for earthworm experiment (following guidance key in Section 3.1 of SP2) *continued*

	No. of individuals per dose rate (e.g. 10)	8 individuals per tank; 14 tanks per dose rate	70	7.1.2	–
k	**Statistical requirements**		69	7	–
	• **Tier 1**	Graphs – check data normally distributed			
	• **Tier 2** (e.g. test used)	F_{max} test			
	• **Tier 3** (e.g. test used)	Analysis of variance (ANOVA)			
l	**Further literature search conducted?** If yes, then list	YES OECD (2000) and ASTM protocols For further details of references consulted see reference section of P3-101/SP7			
Further justification of decisions made to complete the pro-forma (to be completed when decisions are not based on information in the guidance document that can be referred to by page/section/table)		The dose rates were calculated based on the dimensions of the irradiation facility in conjunction with consulting relevant literature.			
Notes (general)		Endpoints assessed every two weeks for 16 weeks week 8 – Interim kill of half the worms (six tanks in each dose group). Proportion of these worms used for histopathological analysis. week 16 – Final kill of half the worms (six tanks in each dose group). Proportion of these worms used for histopathological analysis.			

Table A1.2 | Checklist for data reporting – *Eisenia fetida*

	Tick box
Authors	✓
Article title	✓
Reference details	✓
Keywords	
Type of study (laboratory, field, controlled field)	✓
Radiation type (alpha, beta, gamma or mixed)	✓
Exposure type (internal, external, mixed)	✓
Ecosystem	
Wildlife group	✓
Species name (Latin and common)	✓
Source of organisms (supplier)	✓
Life-stage of organisms	✓
Maintenance of organisms prior to and during the experiments	✓
Umbrella endpoint(s)	✓
Specific endpoint being studied(s)	✓
Frequency and timing of specific endpoint measurements	✓
Dose rate(s)	✓
Notes on how the dose or dose rate was calculated	✓
Activity concentrations for internal exposures	
Dose(s)	✓
No of individuals per treatment group (including control)	✓
Duration of exposure(s)	✓
Result(s)	✓
Statistical analysis	
Any relevant notes	
Production of a data sheet to record results	✓

Appendix A2:

Raw data for *Eisenia fetida*

Table A2.1 | Total weight measurements taken from *Eisenia fetida* during the study

DOSE GROUP		BACKGROUND								
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0.42	0.52	0.32	0.36	0.38	0.39	0.43	0.46	0.43
	2	0.40	0.40	0.37	0.41	0.36	0.40	0.40	0.43	0.39
	3	0.41	0.45	0.39	0.43	0.45	0.43	0.40	0.43	0.40
	4	0.41	0.44	0.43	0.55	0.51				
	5	0.38	0.57	0.30	0.30	0.34	0.30	0.34	?/7	0.31
	6	0.37	0.30	0.32	0.30	0.26	0.30	0.35	0.34	0.30
	7	0.43	0.40	0.34	0.33	0.31	0.34	0.39	0.35	0.36
	1b	0.43	0.49	0.34	0.35	0.37				
	2b	0.39	0.51	0.32	0.34	0.36				
	3b	0.43	0.51	0.37	0.44	0.44				
	4b	0.43	0.57	0.39	0.39	0.37	0.34	0.39	0.41	0.40
	5b	0.47	0.41	0.37	0.36	0.34				
	6b	0.28	0.27	0.30	0.36	0.33				
	7b	0.32	0.41	0.27	0.32	0.25				

DOSE GROUP		0.2								
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0.28	0.55	0.31	0.30	0.26	0.28	0.28	0.36	0.30
	2	0.43	0.46	0.30	0.28	0.29	0.33	0.37	0.36	0.33
	3	0.45	0.45	0.36	0.40	0.37	0.37	0.38	0.39	0.37
	4	0.48	0.54	0.42	0.39	0.36	0.38	0.37	0.42	0.38
	5	0.42	0.42	0.36	0.32	0.33	0.36	0.38	0.40	0.33
	6	0.43	0.45	0.34	0.35	0.36	0.38	0.36	0.40	0.35
	7	0.40	0.41	0.37	0.33	0.33	0.35	0.35	0.40	0.36
	1b	0.40	0.51	0.33	0.34	0.30				
	2b	0.40	0.43	0.31	0.30	0.31				
	3b	0.40	0.57	0.29	0.33	0.27				
	4b	0.40	0.58	0.32	0.29	0.29				
	5b	0.33	0.37	0.28	0.23	0.24				
	6b	0.42	0.47	0.43	0.36	0.30				
	7b	0.37	0.51	0.34	0.28	0.32				

Table A2.1 | Total weight measurements taken from *Eisenia fetida* during the study *continued*

DOSE GROUP		0.4								
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0.30	0.46	0.29	0.26	0.29	0.30	0.32	0.34	0.29
	2	0.33	0.39	0.38	0.40	0.31	0.34	0.31	0.34	0.30
	3	0.35	0.49	0.43	0.36	0.27	0.31	0.31	0.31	0.31
	4	0.37	0.53	0.26	0.37	0.32	0.37	0.41	0.38	0.39
	5	0.31	0.45	0.34	0.29	0.29	0.20	0.30	0.33	0.31
	6	0.32	0.53	0.37	0.36	0.34	0.28	0.28	0.31	0.31
	7	0.39	0.43	0.31	0.33	0.28	0.31	0.32	0.29	0.27
	1b	0.34	0.42	0.34	0.28	0.28				
	2b	0.33	0.48	0.33	0.32	0.29				
	3b	0.35	0.48	0.38	0.33	0.30				
	4b	0.35	0.62	0.32	0.34	0.34				
	5b	0.36	0.61	0.34	0.35	0.27				
	6b	0.36	0.43	0.39	0.33	0.32				
	7b	0.27	0.53	0.33	0.38	0.29				

DOSE GROUP		1.4								
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0.27	0.44	0.27	0.27	0.25	0.30	0.31	0.35	0.33
	2	0.48	0.39	0.39	0.33	0.31	0.40	0.32	0.35	0.36
	3	0.35	0.30	0.29	0.30	0.33	0.32	0.34	0.33	0.27
	4	0.41	0.50	0.34	0.35	0.27	0.33	0.31	0.34	0.34
	5	0.32	0.42	0.34	0.34	0.27	0.33	0.35	0.33	0.37
	6	0.32	0.36	0.31	0.31	0.24	0.23	0.22	0.33	0.25
	7	0.31	0.42	0.37	0.37	0.32	0.36	0.36	0.36	0.33
	1b	0.34	0.40	0.32	0.34	0.32				
	2b	0.39	0.48	0.36	0.31	0.35				
	3b	0.38	0.39	0.28	0.26	0.27				
	4b	0.40	0.38	0.35	0.41	0.26				
	5b	0.35	0.49	0.38	0.29	0.32				
	6b	0.34	0.59	0.36	0.34	0.38				
	7b	0.40	0.38	0.35	0.37	0.34				

DOSE GROUP		2.7								
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0.31	0.30	0.29	0.33	0.27	0.26	0.33	0.35	0.32
	2	0.34	0.42	0.36	0.37	0.29	0.28	0.33	0.37	0.32
	3	0.30	0.43	0.33	0.34	0.32	0.26	0.37	0.39	0.37
	4	0.30	0.34	0.35	0.27	0.27	0.24	0.28	0.32	0.26
	5	0.40	0.58	0.39	0.33	0.33	0.29	0.32	0.35	0.31
	6	0.45	0.58	0.37	0.37	0.32	0.27	0.31	0.35	0.35
	7	0.40	0.43	0.35	0.29	0.28	0.30	0.22	0.29	0.26
	1b	0.34	0.41	0.33	0.36	0.33				
	2b	0.30	0.32	0.29	0.27	0.25				
	3b	0.41	0.40	0.40	0.31	0.33				
	4b	0.37	0.38	0.32	0.32	0.35				
	5b	0.29	0.44	0.30	0.28	0.27				
	6b	0.33	0.47	0.35	0.35	0.37				
	7b	0.37	0.44	0.40	0.32	0.35				

Table A2.1 | Total weight measurements taken from *Eisenia fetida* during the study *continued*

DOSE GROUP		8.5								
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0.40	0.51	0.32	0.35	0.37	0.39	0.38	0.36	0.33
	2	0.48	0.57	0.35	0.42	0.37	0.42	0.44	0.47	0.49
	3	0.47	0.55	0.37	0.39	0.37	0.36	0.37	0.37	0.34
	4	0.47	0.45	0.35	0.29	0.33	0.35	0.36	0.39	0.36
	5	0.37	0.73	0.36	0.38	0.33	0.37	0.32	0.32	0.29
	6	0.40	0.59	0.32	0.38	0.36	0.36	0.36	0.39	0.37
	7	0.38	0.53	0.36	0.38	0.32	0.35	0.35	0.40	0.36
	1b	0.33	0.51	0.37	0.32	0.34				
	2b	0.26	0.42	0.28	0.20	0.25				
	3b	0.29	0.48	0.26	0.25	0.29				
	4b	0.37	0.40	0.34	0.41	0.31				
	5b	0.37	0.45	0.32	0.31	0.34				
	6b	0.39	0.54	0.36	0.39	0.29				
	7b	0.40	0.45	0.35	0.35	0.36				

Table A2.2 | Histopathological findings – *Eisenia fetida*

Worm Pathology			No abnormalities discovered (NAD)	
Interim	Dose Gp.	Animal No.		Seminal vesicle lobe(s)
LIV 1	B	1	NAD	Minimal background Monocystis infection
LIV 1	B	2	NAD	Marked background Monocystis infection
LIV 1	B	3	NAD	Trace background Monocystis infection
LIV 1	B	4	NAD	Trace background Monocystis infection
LIV 1	B	5	NAD	Trace background Monocystis infection
LIV 1	B	6	NAD	Minimal background Monocystis infection
LIV 1	B	7	NAD	Moderate background Monocystis infection
LIV 1	B	8	NAD	Minimal background Monocystis infection
LIV 1	1.4	1	NAD	Moderate background Monocystis infection
LIV 1	1.4	2	NAD	Trace background Monocystis infection
LIV 1	1.4	3	NAD	Trace background Monocystis infection
LIV 1	1.4	4	NAD	Minimal background Monocystis infection
LIV 1	1.4	5	NAD	Moderate background Monocystis infection
LIV 1	1.4	6	NAD	Moderate background Monocystis infection
LIV 1	1.4	7	NAD	Moderate background Monocystis infection
LIV 1	1.4	8	NAD	Moderate background Monocystis infection
LIV 1	8.4	1	NAD	Trace background Monocystis infection
LIV 1	8.4	2	NAD	Minimal background Monocystis infection
LIV 1	8.4	3	NAD	Minimal background Monocystis infection
LIV 1	8.4	4	NAD	Trace background Monocystis infection
LIV 1	8.4	5	NAD	Trace background Monocystis infection
LIV 1	8.4	6	NAD	Minimal background Monocystis infection
LIV 1	8.4	7	NAD	Moderate background Monocystis infection
LIV 1	8.4	8	NAD	Minimal background Monocystis infection

Table A2.2 | Histopathological findings – *Eisenia fetida*
continued

Terminal	Dose Gp.	Animal No.		Seminal vesicle lobe(s)
LIV 1	B	9	NAD	Minimal background Monocystis infection
LIV 1	B	10	NAD	Moderate background Monocystis infection
LIV 1	B	11	NAD	Not present
LIV 1	B	12	NAD	Trace background Monocystis infection
LIV 1	B	13	NAD	Trace background Monocystis infection
LIV 1	B	14	NAD	Moderate background Monocystis infection
LIV 1	B	15	NAD	Minimal background Monocystis infection
LIV 1	B	16	NAD	Minimal background Monocystis infection
LIV 1	0.2	9	NAD	Minimal background Monocystis infection
LIV 1	0.2	10	NAD	Moderate background Monocystis infection
LIV 1	0.2	11	NAD	Moderate background Monocystis infection
LIV 1	0.2	12	NAD	Minimal background Monocystis infection
LIV 1	0.2	13	NAD	Minimal background Monocystis infection
LIV 1	0.2	14	NAD	Trace background Monocystis infection
LIV 1	0.2	15	NAD	Minimal background Monocystis infection
LIV 1	0.2	16	NAD	Moderate background Monocystis infection
LIV 1	0.4	9	NAD	Trace background Monocystis infection
LIV 1	0.4	10	NAD	Moderate background Monocystis infection
LIV 1	0.4	11	NAD	Minimal background Monocystis infection
LIV 1	0.4	12	NAD	Trace background Monocystis infection
LIV 1	0.4	13	NAD	Trace background Monocystis infection
LIV 1	0.4	14	NAD	Trace background Monocystis infection
LIV 1	0.4	15	NAD	Trace background Monocystis infection
LIV 1	0.4	16	NAD	Trace background Monocystis infection
LIV 1	1.4	9	NAD	Trace background Monocystis infection
LIV 1	1.4	10	NAD	Minimal background Monocystis infection
LIV 1	1.4	11	NAD	Minimal background Monocystis infection
LIV 1	1.4	12	NAD	Minimal background Monocystis infection
LIV 1	1.4	13	NAD	Minimal background Monocystis infection
LIV 1	1.4	14	NAD	Moderate background Monocystis infection
LIV 1	1.4	15	NAD	Minimal background Monocystis infection
LIV 1	1.4	16	NAD	Minimal background Monocystis infection
LIV 1	2.7	9	NAD	Minimal background Monocystis infection
LIV 1	2.7	10	NAD	Minimal background Monocystis infection
LIV 1	2.7	11	NAD	Trace background Monocystis infection
LIV 1	2.7	12	NAD	Not present
LIV 1	2.7	13	NAD	Minimal background Monocystis infection
LIV 1	2.7	14	NAD	Not present
LIV 1	2.7	15	NAD	Minimal background Monocystis infection
LIV 1	2.7	16	NAD	Trace background Monocystis infection
LIV 1	8.5	9	NAD	Minimal background Monocystis infection
LIV 1	8.5	10	NAD	Trace background Monocystis infection
LIV 1	8.5	11	NAD	Trace background Monocystis infection
LIV 1	8.5	12	NAD	Minimal/moderate background Monocystis infection
LIV 1	8.5	13	NAD	Moderate background Monocystis infection
LIV 1	8.5	14	NAD	Not present
LIV 1	8.5	15	NAD	Trace background Monocystis infection
LIV 1	8.5	16	NAD	Not present

Table A2.3 | Number of mortalities amongst *Eisenia fetida* during the study

DOSE GROUP		BACKGROUND								
No. of mortalities		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0				
	5	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0
	1b	0	0	0	0	0				
	2b	0	0	0	0	0				
	3b	0	0	0	0	0				
	4b	0	0	0	0	0	0	0	0	0
	5b	0	0	0	0	0				
	6b	0	0	0	0	0				
	7b	0	0	0	0	0				

DOSE GROUP		0.2								
No. of mortalities		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0
	1b	0	0	0	0	0				
	2b	0	0	0	0	0				
	3b	0	0	0	0	0				
	4b	0	0	0	0	0				
	5b	0	0	0	0	0				
	6b	0	0	0	0	0				
	7b	0	0	0	0	0				

DOSE GROUP		0.4								
No. of mortalities		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0
	1b	0	0	0	0	0				
	2b	0	0	0	0	0				
	3b	0	0	0	0	0				
	4b	0	0	0	0	0				
	5b	0	0	0	0	0				
	6b	0	0	0	0	0				
	7b	0	0	0	0	0				

Table A2.3 | Number of mortalities amongst *Eisenia fetida* during the study *continued*

DOSE GROUP		1.4								
No. of mortalities		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0
	1b	0	0	0	0	0				
	2b	0	0	0	0	0				
	3b	0	0	0	0	0				
	4b	0	0	0	0	0				
	5b	0	0	0	0	0				
	6b	0	0	0	0	0				
	7b	0	0	0	0	0				

DOSE GROUP		2.7								
No. of mortalities		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0
	1b	0	0	0	0	0				
	2b	0	0	0	0	0				
	3b	0	0	0	0	0				
	4b	0	0	0	0	0				
	5b	0	0	0	0	0				
	6b	0	0	0	0	0				
	7b	0	0	0	0	0				

DOSE GROUP		8.5								
No. of mortalities		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	0	0	0	0	0	0
	2	0	1	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0
	1b	0	0	0	0	0				
	2b	0	0	0	0	0				
	3b	0	0	0	0	0				
	4b	0	0	0	0	0				
	5b	0	0	0	0	0				
	6b	0	0	0	0	0				
	7b	0	0	0	0	0				

Table A2.4 | Number of cocoons produced by *Eisenia fetida* during the study

DOSE GROUP		BACKGROUND								
No. of cocoons		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	6	7	3	0	1	1	3	0
	2	0	5	3	11	10	5	3	2	3
	3	0	5	10	5	10	5	4	0	4
	4	0	9	2	0	0				
	5	0	6	11	2	6	5	5	0	2
	6	0	9	10	4	8	4	1	0	0
	7	0	8	10	3	10	4	0	0	0
	1b	0	9	4	8	2				
	2b	0	8	3	3	3				
	3b	0	7	13	1	2				
	4b	0	5	4	4	14	5	1	0	4
	5b	0	6	7	4	5				
	6b	0	9	7	6	9				
	7b	0	7	7	10	2				

DOSE GROUP		0.2								
No. of cocoons		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	5	7	2	3	3	1	0	7
	2	0	6	7	6	5	4	2	2	3
	3	0	10	9	17	3	5	3	1	0
	4	0	9	14	16	13	8	6	1	4
	5	0	7	14	9	4	2	1	0	0
	6	0	11	6	9	12	5	3	3	1
	7	0	11	14	8	4	2	2	0	2
	1b	0	11	3	8	1				
	2b	0	8	7	1	1				
	3b	0	11	4	8	6				
	4b	0	6	2	1	3				
	5b	0	10	19	5	18				
	6b	0	8	13	16	15				
	7b	0	6	8	5	18				

DOSE GROUP		0.4								
No. of cocoons		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	7	2	3	5	3	3	1	2
	2	0	1	14	21	12	6	1	0	5
	3	0	8	6	13	6	1	0	2	2
	4	0	12	7	5	1	1	1	1	3
	5	0	5	3	13	4	6	2	3	2
	6	0	4	9	15	18	6	2	4	3
	7	0	4	7	15	30	11	2	0	1
	1b	0	4	2	0	1				
	2b	0	8	11	8	18				
	3b	0	3	11	12	13				
	4b	0	2	7	7	9				
	5b	0	1	17	18	5				
	6b	0	6	10	4	20				
	7b	0	6	17	11	10				

Table A2.4 | Number of cocoons produced by *Eisenia fetida* during the study *continued*

DOSE GROUP		1.4								
No. of cocoons		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	10	5	3	0	2	0	0	0
	2	0	7	1	1	1	0	1	1	0
	3	0	7	7	2	3	2	2	0	2
	4	0	8	7	13	2	3	5	2	1
	5	0	7	4	9	3	5	3	0	0
	6	0	3	5	7	8	5	1	0	4
	7	0	5	5	3	1	3	1	1	1
	1b	0	10	4	1	3				
	2b	0	10	7	1	19				
	3b	0	7	4	0	4				
	4b	0	11	13	7	2				
	5b	0	7	11	3	2				
	6b	0	7	7	3	4				
	7b	0	5	4	4	6				

DOSE GROUP		2.7								
No. of cocoons		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	6	2	9	0	3	1	0	7
	2	0	13	6	14	2	8	4	0	3
	3	0	4	9	8	5	0	0	0	2
	4	0	5	20	11	7	8	1	0	5
	5	0	11	14	16	15	6	2	0	7
	6	0	10	20	7	4	4	2	4	4
	7	0	8	8	8	15	7	0	0	6
	1b	0	6	4	1	0				
	2b	0	4	1	3	1				
	3b	0	10	18	10	6				
	4b	0	10	15	7	11				
	5b	0	5	7	6	5				
	6b	0	5	4	4	12				
	7b	0	9	4	4	4				

DOSE GROUP		8.5								
No. of cocoons		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	8	10	13	3	5	3	1	3
	2	0	13	9	5	3	3	0	1	0
	3	0	10	4	9	4	4	0	3	2
	4	0	6	7	11	2	3	5	7	4
	5	0	10	9	4	3	3	8	7	4
	6	0	10	5	10	2	7	4	7	5
	7	0	6	8	12	13	11	2	6	4
	1b	0	2	7	4	12				
	2b	0	8	4	13	26				
	3b	0	4	13	4	7				
	4b	0	4	5	5	16				
	5b	0	11	14	3	5				
	6b	0	8	12	14	4				
	7b	0	5	6	3	3				

Table A2.5 | Number of offspring produced by *Eisenia fetida* during the study

DOSE GROUP		BACKGROUND								
No. of offspring		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	8	17	23	40	51	54
	2	0	0	1	5	19	19	51	44	67
	3	0	0	0	6	17	21	36	41	50
	4	0	0	0	0	4				
	5	0	0	0	8	25	24	47	58	70
	6	0	0	0	5	14	30	37	38	43
	7	0	0	0	3	5	30	39	42	64
	1b	0	0	1	8	15				
	2b	0	0	2	10	16				
	3b	0	0	0	6	9				
	4b	0	0	1	14	34	30	40	37	47
	5b	0	0	3	6	22				
	6b	0	0	0	4	22				
	7b	0	0	0	7	14				

DOSE GROUP		0.2								
No. of offspring		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	8	21	39	53	65	86
	2	0	0	1	4	27	47	72	82	106
	3	0	0	0	5	15	78	78	85	89
	4	0	0	4	16	50	62	64	68	93
	5	0	0	1	8	60	25	50	34	85
	6	0	0	13	15	54	59	74	92	94
	7	0	0	2	0	25	29	37	43	73
	1b	0	0	1	2	12				
	2b	0	0	0	1	12				
	3b	0	0	3	5	51				
	4b	0	0	3	5	16				
	5b	0	0	0	2	28				
	6b	0	0	0	9	31				
	7b	0	0	0	2	36				

DOSE GROUP		0.4								
No. of offspring		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	2	5	23	33	51	40	60
	2	0	0	2	9	28	64	83	90	151
	3	0	0	1	11	32	38	61	45	64
	4	0	0	2	2	13	27	42	44	61
	5	0	0	0	13	26	38	43	61	100
	6	0	0	0	10	32	43	46	74	66
	7	0	0	0	12	41	59	83	84	101
	1b	0	0	0	7	15				
	2b	0	0	0	14	30				
	3b	0	0	0	5	42				
	4b	0	0	0	1	22				
	5b	0	0	0	10	36				
	6b	0	0	1	14	53				
	7b	0	0	0	8	30				

Table A2.5 | Number of offspring produced by *Eisenia fetida* during the study

continued

DOSE GROUP		1.4								
No. of offspring		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	2	4	12	31	39	46
	2	0	1	2	2	8	27	21	46	56
	3	0	1	1	8	33	36	67	63	92
	4	0	0	4	10	16	25	40	67	81
	5	0	0	1	6	27	41	49	63	62
	6	0	0	0	7	19	27	36	53	61
	7	0	0	0	12	41	51	49	64	69
	1b	0	0	2	7	24				
	2b	0	0	0	2	15				
	3b	0	0	2	5	25				
	4b	0	0	2	17	45				
	5b	0	0	0	7	11				
	6b	0	0	1	4	32				
	7b	0	0	3	3	20				

DOSE GROUP		2.7								
No. of offspring		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	5	5	14	34	58	72
	2	0	0	3	6	14	25	36	42	76
	3	0	0	0	0	12	13	28	37	44
	4	0	0	2	25	62	77	88	88	133
	5	0	0	1	18	54	64	70	62	117
	6	0	0	1	25	51	68	81	84	82
	7	0	0	3	15	40	71	77	100	119
	1b	0	0	0	1	11				
	2b	0	0	0	3	0				
	3b	0	0	6	10	49				
	4b	0	0	2	7	25				
	5b	0	0	0	6	23				
	6b	0	0	0	4	26				
	7b	0	0	1	4	27				

DOSE GROUP		8.5								
No. of offspring		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
TANK	1	0	0	0	11	22	36	51	56	75
	2	0	0	1	8	11	24	42	35	44
	3	0	0	2	9	21	43	46	48	52
	4	0	0	2	14	20	45	53	58	70
	5	0	0	0	9	28	49	52	75	82
	6	0	0	1	1	19	28	37	37	42
	7	0	0	1	11	26	47	58	63	77
	1b	0	0	0	6	8				
	2b	0	0	0	20	34				
	3b	0	0	0	2	11				
	4b	0	0	2	9	29				
	5b	0	0	1	12	10				
	6b	0	0	0	3	5				
	7b	0	0	0	2	6				

Appendix A3:

Table A3.1 | Pro-forma for woodlice experiment (following guidance key in Section 3.1 of SP2)

Main experiment (delete as appropriate)					
Key instruction			Page	Section	Table
a	**Umbrella endpoint of interest** (e.g. reproduction)	Reproduction	53 57	5.1.2 5.2.1	5.1+5.2
b	**Wildlife group and species**	Soil fauna *Porcellio scaber* (woodlouse)	44	4.17	4.13a
c	**Maintenance conditions** (e.g. temperature, light regime, diet)	Room temperature, continual light bark compost bran	44	4.17	4.13 a,b,c
d	• **Specific endpoint(s) to study** (e.g. No. of eggs produced)	No. of juveniles	53	5.2	5.1+5.2
	• **Compulsory measurements to record**	Weight Length Growth rate No. of mortalities occurring			
e	**Irradiation type** (internal/external/mixed)	External	59	6.2	–
f	**Facilities required** (e.g. Cs-137 source)	Cs-137 source	68	6.7.1	–
g	**Dose rates to use** (e.g. Background, 10, 20, 40, 80, 160, 320, etc. Gy or µGy/hour). Where known also indicate, in the brackets, the total dose received at each dose rate.	Background = minimal Dose rate 1 = 0.2 mGy/hour (0.3 Gy) Dose rate 2 = 0.4 mGy/hour (1.0 Gy) Dose rate 3 = 1.5 mGy/hour (4.0 Gy) Dose rate 4 = 4.0 mGy/hour (10 Gy) Dose rate 5 = 8.0 mGy/hour (20 Gy)	66	6.5.6	6.9
h	**Need for a pilot experiment?**	No	67	6.6	–
	• No. of dose rates, including control				
	• No. of individuals per dose rates				
	• Dose/dose rates used				
	• Duration of irradiation				
	• Duration of experiment				
	• **Notes/other considerations**				
i	**Duration of irradiation** (e.g. list daily time period for irradiation – 20 hours)	Continual – irradiation only interrupted for assessing endpoints			
	Duration of experiment	108 days			
j	**No. of dose rates, including control**	6			

Table A3.1 | Pro-forma for woodlice experiment (following guidance key in Section 3.1 of SP2) *continued*

	No. of individuals per dose rate (e.g. 10)	15 individuals per tank; 12 tanks per dose rate			
k	**Statistical requirements**				
	• **Tier 1**	Graphs – check data normally distributed			
	• **Tier 2** (e.g. test used)	F_{max} test			
	• **Tier 3** (e.g. test used)	Analysis of variance (ANOVA)			
l	**Further literature search conducted?** If yes, then list	YES For further details of references consulted see reference section of P3-101/SP7			
Further justification of decisions made to complete the pro-forma (to be completed when decisions are not based on information in the guidance document that can be referred to by page/section/table)		The dose rates were calculated based on the dimensions of the irradiation facility in conjunction with consulting relevant literature.			
Notes (general)		Endpoints assessed every two weeks for 14 weeks week 8 – Interim kill of half the woodlice (six tanks in each dose group). Proportion of these woodlice used for histopathological analysis. week 14 – Final kill of half the woodlice (six tanks in each dose group). Proportion of these worms used for histopathological analysis.			

Table A3.2 | Checklist for data reporting – *porcellio scaber*

	Tick box
Authors	✓
Article title	✓
Reference details	✓
Keywords	
Type of study (laboratory, field, controlled field)	✓
Radiation type (alpha, beta, gamma or mixed)	✓
Exposure type (internal, external, mixed)	✓
Ecosystem	
Wildlife group	✓
Species name (Latin and common)	✓
Source of organisms (supplier)	✓
Life-stage of organisms	✓
Maintenance of organisms prior to and during the experiments	✓
Umbrella endpoint(s)	✓
Specific endpoint being studied(s)	✓
Frequency and timing of specific endpoint measurements	✓
Dose rate(s)	✓
Notes on how the dose or dose rate was calculated	✓
Activity concentrations for internal exposures	
Dose(s)	✓
No of individuals per treatment group (including control)	✓
Duration of exposure(s)	✓
Result(s)	✓
Statistical analysis	
Any relevant notes	
Production of a data sheet to record results	✓

Appendix A4:

Raw data for Porcellio scaber

Table A4.1 | Total weight measurements taken from *Porcellio scaber* during the study

DOSE GROUP		BACKGROUND							
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0.05	0.04	0.05	0.06	0.06	0.07	0.07	0.07
	2	0.03	0.04	0.05	0.05	0.05	0.05	0.06	0.06
	3	0.06	0.04	0.04	0.05	0.06	0.06	0.06	0.06
	4	0.01	0.03	0.03	0.03	0.04	0.04	0.05	0.05
	5	0.05	0.03	0.04	0.04	0.04	0.05	0.05	0.05
	6	0.03	0.03	0.03	0.04	0.04	0.04	0.05	0.05
	1b	0.04	0.04	0.05	0.06				
	2b	0.04	0.04	0.05	0.06				
	3b	0.04	0.04	0.05	0.05				
	4b	0.11	0.05	0.05	0.06				
	5b	0.03	0.03	0.04	0.05				
	6b	0.01	0.03	0.03	0.04				

DOSE GROUP		0.2							
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0.22	0.04	0.05	0.05	0.06	0.06	0.07	0.07
	2	0.12	0.04	0.04	0.05	0.05	0.06	0.06	0.07
	3	0.03	0.04	0.05	0.05	0.05	0.06	0.06	0.05
	4	0.04	0.04	0.05	0.05	0.05	0.05	0.06	0.06
	5	0.04	0.05	0.05	0.06	0.06	0.06	0.06	0.07
	6	0.07	0.06	0.06	0.06	0.06	0.06	0.07	0.07
	1b	0.03	0.04	0.04	0.05				
	2b	0.04	0.03	0.04	0.05				
	3b	0.03	0.03	0.04	0.05				
	4b	0.06	0.05	0.05	0.06				
	5b	0.02	0.04	0.05	0.05				
	6b	0.05	0.03	0.04	0.05				

Table A4.1 | Total weight measurements taken from *Porcellio scaber* during the study *continued*

DOSE GROUP		0.4							
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0.05	0.04	0.05	0.06	0.05	0.06	0.07	0.06
	2	0.03	0.03	0.04	0.05	0.05	0.06	0.06	0.07
	3	0.04	0.03	0.04	0.04	0.05	0.05	0.05	0.05
	4	0.01	0.04	0.04	0.05	0.05	0.05	0.06	0.06
	5	0.06	0.04	0.04	0.05	0.05	0.06	0.06	0.06
	6	0.02	0.03	0.04	0.04	0.04	0.04	0.05	0.05
	1b	0.08	0.04	0.04	0.05				
	2b	0.03	0.04	0.04	0.05				
	3b	0.02	0.02	0.03	0.04				
	4b	0.01	0.03	0.04	0.05				
	5b	0.03	0.04	0.05	0.05				
	6b	0.04	0.04	0.05	0.05				

DOSE GROUP		1.5							
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0.04	0.04		0.05	0.05	0.05	0.06	0.05
	2	0.04	0.05	0.05	0.06	0.05	0.06	0.06	0.07
	3	0.03	0.04	0.04	0.04	0.05	0.05	0.05	0.06
	4	0.01	0.03	0.04	0.04	0.05	0.05	0.06	0.06
	5	0.03	0.04	0.04	0.04	0.05	0.05	0.05	0.06
	6	0.06	0.05	0.05	0.06	0.07	0.07	0.08	0.07
	1b	0.04	0.04	0.07	0.05				
	2b	0.04	0.04	0.04	0.06				
	3b	0.04	0.05	0.05	0.05				
	4b	0.05		0.06	0.06				
	5b	0.05	0.04	0.04	0.04				
	6b	0.00	0.03	0.04	0.05				

Table A4.1 | Total weight measurements taken from *Porcellio scaber* during the study *continued*

DOSE GROUP		1.5							
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0.04	0.04		0.05	0.05	0.05	0.06	0.05
	2	0.04	0.05	0.05	0.06	0.05	0.06	0.06	0.07
	3	0.03	0.04	0.04	0.04	0.05	0.05	0.05	0.06
	4	0.01	0.03	0.04	0.04	0.05	0.05	0.06	0.06
	5	0.03	0.04	0.04	0.04	0.05	0.05	0.05	0.06
	6	0.06	0.05	0.05	0.06	0.07	0.07	0.08	0.07
	1b	0.04	0.04	0.07	0.05				
	2b	0.04	0.04	0.04	0.06				
	3b	0.04	0.05	0.05	0.05				
	4b	0.05		0.06	0.06				
	5b	0.05	0.04	0.04	0.04				
	6b	0.00	0.03	0.04	0.05				

DOSE GROUP		2.8							
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0.03	0.03	0.04	0.04	0.05	0.05	0.06	0.07
	2	0.03	0.03	0.04	0.04	0.04	0.05	0.05	0.06
	3	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	4	0.03	0.05	0.05	0.05	0.06	0.06	0.06	0.06
	5	0.03	0.04	0.05	0.05	0.05	0.05	0.06	0.06
	6	0.06	0.05	0.05	0.07	0.06	0.07	0.07	0.07
	1b	0.06	0.04	0.04	0.05				
	2b	0.02	0.04	0.05	0.05				
	5b	0.03	0.03	0.04	0.04				
	6b	0.03	0.03	0.04	0.04				

DOSE GROUP		8.9							
AVERAGE WEIGHT		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0.04	0.03	0.04	0.04	0.05	0.05	0.06	0.06
	2	0.03	0.03	0.04	0.04	0.04	0.04	0.05	0.05
	3	0.03	0.04	0.11	0.04	0.05	0.05	0.05	0.05
	4	0.04	0.03	0.04	0.04	0.05	0.05	0.05	0.06
	5	0.03	0.03	0.04	0.05	0.05	0.05	0.05	0.05
	6	0.03	0.04	0.05	0.05	0.06	0.06	0.05	N/A
	1b	0.03	0.03	0.04	0.05				
	2b	0.02	0.03	0.03	0.04				
	3b	0.04	0.04	0.05	0.05				
	4b	0.05	0.05	0.05	0.06				
	5b	0.01	0.04	0.04	0.05				
	6b	0.05	0.03	0.05	0.05				

Table A4.2 | Histopathological findings – *Porcellio scaber*

Wood lice Pathology				No abnormalities discovered (NAD)
Interim	Dose Gp.	Animal No.	Sex	
LIV 2	B	1	f	NAD
LIV 2	B	2	f	NAD
LIV 2	B	3	m	NAD
LIV 2	B	4	f	Late vitellogenic oocytes
LIV 2	B	5	f	NAD
LIV 2	B	6	m	NAD
LIV 2	B	7	f	Early vitellogenic oocytes
LIV 2	B	8	m	NAD
LIV 2	1.5	1	f	NAD
LIV 2	1.5	2	f	NAD
LIV 2	1.5	3	f	NAD
LIV 2	1.5	4	m	NAD
LIV 2	1.5	5	f	NAD
LIV 2	1.5	6	f	Very early vitellogenic oocytes
LIV 2	1.5	7	f	NAD
LIV 2	1.5	8	f	NAD
LIV 2	8.9	1	*	Tissues lost during processing as the cassette was found open.
LIV 2	8.9	2	f	NAD
LIV 2	8.9	3	m	NAD
LIV 2	8.9	4	m	NAD
LIV 2	8.9	5	f	NAD
LIV 2	8.9	6	f	NAD
LIV 2	8.9	7	f	NAD
LIV 2	8.9	8	f	Late vitellogenic oocytes
Terminal				
LIV 2	B	9	f	NAD
LIV 2	B	10	m	NAD
LIV 2	B	11	f	Early vitellogenic oocytes
LIV 2	B	12	f	Late vitellogenic oocytes
LIV 2	B	13	f	Early vitellogenic oocytes
LIV 2	B	14	f	Late vitellogenic oocytes
LIV 2	B	15	m	NAD
LIV 2	B	16	m	NAD
LIV 2	0.2	9	m	NAD
LIV 2	0.2	10	m	NAD
LIV 2	0.2	11	f	Moderate increase in adipocytes
LIV 2	0.2	12	m	NAD
LIV 2	0.2	13	m	NAD
LIV 2	0.2	14	f	NAD
LIV 2	0.2	15	m	NAD
LIV 2	0.2	16	m	NAD
LIV 2	0.4	9	m	NAD
LIV 2	0.4	10	f	NAD
LIV 2	0.4	11	f	NAD
LIV 2	0.4	12	f	Anterior hind gut focus of epithelia stratificiation
LIV 2	0.4	13	m	NAD
LIV 2	0.4	14	f	NAD
LIV 2	0.4	15	f	Early vitellogenic oocytes
LIV 2	0.4	16	f	NAD
LIV 2	1.5	9	f	Early vitellogenic oocytes
LIV 2	1.5	10	m	NAD
LIV 2	1.5	11	m	NAD
LIV 2	1.5	12	m	Anterior hind gut focus of epithelia stratificiation
LIV 2	1.5	13	m	NAD

Table A4.2 | Histopathological findings – *Porcellio scaber*

continued

LIV 2	1.5	14	f	Very early vitellogenic oocytes
LIV 2	1.5	15	f	Moderate increase in adipocytes
LIV 2	1.5	16	f	Early vitellogenic oocytes
LIV 2	2.8	9	f	NAD
LIV 2	2.8	10	m	NAD
LIV 2	2.8	11	m	NAD
LIV 2	2.8	12	f	Moderate increase in adipocytes
LIV 2	2.8	13	f	Very early vitellogenic oocytes
LIV 2	2.8	14	f	Early vitellogenic oocytes
LIV 2	2.8	15	f	Very early vitellogenic oocytes
LIV 2	2.8	16	m	Moderate increase in adipocytes
LIV 2	8.9	9	U	Gonads not in section
LIV 2	8.9	10	U	Gonads absent.The individual appears to be malnourished
LIV 2	8.9	11	m	NAD
LIV 2	8.9	12	m	NAD
LIV 2	8.9	13	f	NAD
LIV 2	8.9	14	m	NAD
LIV 2	8.9	15	f	NAD
LIV 2	8.9	16	f	NAD

Table A4.3 | Number of total mortalities amongst *Porcellio scaber* during the study

DOSE GROUP		BACKGROUND							
NO. OF MORTALITIES		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	1	0	0	0	0	1	0
	2	0	1	2	0	1	0	0	0
	3	0	0	0	1	1	0	0	1
	4	0	0	0	0	1	0	0	0
	5	0	4	0	1	0	0	0	0
	6	0	0	2	0	0	0	0	0
	1b	0	1	1	0				
	2b	0	0	0	0				
	3b	0	0	0	0				
	4b	0	1	0	0				
	5b	0	0	0	0				
	6b	0	3	0	0				

DOSE GROUP		0.2							
NO. OF MORTALITIES		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	0	0	0	2	0	1	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	1
	4	0	0	1	1	0	0	1	2
	5	0	0	0	1	0	0	1	0
	6	0	0	0	0	2	1	0	0
	1b	0	0	0	0				
	2b	0	1	0	0				
	3b	0	0	0	0				
	4b	0	2	0	0				
	5b	0	0	1	1				
	6b	0	0	1	0				

DOSE GROUP		0.4							
NO. OF MORTALITIES		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	0	0	0	0	1	1	0
	2	0	1	0	0	0	2	2	1
	3	0	0	0	0	0	0	0	0
	4	0	2	3	0	0	1	0	0
	5	0	0	2	0	0	0	0	0
	6	0	0	2	0	0	1	1	0
	1b	0	0	0	1				
	2b	0	0	0	0				
	3b	0	1	0	0				
	4b	0	0	3	1				
	5b	0	0	0	1				
	6b	0	0	0	0				

Table A4.3 | Number of total mortalities amongst *Porcellio scaber* during the study *continued*

DOSE GROUP		1.5							
NO. OF MORTALITIES		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	1	0	0	0	0	0	0
	2	0	0	1	0	2	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	1
	5	0	0	1	0	0	0	0	1
	6	0	1	0	0	0	0	0	1
	1b	0	0	0	0				
	2b	0	0	0	0				
	3b	0	0	0	0				
	4b	0	0	0	1				
	5b	0	0	0	1				
	6b	0	1	0	0				

DOSE GROUP		2.8							
NO. OF MORTALITIES		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	1	0	0	0	2	0	1
	2	0	1	1	0	0	0	1	0
	3	0	0	2	1	0	0	1	0
	4	0	1	0	1	0	2	0	0
	5	0	0	0	0	0	1	0	2
	6	0	0	0	0	1	0	0	2
	1b	0	2	0	0				
	2b	0	0	0	1				
	3b	0	0	1	1				
	4b	0	2	0	0				
	5b	0	1	0	0				
	6b	0	0	0	0				

DOSE GROUP		8.9							
NO. OF MORTALITIES		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	2	0	1	0	0	0	0
	2	0	0	0	0	1	0	2	2
	3	0	0	2	1	0	0	0	0
	4	0	2	1	0	0	0	1	1
	5	0	0	0	0	1	0	2	0
	6	0	4	0	0	1	1	0	3
	1b	0	0	0	0				
	2b	0	0	0	0				
	3b	0	1	0	0				
	4b	0	0	0	0				
	5b	0	0	0	0				
	6b	0	0	1	1				

Table A4.4 | Number of offspring produced by *Porcellio scaber* during the study

DOSE GROUP		BACKGROUND							
NO. OF OFFSPRING		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	15	43	17	20	25	33	64
	2	0	30	17	11	27	23	26	37
	3	0	2	0	0	0	6	0	23
	4	0	28	20	16	14	11	10	21
	5	0	0	0	1	1	2	0	0
	6	0	38	14	8	12	14	7	9
	1b	0	9	15	26				
	2b	0	44	35	29				
	3b	0	47	77	43				
	4b	0	11	34	30				
	5b	0	40	44	27				
	6b	0	13	26	28				

DOSE GROUP		0.2 (written as 0.1 on tanks)							
NO. OF OFFSPRING		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	55	74	29	26	13	18	19
	2	0	18	22	18	11	13	15	17
	3	0	16	31	31	18	14	20	32
	4	0	29	33	28	36	27	20	39
	5	0	59	75	47	19	22	33	31
	6	0	39	53	32	27	28	28	29
	1b	0	42	64	49				
	2b	0	4	2	7				
	3b	0	4	5	2				
	4b	0	37	13	6				
	5b	0	38	32	69				
	6b	0	30	36	26				

DOSE GROUP		0.4							
NO. OF OFFSPRING		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	0	28	16	12	21	17	25
	2	0	52	21	24	31	9	12	57
	3	0	5	5	10	21	8	18	77
	4	0	51	34	23	33	23	23	30
	5	0	35	55	39	46	20	30	40
	6	0	19	4	5	9	10	7	28
	1b	0	28	13	8				
	2b	0	13	5	2				
	3b	0	0	0	0				
	4b	0	29	28	26				
	5b	0	39	36	27				
	6b	0	65	114	67				

Table A4.4 | Number of offspring produced by *Porcellio scaber* during the study *continued*

DOSE GROUP		1.5							
NO. OF OFFSPRING		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	31	22	17	20	12	19	17
	2	0	41	32	24	31	15	22	52
	3	0	69	70	40	43	18	24	29
	4	0	27	30	15	33	9	8	19
	5	0	33	57	19	46	38	26	54
	6	0	0	0	0	9	0	0	0
	1b	0	70	80	66				
	2b	0	78	59	5				
	3b	0	95	91	78				
	4b	0	49	44	39				
	5b	0	23	33	19				
	6b	0	12	9	2				

DOSE GROUP		2.8							
NO. OF OFFSPRING		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	13	11	10	3	12	3	7
	2	0	15	18	11	11	15	7	14
	3	0	63	110	73	51	42	51	53
	4	0	59	51	43	15	16	21	18
	5	0	15	40	30	38	18	15	15
	6	0	0	0	0	16	6	4	5
	1b	0	11	18	7				
	2b	0	0	0	0				
	3b	0	88	61	59				
	4b	0	23	10	17				
	5b	0	15	10	6				
	6b	0	19	19	21				

DOSE GROUP		8.9							
NO. OF OFFSPRING		WK 0	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14
TANK	1	0	38	64	40	23	19	24	27
	2	0	9	12	11	10	10	3	6
	3	0	48	49	43	25	24	45	27
	4	0	14	19	12	17	9	9	4
	5	0	6	10	6	4	1	1	3
	6	0	27	31	10	13	9	18	14
	1b	0	15	14	14				
	2b	0	5	26	18				
	3b	0	30	66	79				
	4b	0	23	27	30				
	5b	0	29	37	31				
	6b	0	27	22	20				

Glossary of terms

Aberrant cell
Cell deviating from the normal cell type.

Absorbed dose
Quantity of energy imparted by ionising radiation to unit mass of matter such as tissue. Unit: Gray, symbol Gy. 1 Gy = 1 joule per kilogram.

Acute exposure
Exposure received within a short period of time. Normally used to refer to exposure of sufficiently short duration that the resulting dose can be treated as instantaneous (e.g. less than an hour).

Alimentary canal
Tubular passage of mucous membrane and muscle extending from the mouth to the anus; functions in digestion and elimination.

Biomarker
A biological response to an environmental pollutant, which gives a measure of exposure. The response may be molecular, cellular or whole organism.

Chronic exposure
Exposure persisting in time. Normally used to refer to continuous exposures to low concentrations of pollutants.

Comet assay
A technique that detects DNA strand breaks in cells through the use of a series of buffers, electrophoresis and fluorescent dyes. Also known as single cell gel electrophoresis.

Cuticle
Protective layer covering the epidermis of an invertebrate.

Dose
General term for quantity of ionising radiation.

Dose rate
Dose released over a specified unit of time.

Endpoint
The characteristic of the biological unit under investigation that is being assessed in relation to different dose rate regimes.

Experimental (or specific) endpoints
Quantifiable characteristics of an organism or its progeny that can be used to investigate the effects of a contaminant on a particular umbrella endpoint.

Ganglia
A number of nerve cells that form a swelling on a nerve fibre.

Gonad
An organ that produces gametes; a testes or an ovary.

Habitat
The place in which a plant or animal lives.

Hyperplasia
The enlargement of an organ or tissue caused by an increase in the reproduction rate of its cells.

Ionising radiation
Radiation that produces ionisation in matter. Examples include alpha particles, gamma rays, X-rays and neutrons.

Morbidity
The state of being diseased.

Mortality
The number of deaths in a given period.

Multinucleated
Containing many nuclei.

Musculo-skeletal system
Linkage of muscles and skeleton.

Mutation
A change in the genetic material of an organism. This can be spontaneous or induced by chemicals or radiation.

Necrosis
The death of most or all of the cells in a tissue due to disease, injury or lack of blood supply.

Nephridia
Small tube involved in excretion and osmoregulation.

Oocytes
Cells that become ova.

Pleopods
Male sex hormone glands.

Radionuclide
An unstable nuclide that emits ionising radiation.

Reproduction
The formation of new individuals by sexual or non-sexual means.

Seminal vesicle
The organ that stores sperm in the male.

Spermatozoa
Male gamete.

Umbrella endpoints
A descriptive term that is used to group biological effects of particular types, e.g. morbidity, mortality, mutation and reproduction.

Vasa deferentia
Ducts that convey sperm.

List of abbreviations

ANOVA	Analysis of variance
CF	Concentration factor
EQS	Environmental Quality Standard
FASSET	EC Fifth Framework Programme (FP5) project (Framework for ASSessment of Environmental impacT) Contract FIGE-CT-2000-00102
FRED	FASSET Radiation Effects Database
GPG	Good Practice Guide
LOD	Limit of detection
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic aromatic hydrocarbon
R&D	Research and development
SD	Standard deviation
TLD	Thermoluminescent dosimeter
US EPA	US Environmental Protection Agency

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