Environmental Assessment

Safe-Guard Aquasol for Swine

(20% Fenbendazole Suspension)

Date	**January 15, 2015**
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Glossary of Abbreviations and Definition of Terms

AF	assessment factor
API	active pharmaceutical ingredient
BW	body weight
C	carbon
CAKE	Computer Assisted Kinetic Evaluation
$CaCl_2$	calcium chloride
d	day(s)
DT_{50}	disappearance times half-lives; time to degradation of 50% of original concentration of the test substance
DT_{90}	time to degradation of 90% of original concentration of the test substance
EA	environmental assessment
EC_{50}	concentration of the test substance which results in 50% of the test organisms being adversely affected
EPA	U.S. Environmental Protection Agency
EPI	Estimation Program Interface
EU	European Union
FAO	Food and Agriculture Organization
FBZ	fenbendazole
FBZ-SO_2	fenbendazole sulfone
FDA	U.S. Food and Drug Administration
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
GL	guideline
GLP	Good Laboratory Practice
h	hour(s)
HPLC	high performance liquid chromatography
K_d	distribution coefficient
K_f	Freundlich adsorption coefficient
kg	kilogram
K_{OC}	adsorption/desorption partition co-efficient normalized to the organic carbon content of soil
K_{OW}	octanol/water partition coefficient
L	liter(s)
LC_{50}	concentration of the test substance which results in death in 50% of the test organisms
lb	pound
LOEC	lowest observed effect concentration
LSC	liquid scintillation counting
m	meter
m^2	square meter
mg	milligram
NADA	New Animal Drug Application
NOEC	no-observed effect concentration
OC	organic carbon

OECD	Organization for Economic Cooperation and Development
OM	organic matter
OXF	oxfendazole
PC	physical-chemical
PEC_{soil}	predicted environmental concentration in soil
$PEC_{groundwater}$	predicted environmental concentration in groundwater
$PEC_{sediment}$	predicted environmental concentration in sediment
$PEC_{surfacewater}$	predicted environmental concentration in surface water
PNEC	predicted no effect concentration
SFO	simple first order
SPE	solid phase extraction
TAD	Technical Assistance Document
TLC	thin layer chromatography
TWM	time-weighted mean
UV	ultraviolet light
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
VMP	veterinary medicinal product

1. Purpose and Need

A new animal drug approval has been requested for the use of Safe-Guard Aquasol for Swine (20% Fenbendazole Suspension) (Safe-Guard Aquasol). This product contains fenbendazole (FBZ) in a modified form as the single active pharmaceutical ingredient (API). FBZ belongs to the group of benzimidazoles anthelmintics[1]. Safe-Guard Aquasol for Swine is being developed for the removal and control of parasites listed below:

- Lungworms *(Metastrongylus apri, Metastrongylus pudendotectus)*,
- Gastrointestinal worms, adult and larvae (L3, L4 stages, liver, lung, intestinal forms)
    - large roundworms *(Ascaris suum)*
    - nodular worms *(Oesophagostomum dentatum, O. quadrispinulatum)*
    - small stomach worms *(Hyostrongylus rubidus)*
    - whipworms, adult and larvae (L2, L3, L4 stages - intestinal mucosal forms), *(Trichuris suis)*.
- Kidney worms: Adult and larvae *(Stephanurus dentatus)*

Safe-Guard Aquasol for Swine is intended to be administered orally with drinking water on 3 consecutive days (d) at a dose of 2.2 mg/kg bodyweight (BW)/d.

The purpose of the environmental assessment (EA) is to evaluate whether the approval of the product will cause significant environmental impacts. The EA is prepared in support of the New Animal Drug Application (NADA) for the use of the drug product.

The environmental impact assessment approach in this EA follows the process described in CVM Guidance for Industry #166 [Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) – Phase II (CVM, 2006; VICH, 2004)][2] combined with EMEA Guidance (EMEA, 2008).

A preliminary assessment was made following the Phase I decision tree as outlined in CVM Guidance for Industry #89 [(Environmental Impact Assessment (EIA's) for Veterinary Medicinal Products (VMP's) – Phase I Guidance (CVM, 2001; VICH, 2000)]. Utilizing the Phase I decision tree the following points have been raised. Safe-Guard Aquasol for Swine:

[1] http://www.whocc.no/atcvet/atcvet_index/?code=QP52AC13

[2] Referred to throughout this document as VICH GL or VICH GL 38

- is not exempt from the need for an environmental impact assessment by legislation and/or regulation;
- is not a natural substance;
- will not be used only in non-food animals;
- is not intended for use in a minor species;
- will not be used to treat a small number of animals in a flock or herd;
- is not extensively metabolized in the treated animal;
- will be used to treat terrestrial organisms not reared on pasture.

Accordingly, the initial predicted environmental concentration in soil (PEC_{soil}) is calculated. As the initial PEC_{soil} is not exceeding the trigger value of 100 µg/kg, the environmental effects per definition are considered limited. Accordingly, the environmental impact assessment principally stops with VICH Phase I without the need to present experimental data on physical-chemical (PC) properties, or fate and effects in the environment.

Safe-Guard Aquasol for Swine is another formulation of the approved to Safe-Guard 20% Type A Medicated Article (Safe-Guard 20%) (NADA 131-675), containing modified FBZ. Accordingly, Safe-Guard Aquasol for Swine is principally eligible for categorical exclusion under 21 CFR 25.33 (a)(3). However, extraordinary circumstances were identified as described under 21 CFR 25.21(a) as available data establish that at the expected level of exposure, there is a potential for serious harm to the environment as follows:

- Based on initial calculations using a predicted environmental concentration in surface water ($PEC_{surfacewater}$) of 0.37 µg/L (determined using assumptions and equations traditionally accepted by CVM) and a predicted no effects concentrations (PNEC) value of 0.012 µg/L (based on unmodified FBZ) determined from acute *Daphnia magna* data reported in the EA for Safe-Guard 20%, the PEC/PNEC ratios for FBZ are well above the trigger value of 1 recommended in VICH GL 38 (PEC/PNEC ratio of 30), indicating a potential for environmental impacts.
- The modification of FBZ for Safe-Guard Aquasol involves reducing the particle size of the FBZ. This process does not make FBZ more water soluble, but rather decreases the median drug particle size and increases the surface area to ensure that the drug remains in suspension to allow administration in drinking water. As discussed in Chapter 4.1.1 of this EA, modifying FBZ results in an increased bioavailability within swine as compared to the unmodified FBZ and, therefore, will be administered at a lower dose (2.2 mg/kg BW for 3 d) than the unmodified FBZ product (3 mg/kg BW for 3 d). While the administered dose will be reduced, there is still the potential for an increase in bioavailability and toxicity of the drug in non-target organisms because of the reduced particle size.

Accordingly, neither the VICH Phase I stop criteria apply, nor the categorical exclusion under 21 CFR 25.33 (a)(3). Therefore, a VICH Phase II assessment is conducted to demonstrate that the approval of the product will not cause significant environmental impacts.

The EA does not consider the API FBZ only but also the transformation products in soil, namely oxfendazole (OXF) and fenbendazole sulfone (FBZ-SO_2). With a potential to accumulate in soil and an increased run-off and leaching potential, OXF is considered to be the main compound to

be considered in the environmental impact assessment. Also, OXF itself is an API. Accordingly, separate exposure assessments are conducted for FBZ and OXF. This procedure does not follow the total residue approach initially proposed by VICH for consideration of metabolites/degradates. However, due to significant differences in essential PC and fate properties (e.g. water solubility, soil adsorption and degradation) the more complex approach is selected. For FBZ-SO_2 however, which is generated by degradation of OXF, the total residue principle is followed as the essential PC and fate properties are similar to OXF. Effect data are presented for FBZ and OXF, while for FBZ-SO_2 it is assumed that it is equally toxic as OXF. Finally, the risk characterization is conducted in a first step for FBZ and OXF/FBZ-SO_2 independently. In a second step, individual PEC/PNEC ratios are summed up and compared to the trigger value of 1. In case the combined PEC/PNEC ratio is <1, risk is per definition absent for the parallel exposure of non-target organisms to both compounds. A schematic overview on how the exposure and effect assessment and finally the risk characterization are conducted is presented in Figure 1-1.

All calculations were performed by using Microsoft Excel 2010 in full precision mode. The values presented in the EA were rounded. Manual calculations using the rounded values may produce slightly different results.

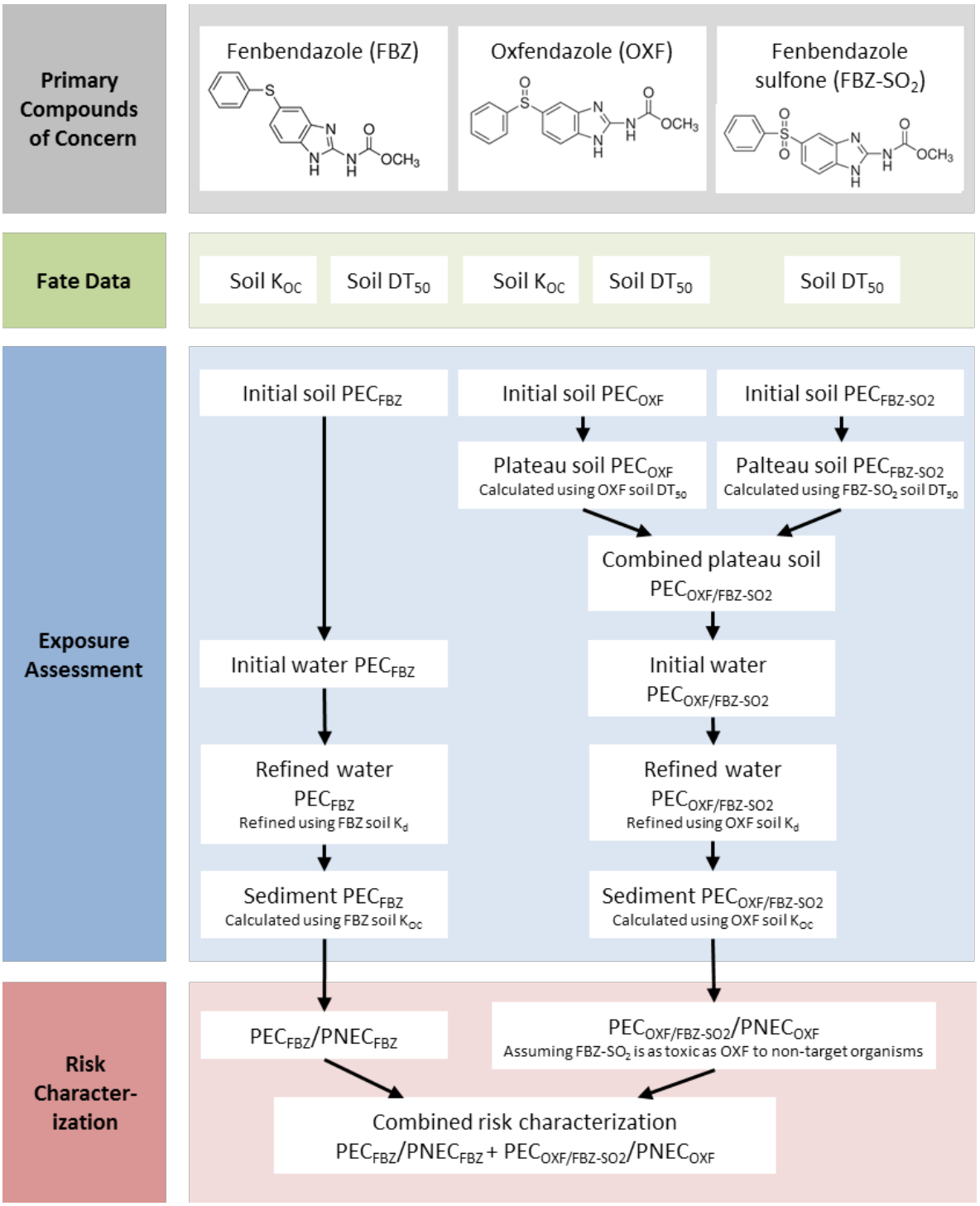

Figure 1-1: Schematic overview on how the exposure and effect assessment and finally the risk characterization are conducted.

2. Identification of Substances that are Subject of the proposed Action

Information which allow for the identification of substances that are subject of the proposed action are summarized in Table 2-1 and Figure 2-1.

Table 2-1: Identification of substances that are subject of the proposed action

Established name	Fenbendazole	Oxfendazole	Fenbendazole sulfone
Chemical name	-	-	-
IUPAC name	Methyl N-(6-phenylsulfanyl-1H-benzimidazol-2-yl)carbamate	Methyl N-[6-(benzenesulfinyl)-1H-benzimidazol-2-yl]carbamate	Methyl N-[6-(benzenesulfonyl)-1H-benzimidazol-2-yl]carbamate
Intervet name (alternative)	FBZ	OXF Fenbendazole sulfoxide	FBZ-SO_2 Oxfendazole sulfone MPBC Methyl-5-phenylsulfonyl-2-benzimidazole carbamate
CAS number	43210-67-9	53716-50-0	54029-20-8
Molecular formula	$C_{15}H_{13}N_3O_2S$	$C_{15}H_{13}N_3O_3S$	$C_{15}H_{13}N_3O_4S$
Molecular weight	299.35 g/mol	315.35 g/mol	331.35 g/mol
Structural formula	See Figure 2-1	See Figure 2-1	See Figure 2-1

The API of Safe-Guard Aquasol for Swine is FBZ. Compared to FBZ used in the already licensed Safe-Guard 20%, the FBZ in Safe-Guard Aquasol for Swine has a modified particle size. Accordingly, the terms unmodified and modified FBZ are used to differentiate between the two physical forms of FBZ.

OXF and FBZ-SO_2 are the primary and secondary metabolites/degradates of FBZ, respectively. OXF itself is an API of the benzimidazole anthelmintics (ATCvet Index[3]). Accordingly, both compounds are considered in the environmental impact assessment next to FBZ.

The formulation of Safe-Guard Aquasol for Swine consists of 200 mg FBZ, 100 mg Polysorbate 80 (Tween 80), 5 mg simethicone emulsion (30%), 20 mg benzyl alcohol and water (added to 1 mL). Excipients in the formulation will not affect the toxicity or environmental persistence of FBZ and its metabolites/degradates. Also they are not considered to have the potential to cause an environmental risk of their own. Accordingly, they are not considered in the environmental impact assessment.

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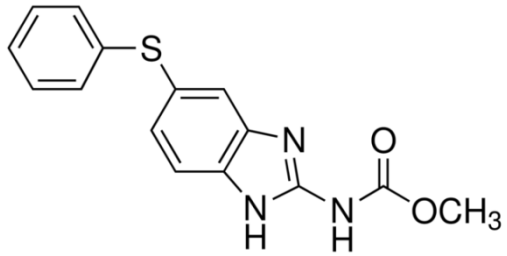
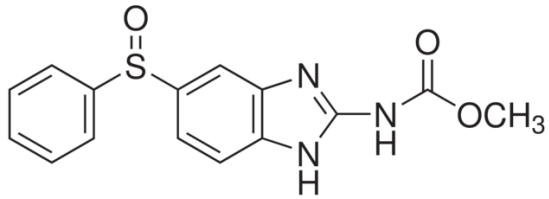
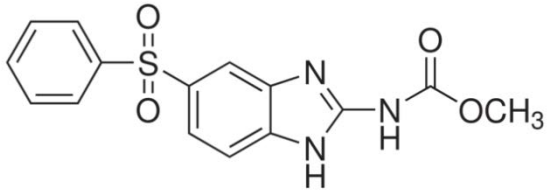
[3] http://www.whocc.no/atcvet/atcvet_index/?code=QP52AC02



(A)



(B)



(C)

Figure 2-1: Structural formulas of FBZ (A), OXF (B) and FBZ-SO_2 (C).

3. Ecosystem at the Site of Introduction

Safe-Guard Aquasol for Swine will be used in swine farms throughout the US. As swine are typically held in enclosed buildings (not pasture) (U.S. EPA, 2012a), the general route by which FBZ residues might enter the environment is through the application of manure on agricultural land. Accordingly, ecosystems potentially at risk are soil and freshwater aquatic environments (exposed directly via run-off from agricultural land and indirectly via entry of groundwater). Swine are generally intensively reared animals, not pasture animals, and their manure is held in liquid form that is typically applied to agricultural lands by injection. Therefore, dung beetle and dung fly larvae are not considered to be at risk.

4. Metabolism and Environmental Data

Internal studies conducted to generate environmental data are generally rated valid if they are conducted according to OECD protocols as requested in VICH GL 38. This is also true for studies which were not performed according to OECD GL if they were completed before implementation of VICH GL 38 and if they were conducted according to test GL in place at this time, such as FDA Technical Assistance Document (TAD) and EPA GLs.

4.1 Absorption, Distribution, Metabolism and Excretion

After absorption, FBZ is rapidly metabolized by liver microsomes. The major metabolite is OXF produced by sulfoxidation. This metabolism step is reversible, but OXF is further sulfoxidated to FBZ-SO_2. FBZ can also undergo a demethoxycarbonylation to FBZ-amine or a hydroxylation to p-hydroxy-FBZ (Short et al., 1988) (Figure 4-1).

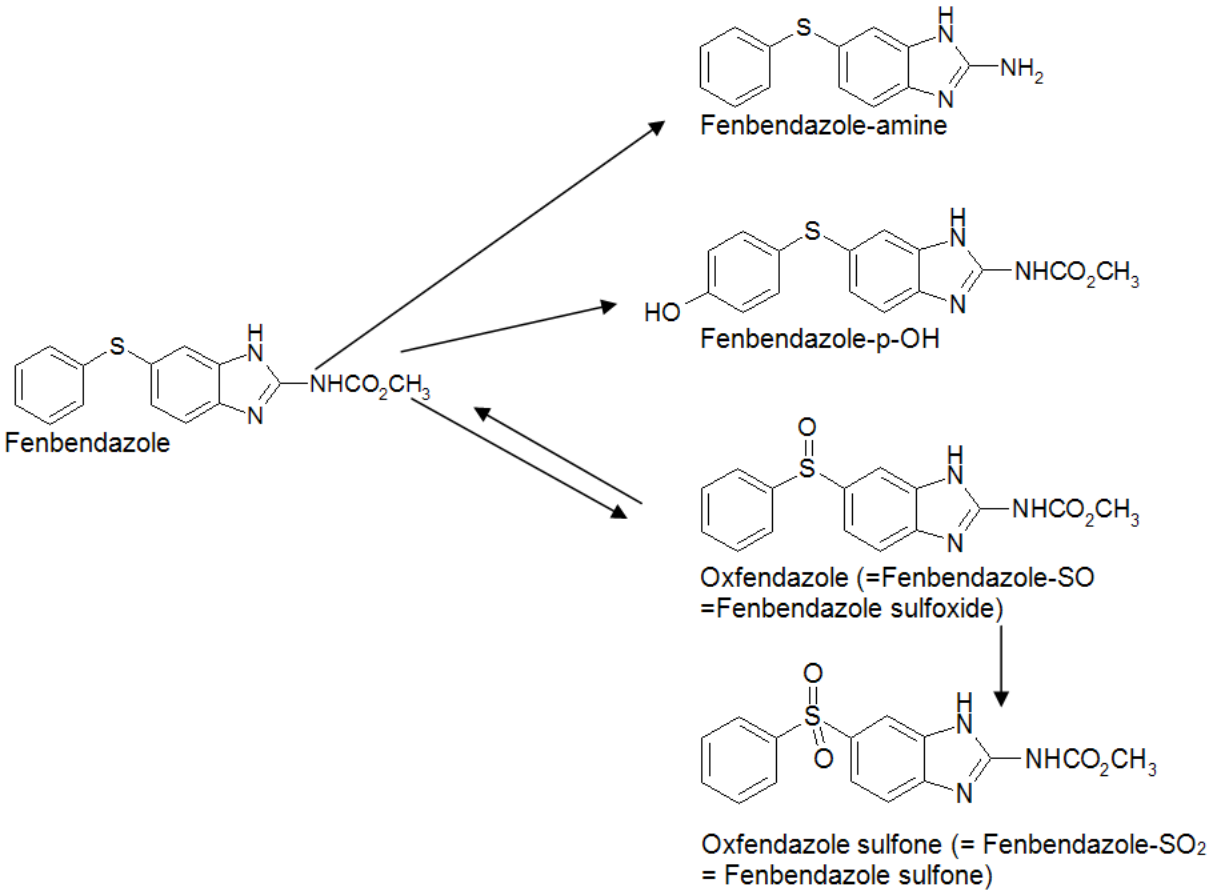


Figure 4-1 : Diagram of known pathways of oxidative metabolism of FBZ (from Short at al., 1988).

4.1.1 *Bioequivalence study with modified fenbendazole*

A pivotal bioequivalence study was conducted in swine to demonstrate bioequivalence between Safe-Guard Aquasol at a dose of 2.2 mg/kg and the pioneer product Safe-Guard 20% administered at a dose of 3 mg/kg, which is the total daily dose in a 3 day label claim (Sczesny, 2010). The study was performed in compliance with GLP, to satisfy the general principles of CVMs Guidance for Industry # 35 (Bioequivalence guidance, FDA, CVM, November 8, 2006) with the exception that the hematology and blood chemistry investigations were not conducted in a GLP certified laboratory and the pathology investigation was not conducted according to GLP.

For the design of the pivotal study, results from three non-pivotal studies were taken into consideration. Based on the outcome of these studies, it was assumed that the Safe-Guard Aquasol dose that is bioequivalent to Safe-Guard 20% at a dose of 3 mg/kg is between 2 and 2.5 mg/kg. Hence, the pivotal study was performed to demonstrate that Safe-Guard Aquasol for Swine at a dose of 2.2 mg/kg is bioequivalent to Safe-Guard 20% at a dose of 3 mg/kg.

To demonstrate bioequivalence, the FBZ concentration in the plasma of pigs collected at consecutive time points after administration of either Safe-Guard Aquasol or Safe-Guard 20% was determined. Based on the plasma concentrations, the pharmacokinetic variables were determined and statistically compared. The outcome of the pivotal bioequivalence study demonstrated that Safe-Guard Aquasol administered at a dose of 2.2 mg/kg is bioequivalent to pioneer product Safe-Guard 20% at a dose of 3 mg/kg.

The bioequivalence study of Sczesny (2010) demonstrates that the modified FBZ has a 0.3 times increased bioavailability compared to the unmodified form. Assuming that the modified FBZ is excreted and land applied as particles with reduced size (and not in soluble form, assuming that passage through the swine and storage of porcine manure will not have an impact on particle size), it will exert a 0.3 fold increased bioavailability (compared to the unmodified FBZ) also to terrestrial non-target organisms.

4.2 Physical-chemical Properties

4.2.1 *Fenbendazole*

For FBZ the PC properties were investigated according to OECD GLs by comparing unmodified and modified compound (Holzer, 2012). The objective of this study was the physical and chemical characterization of modified in comparison to unmodified FBZ, in order to evaluate if the modification process and therefore the reduction of the particle size, has an impact on the PC properties. FBZ was modified by mimicking the production process, which includes the addition of excipients. The OECD GLs followed were: OECD 105 for determination of water solubility, OECD 112 for determination of dissociation constant in water, OECD 101 for the determination of UV-visible absorption spectrum (the test GL is not mentioned in the main part of the report but in Appendix 14.11 and 14.12 of Holzer, 2012), OECD 102 for determination of melting point, OECD 117 for determination of n-octanol/water partition coefficient, and OECD 104 for determination of vapor pressure. The experiments were not conducted according to GLP

but in GLP compliant facilities. The PC properties of unmodified and modified FBZ are summarized in Table 4-1.

Table 4-1: Physical-chemical properties for FBZ

Property	unmodified	modified	OECD GL	Reference
Water solubility	0.08 mg/L	0.08 mg/L	105	Holzer (2012)
Dissociation constant in water	n.d.	n.d.	112	Holzer (2012)
	pKa: 5.25, 10.80	n.d.	-	ACD/Labs
UV-visible absorption spectrum	220 nm	219 nm	101	Holzer (2012)
	245 nm	243 nm		
	290 nm	296 nm		
Melting point	238-243 °C	236-238 °C	102	Holzer (2012)
n-octanol/water partition coefficient	Log K_{OW}: 3.32	Log K_{OW}: 3.40	117	Holzer (2012)
Vapor pressure	5.6 x 10^{-9} Pa	8.0 x 10^{-11} Pa	104	Holzer (2012)
	4.2 x 10^{-11} Torr	6.0 x 10^{-13} Torr		

n.d. – not determined

The dissociation constant pKa could not be determined for both forms of FBZ, neither by means of the spectrometric method nor by means of the titration method. The spectrometric method revealed only very slight differences in the absorption spectra at pH values ranging from 0.8 to 13.5. Thus it was impossible to estimate at which pH-value the compound is dissociated and at which not. For the titration method, two different aqueous solutions of the test item, originally being neutral with pH 6, were titrated either with HCl (to pH 4) or NaOH (to pH 8). Both titrations proceeded without any inflexion point up to a pH of approximately 8 and down to a pH of approximately 4. A calculation with the Advanced Chemistry Development (ACD/Labs) Software V11.02 revealed a pKa of 5.25 and 10.80 for unmodified FBZ.

For the melting point, vapor pressure and octanol water partition coefficient, slightly different values were measured for unmodified and modified FBZ. These differences were caused by the presence of the excipient following the modification. Instead of extensive washing, the excipient could not be removed completely. For the melting point and vapor pressure, the presence of excipient will generally cause a decrease. This theoretical prediction has been confirmed by measured values. In conclusion it can be assumed that these slight differences would not have been observed if the modified FBZ sample had been free of excipient. For the water solubility, measured values are identical for unmodified and modified FBZ. Accordingly, the PC properties of modified FBZ, including water solubility and the octanol/water partition coefficient, remain identical or at least substantially equivalent compared to unmodified FBZ.

The log K_{OW} was determined to be 3.32 and 3.40 for unmodified and modified FBZ. According to the criteria presented in VICH, substances with a log Kow of <4.0 are not considered bioaccumulative.

4.2.2 *Oxfendazole*

The PC properties of OXF were determined in several studies by according to FDA TAD protocols (Das, 1986a; Das, 1986b; Das, 1986c; Marple, 1987) (Table 4-2).

Table 4-2: Physical-chemical properties for OXF

Property		Reference
Water solubility	3.87 mg/L	Marple (1987)
Dissociation constant in water	pKa: 4.47, 10.27	ACD/Labs
UV-visible absorption spectrum	228 and 295 nm	Das (1986a)
Melting point	241.98 °C	MPBWIN
n-octanol/water partition coefficient	Log K_{OW}: 1.953	Das (1986b)
Vapor pressure	2.9 x 10^{-4} Pa 2.2 x 10^{-6} Torr	Das (1986c)

The water solubility was determined by Marple (1987) according to FDA TAD 3.01 and by procedures in IPS Letter No. 52,654. Approaching saturation from under- and supersaturation levels, Method A (FDA TAD 10.01), revealed solubilities ranging from 1.08 to 6.9 mg/L. Approaching from undersaturation over a prolonged period, Method C (FDA TAD 3.01), revealed solubilities ranging from 8.5 to 9.0 mg/L when the OXF deposit was formed by evaporation of a methanol solution, and solubilities ranging from 6.8 to 9.7 mg/L when the deposit was formed by evaporation of an acetone solution. When the solubility of OXF was measured by IPS Procedure No. 52,654 the mean solubility was 5.11 mg/L. A review of all of the aqueous solubility values shows that they cluster between 3 and 5 mg/L. The best values appear to be given by Method A (FDA TAD 10.01), approaching from unsaturation. The mean solubility by Method A is 3.87 mg/L.

No experimental determination of the dissociation constant was performed. Accordingly, the dissociation constant is calculated using ACD/Labs Software V11.02. The pKa for OXF is calculated to be 4.47.and 10.27.

The UV-visible absorption spectrum of OXF was determined by Das (1986a). Two concentrations of OXF in water were studied in a scanning UV-visible spectrophotometer for its property of absorbance of various light energies in the range of 190-750 nm. Absorbance peaks were found at 228 and 295 nm.

The n-octanol/water partition coefficient of OXF was determined by Das (1986b) according to FDA TAD 10.02 at 25 °C. Initial concentrations of OXF in n-octanol were 0.5 and 5.0 µg/L. The mean log K_{OW} was determined to be 1.953. According to the criteria presented in VICH, substances with a log Kow of <4.0 are not considered bioaccumulative.

The vapor pressure of OXF was determined by Das (1986c) by the headspace method. The test chemical was coated onto the inner wall of a glass bottle. After flushing out all the air with nitrogen, the vial was sealed with a rubber stopper and equilibrated for 24 h at 23, 50 and 65 °C. Vapor pressure was calculated with the Ideal Gas Law by measuring the OXF content in the

headspace. The vapor pressure was found to be 2.2 x 10^{-6} Torr at 25 °C, which equals 2.9 x 10^{-4} Pa.

No experimental determination of the melting point was performed. Accordingly, the melting point is estimated by using EPA's Estimation Program Interface (EPI) Suite (v4.11) program MPBWIN (v1.43)[4] (Appendix 14.1). For OXF the melting point proposed by MPBWIN is 241.98 °C

4.2.3 *Fenbendazole sulfone*

For FBZ-SO2, PC properties were not determined experimentally. Accordingly, they were calculated with different EPI Suite programs (water solubility, melting point, n-octanol/water partition coefficient, and vapor pressure) (Appendix 14.1) and ACD/Labs Software (dissociation constant in water). The calculated PC properties and software tools used are presented in Table 4-3.

Table 4-3: Physical-chemical properties for FBZ-SO_2

Property	FBZ-SO_2	Reference
Water solubility	13.58 mg/L	WATERNT (v1.01)
Dissociation constant in water	pKa: 4.04, 10.14	ACD/Labs
UV-visible absorption spectrum	n.d.	-
Melting point	245.8 °C	MPBWIN (v1.43)
n-octanol/water partition coefficient	Log K_{OW}: 2.17	KOWWIN
Vapor pressure	2.23 x 10^{-10} Pa 1.68 x 10^{-12} Torr	MPBWIN (v1.43)

n.d. – not determined

The log K_{OW} was calculated to be 2.17. According to the criteria presented in VICH, substances with a log Kow of <4.0 are not considered bioaccumulative.

4.3 Environmental Fate

4.3.1 *Soil Adsorption/Desorption*

4.3.1.1 Fenbendazole

A soil adsorption/desorption study in three soils (clay loam, loamy sand and sandy loam) was conducted by Mackie and Ayliffe (1999) in accordance with GLP and OECD GL 106. For each soil, solutions of [^{14}C]-FBZ at four concentrations (40, 190, 991, and 4970 µg/kg) in aqueous 0.01 M $CaCl_2$ were added to the soil and shaken for a predetermined equilibration time (4 h).

[4] http://www.epa.gov/opptintr/exposure/pubs/episuite.htm

Following centrifugation and separation, the concentration of [^{14}C]-FBZ in each supernatant was determined by liquid scintillation counting (LSC).

FBZ demonstrated potential to adsorb to each of the soils, independent from the initial concentration selected. This adsorption was deemed irreversible (based on the results of the screening test). Calculation of the Freundlich adsorption coefficient (K_f) demonstrated that the extent of adsorption of FBZ to each of the 3 soil types was in the order clay loam (K_f = 92) < loamy sand (K_f = 137) < sandy loam (K_f = 181). Individual test vessel replicate results of soil adsorption/desorption partition coefficients (normalized to the organic content of soil, K_{OC}) ranged from 6438 to 46563 L/kg, 6556 to 22667 L/kg, and 10250 to 45125 L/kg in clay loam, loamy sand and sandy loam, respectively, depending on the initial FBZ concentration. Average K_{OC} values for the soil types were not reported. Based on these findings, FBZ is rated as slightly mobile to hardly mobile according to Food and Agriculture Organization (FAO) Mobility Classification Table [5].

Because average K_{OC} values were not calculated in Mackie and Ayliffe (1999), average K_{OC} values are calculated in this EA by first estimating the average adsorption distribution coefficient (K_d) for each soil type. The average K_d for each soil type is obtained by fitting the adsorbed soil concentration versus the solution concentration data pairs and performing linear regression analyses (Appendix 14.2). The average K_{OC} values are calculated by inserting the K_d values in to Equation 4-1. Although Mackie and Ayliffe (1999) tested four concentrations, the results of the highest test concentration (4970 µg/kg) was excluded from the analyses because the test concentration was 150-fold greater than the PEC_{soil}. This method provided for the best fit for the most environmentally relevant concentrations, resulting in a higher, more conservative, $PEC_{surfacewater}$ and $PEC_{groundwater}$.

Equation 4-1

$$K_{OC}\ [L/kg] = K_d\ [L/kg] \times \frac{100}{OC\ [\%]}$$

For clay loam as an example, Equation 4-1 becomes:

$$K_{OC}\ [L/kg] = 258.18\ L/kg \times \frac{100}{1.6\%} = 16136\ L/kg$$

The calculation of average K_{OC} values for all soil types is summarized in Table 4-4:

[5] http://www.epa.gov/pesticides/science/efed/policy_guidance/team_authors/endangered_species_reregistration_workgroup/esa_reporting_fate.htm#II_C

Table 4-4: Averages soil K_{OC} values for FBZ based on average K_d values obtained by best fit of adsorbed concentration versus the solution concentration data pairs (except the highest test concentration) reported by Mackie and Ayliffe (1999) (Appendix 14.2)

	Soil type		
	Clay loam	Loamy sand	Sandy loam
K_d [L/kg]	258.18	128.63	321.50
OC [%]	1.6	1.8	1.6
K_{OC} [L/kg]	16136	7146	20094

The study of Mackie and Ayliffe (1999) reveals deficiencies when compared to OECD GL 106 but is considered valid nevertheless as follows:

1. Only three soils were investigated rather than five as requested in the current version of OECD GL 106. However, this new version was implemented after conduct of this study. Accordingly, this deviation is acceptable.

2. There is a lack of variability in the OC content within the soils. The OC content of the soils was relatively low (1.6-1.8%). Because of the strong affinity of FBZ to the soils and its low water solubility (0.08 mg/L), it is likely that even with a reduced OC content, the majority of FBZ, if not all, would strongly bind to soils. In addition, if the OC content was reduced to levels to which FBZ would saturate the soil binding sites, it is likely that FBZ would preferably adsorb to the test vessel rather than remain in solution (based on results of the preliminary tests). Therefore, while it would have been beneficial to have five soil types with varying OC contents investigated, the results are valid nevertheless.

3. A soil/solution ratio preliminary test was not conducted. According to current OECD guideline 106, a soil/solution ratio test with three soil/solution ratios (1:1, 1:5, and 1:25) using 2 soil types should have been conducted. However, the study was conducted using the default ratio of 1:5. Especially for compounds with a high K_d, higher soil/solution ratios (i.e. up to 1: 100) should be considered. The study revealed that with a 1:5 ratio, >98% of FBZ was bound to the soil or test apparatus. A higher ratio may have allowed for more FBZ to remain in solution (i.e., potentially saturate binding sites on the soil), but based on the results of the preliminary and screening tests, it is more likely that the FBZ would bind to the test vessel instead of remaining in solution. Therefore, while it would have been beneficial to investigate at a more appropriate soil/solution ratio, the results are valid nevertheless.

4. The interpretation of the results is incorrect as it was concluded that the adsorption of FBZ was independent of soil organic matter (OM) content. However, because OM content (and OC content) did not vary across soil types, the results indicate that adsorption is independent of soil pH and clay content, not OM content. The extent of adsorption in terms of soil OM was similar for the three soils, as it would be expected, because the OM did not vary among the soils. It is therefore possible that the OM content is the primary factor influencing the adsorption of FBZ in soil.

Mackie and Ayliffe (1999) investigated the unmodified form of FBZ. However, because the PC properties are similar for modified and unmodified FBZ (Chapter 4.2) and the unmodified FBZ was tested in the soluble form (according to OECD GL 106), it is expected that the modified FBZ acts similarly. Therefore, the soil adsorption/desorption study conducted with unmodified FBZ can be used to assess the environmental fate of its modified form.

4.3.1.2 Oxfendazole

A soil adsorption/desorption study in three soils (sandy loam, silt loam, and clay loam) was conducted by Cargile (1985) in accordance with FDA TAD 10.08. For each soil, solutions of [^{14}C]-OXF at four concentrations (0.04, 0.2, 1.0, and 5.0 µg/g) in aqueous 0.01 M $CaCl_2$ were added to the soil and shaken for a predetermined equilibration time (14.25 h). Following sampling, the concentration of [^{14}C]-OXF in each supernatant was determined by LSC.

OXF demonstrated potential to adsorb to each of the soils. Calculation of average K_d by linear regression demonstrated that the extent of adsorption of OXF to each of the 3 soil types was in the order silt loam (K_d = 4.5 L/kg) < sandy loam (K_d = 6.4 L/kg) < clay loam (K_d = 15.7 L/kg). Average K_{OC} values correspond to 544, 546, and 1570 L/kg for, sandy loam, silt loam, and clay loam, respectively. These K_{OC} values are calculated based on OM by applying a correction factor of 1.7. This approach is in line with OECD GL 106. Based on the findings Cargile (1985), OXF is rated as moderately mobile to slightly mobile according to FAO.

The study of Cargile (1985) reveals deficiencies when compared to OECD GL 106 but is considered valid nevertheless as follows:

1. Only three soils were investigated rather than five as requested in the current version of OECD GL 106. However, this new version was implemented after conduct of this study. Accordingly, this deviation is acceptable.

2. There is a lack of variability in the OC content within the soils. The OC content (derived from the reported OM content divided by 1.7) of the soils was relatively low (0.8-1.2%). Investigation of additional soils with a higher OC content would likely reveal higher K_{OC} values. While it would have been beneficial to have five soil types with varying OC contents investigated, the results are valid nevertheless for the purpose of the risk assessment as the K_{OC} values are conservative.

3. A soil/solution ratio preliminary test was not conducted. According to current OECD guideline 106, a soil/solution ratio test with three soil/solution ratios (1:1, 1:5, and 1:25) using 2 soil types should have been conducted. However, the study was conducted using the default ratio of 1:5. As the percentages adsorbed range between 43% and 76% depending on soil type, the recommendation of OECD GL 106 are met (percentage adsorbed should be >20% and preferably >50%. Higher soli/solution ratios (i.e. up to 1: 100) should be considered for especially for compounds with a high K_d, which is not relevant for OXF. Therefore, while it would have been beneficial to investigate at a more appropriate soil/solution ratio, the results are valid nevertheless.

4.3.1.3 Fenbendazole sulfone

For FBZ-SO_2 no experimental determination of K_{OC} values was performed. Accordingly, the K_{OC} is estimated by using EPI Suite program KOCWIN (v2.00)[6] (Appendix 14.1.5). For FBZ-SO_2 the K_{OC} is estimated to 2918 (Sabljic molecular connectivity method with improved correction factors) and 292 (traditional method based on log K_{OW}). FBZ-SO_2 is rated as moderately mobile to slightly mobile according to FAO.

4.3.1.4 Summary of K_{OC} values

Average K_{OC} values reported for FBZ and OXF are summarized in Table 4-5 together with the estimated K_{OC} values for FBZ-SO_2.

Table 4-5: Soil K_{OC} values for FBZ, OXF, and FBZ-SO_2 [L/kg]

Soil type	Compound					
	FBZ	Source	OXF	Source	FBZ-SO_2	Source
Clay loam	16136	Table 4-4	1570	Cargile (1985)	n.d.	-
Loamy sand	7146	Table 4-4	n.d.	-	n.d.	-
Sandy loam	20094	Table 4-4	544	Cargile (1985)	n.d.	-
Silt loam	n.d.	-	546	Cargile (1985)	n.d.	-
not specified	-	-	-	-	2918 [1]	KOCWIN
not specified	-	-	-	-	292 [2]	KOCWIN

n.d. – not determined
[1] - Estimated by Sabljic molecular connectivity method with improved correction factors
[2] - Estimated by the traditional method based on log K_{OW}

Although K_{OC} data is reported for FBZ-SO_2 in Table 4-5, the resulting values are based on modeling software and are an order of magnitude different between the two unidentified soil types. Therefore, because the OXF data is considered to be more reliably (measured K_{OC} data available), only the OXF K_{OC} data are used to refine the $PEC_{surfacewater}$ and $PEC_{groundwater}$.

For the refinement of $PEC_{surfacewater}$ (Chapter 5.2.2) the arithmetic mean K_d and K_{OC} are calculated for FBZ and OXF respectively which is in line with principles set by US EPAs, namely the input guide for the PRZM model (which requests the use of mean K_{OC})[7]. For the calculation of $PEC_{groundwater}$ (using US EPAs SCI-GROW model) (Chapter 5.4) the median K_{OC} is calculated which is in line with principles set by US EPAs input guide for the SCI-GROW model (as measured K_{OC} values do not show greater than a three-fold variation)[8].

The calculation of arithmetic mean K_d and K_{OC} and median K_{OC} are is summarized in Table 4-6:

[6] http://www.epa.gov/opptintr/exposure/pubs/episuite.htm

[7] http://www.epa.gov/oppefed1/models/water/input_parameter_guidance.htm#Przm

[8] http://www.epa.gov/oppefed1/models/water/input_parameter_guidance.htm#Scigrow

Table 4-6: Arithmetic mean K_d and K_{OC} and median K_{OC} [L/kg]

	FBZ				OXF			
	K_d	Source	K_{OC}	Source	K_d	Source	K_{OC}	Source
	258	Table 4-4	16136	Table 4-4	4.5	Cargile (1985)	1570	Cargile (1985)
	129	Table 4-4	7146	Table 4-4	6.4	Cargile (1985)	546	Cargile (1985)
	322	Table 4-4	20094	Table 4-4	15.7	Cargile (1985)	544	Cargile (1985)
Arithmetic mean	236		14459		9		887	
Median	-		16136		-		546	

The arithmetic mean K_d amount to 236 L/kg for FBZ and to 9 L/kg for OXF, the arithmetic mean K_{OC} to 14459 L/kg for FBZ and to 887 L/kg for OXF. The median K_{OC} amount to 16136 L/kg for FBZ and to 546 L/kg for OXF.

4.3.1.5 Additional information

Kreuzig et al. (2007) investigated the soil adsorption/desorption of FBZ in two soils (silty clay and silty sand) in accordance with OECD GL 106. For each soil, a solution of [^{14}C]-FBZ in aqueous 0.01 M $CaCl_2$ was added to the soil (final concentration: 400 µg/kg) and shaken for 24 h. Following centrifugation, the concentration of [^{14}C]-FBZ in supernatant was determined by LSC. K_d values were 63 L/kg for the silty clay and 58 L/kg for the silty sand. Considering the OC content of 1.6% for silty clay and 0.8% for silty sand, K_{OC} values are calculated according to Equation 4-1. The calculation of average K_{OC} values for the two soil types is summarized Table 4-7.

Table 4-7: K_{OC} values for FBZ based on K_d values reported by Kreuzig et al. (2007)

	Soil type	
	Silty clay	Silty sand
K_d [L/kg]	63	58
OC [%]	1.6	0.8
K_{OC} [L/kg]	3938	7250

Considering the findings of Kreuzig et al. (2007), FBZ is rated as slightly mobile according to FAO. Differences in the K_{OC} values determined by Mackie and Ayliffe (1999) and Kreuzig et al. (2007) can likely be attributed to the different soil types used and the fact that Kreuzig et al. (2007) investigated a single test concentration only that was relatively low compared to test concentrations used by Mackie and Ayliffe (1999).

4.3.2 *Soil Biodegradation*

A soil degradation study in three soils (sandy loam, loamy sand and clay loam) was conducted by Mackie and Ayliffe (2000) in accordance with GLP. The degradation rate was investigated in all three soils, while the route was determined in sandy loam only. Soil samples were amended with [^{14}C]-FBZ at an initial concentration of 0.6 mg/kg. The samples were incubated in the dark

at a nominal temperature of 20°C for up to 365 d under aerobic conditions. In principle, the study design follows OECD GL 307.

The total recovery ranged from 94 - 104% applied radioactivity for the route soil (sandy loam). Extractability declined over time in all three soils from 98 to 61% applied radioactivity, 99 to 67% applied radioactivity and 94 to 39% applied radioactivity for sandy loam, loamy sand and clay loam, respectively. FBZ declined rapidly in all soils, representing 94.2 to 99.3% applied radioactivity at beginning of the study based on high performance liquid chromatographic (HPLC) analysis. At termination (365 d), FBZ was absent in sandy loam and loamy sand and represented 1% applied radioactivity in clay loam. The primary degradation product detected in all soils was OXF. Maximum amounts of OXF were 61.9% applied radioactivity in sandy loam (120 d), 62.5% in loamy sand (120 d) and 51.8% in clay loam (32 d). OXF was degraded subsequently with amounts decreasing to 22.6% applied radioactivity, 50.2% applied radioactivity, and 12.9% applied radioactivity in sandy loam, loamy sand and clay loam, respectively, at the end of the study (365 d). The secondary transformation product, generated by degradation of OXF was FBZ-SO_2, which was detected in all soils. Maximum amounts of FBZ-SO_2 were 37.8% applied radioactivity in sandy loam (365 d), 10.3% in loamy sand (365 d) and 25.8% in clay loam (100 d). No other degradation product >10% applied radioactivity was identified.

For FBZ, the time to degradation of 50% of original concentration (DT_{50}) were determined to 4, 12, and 8 d for sandy loam, loamy sand and clay loam, respectively, the corresponding time to degradation of 90% of original concentration (DT_{90}) were 120, 131, and 83 d (Table 4-8). Accordingly, FBZ is classified as non-persistent in soil ($DT_{90} \leq 1$ year). For OXF, DT_{50} were determined to 1424 and 133 d for loamy sand and clay loam (for sandy loam, there were insufficient data points to allow for a DT_{50} estimation with any degree of confidence) (Table 4-8). In absence of reported DT_{90} values for OXF, a classification is difficult. However, OXF is likely persistent, at least in loamy sand.

Table 4-8: Soil biodegradation of FBZ and OXF

Soil type	Compound				Reference
	FBZ		OXF		
	DT_{50}	DT_{90}	DT_{50}	DT_{90}	
Sandy loam	4 d	120 d	n.d.	n.d.	Mackie and Ayliffe (2000)
Loamy sand	12 d	131 d	1424 d	n.d.	Mackie and Ayliffe (2000)
Clay loam	8 d	83 d	133 d	n.d.	Mackie and Ayliffe (2000)

n.d. – not determined

Mackie and Ayliffe (2000) investigated the degradation of FBZ over a period of 365 d, which is clearly exceeding the normal duration proposed in OECD GL 307 (120 d). According to OECD GL 307, exceeding the 120 d study duration may cause a decrease of the soil microbial activity with time in an artificial laboratory system isolated from natural replenishment. In fact, the microbial biomass decreased between 120 d and 365 d (from 58 to 39 mg C/100 g soil, 165 to 78 mg C/100 g soil, and 63 to 50 mg C/100 g soil, for sandy loam, loamy sand and lay loam,

respectively), indicating a decrease in microbial activity. Accordingly, degradation of compounds beyond 120 d took likely place at a lower rate than expected under field conditions.

The initial concentration in the study of Mackie and Ayliffe (2000) of 0.6 mg/kg exceeds the initial PEC_{soil} for FBZ of 33.44 µg/kg by factor 18, and the plateau PEC_{soil} of OXF/FBZ-SO_2 of 72.31 µg/kg by factor 8 (Chapter 5.1).

Mackie and Ayliffe (2000) investigated the unmodified form of FBZ. However, because the PC properties are similar for modified and unmodified FBZ (Chapter 4.2) and the unmodified FBZ was tested in the soluble form (according to OECD GL 307), it is expected that the modified FBZ acts similarly. Therefore, the soil biodegradation study conducted with unmodified FBZ can be used to assess the environmental fate of its modified form.

In the study of Mackie and Ayliffe (2000) DT_{50}/DT_{90} values for FBZ and OXF were estimated by fitting the data to a model developed in 1986[9], while for FBZ-SO_2 no DT_{50}/DT_{90} values were estimated at all. Therefore the data from Mackie and Ayliffe (2000) are re-evaluated by using the Computer Assisted Kinetic Evaluation (CAKE) software (version 1.4)[10] in order to derive DT_{50}/DT_{90} values for FBZ, OXF and FBZ-SO_2. CAKE assists in the construction of kinetic evaluations of chemicals, in line with the European Union (EU) Forum for Co-ordination of Pesticide Fate Models and their Use (FOCUS) GLs.

To allow for DT_{50}/DT_{90} calculations for each compound, percentages of applied radioactivity per sampling interval as presented in Mackie and Ayliffe (2000) for all three compounds, FBZ, OXF, and FBZ-SO_2, were used (Appendix 14.3).

CAKE evaluations (Appendix 14.3) are performed for all three soil types in accordance with principles described by EPA for soil biodegradation studies (OPPTS GL 835.4100), namely by applying the simple first order (SFO) kinetic if R^2 is ≥0.7. This approach is conservative as application of more advanced compartment models, e.g. first order multi-compartment, would cause in a better fit which in turn would result in lower DT_{50}/DT_{90} values. For sandy loam, only FBZ and OXF are considered as the fit failed when including also FBZ-SO_2. The resulting final fit step results for FBZ (parent), OXF (A1) and FBZ-SO_2 (A2) are summarized in Table 4-9.

[9] Timme G, Frehse H, and Laska V (1986) Statistical interpretation and graphic representation of the degradational behavior of pesticide residues. II. *Pflanzenschutz Nachrichten Bayer* 39, 187–203.

[10] http://projects.tessella.com/cake/

Table 4-9: CAKE evaluations applying SFO kinetics for FBZ, OXF, and FBZ-SO_2 based on the results from Mackie and Ayliffe (2000), percentages of applied radioactivity per sampling interval for each compound

Soil type	Compound					
	FBZ		OXF		FBZ-SO_2	
	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}
Sandy loam	9.4 d	31.3 d	319.4 d	1061 d	n.d.	n.d.
Loamy sand	11.4 d	37.9 d	705.7 d	2344 d	186.5 d	619.6 d
Clay loam	7.6 d	25.3 d	123.1 d	408.8 d	221.3 d	735 d

n.d. – not determined

Considering the CAKE evaluations (Table 4-9), both, OXF and FBZ-SO_2 are classified as persistent in soil (DT_{90} >1 year). As the DT_{90} values clearly exceed the trigger value of 1 year, this classification is correct even considering that the DT_{90} values determined for OXF and FBZ-SO_2 are likely conservative due to the decrease in soil microbial activity under artificial laboratory conditions and isolation from natural replenishment for 365 days. Accordingly, degradation of both compounds was slower in this study as it would have been in studies using the compounds directly as test items. However, as the DT_{90} values for both compounds clearly exceed the trigger value of 1 year, the classification as persistent in soil is considered to be valid nevertheless. Studies using the compounds directly as test items would likely reveal DT_{90} values of >1 year, too.

For the calculation of the plateau PEC_{soil} for OXF and FBZ-SO_2 (Chapter 5.1.2), the upper 90th percentile confidence bound on the mean DT_{50} is calculated. Using the upper 90th percentile confidence bound on the mean DT_{50} instead of the mean or highest DT_{50} is in line with principles set by US EPAs, namely the input guide for the PRZM model, which specifies the use of the upper 90th percentile confidence bound on the mean DT_{50} in case that more than one DT_{50} value is available[11]. For the calculation of $PEC_{groundwater}$ (using US EPAs SCI-GROW model) (Chapter 5.4), the arithmetic mean DT_{50} is used. Using the arithmetic mean DT_{50} instead of the median is in line with US EPAs input guide for the SCI-GROW model (as less than four DT_{50} values are available)[12]. Calculations of the upper 90th percentile confidence bound on the mean DT_{50} are conducted according to Equation 4-2:

Equation 4-2

$$CB_{DT50}[d] = AM_{DT50}[d] + \left[\frac{\left(t_{90,n-1} \times SD_{DT50}\ [d]\right)}{n^{1/2}}\right]$$

[11] http://www.epa.gov/oppefed1/models/water/input_parameter_guidance.htm#Przm

[12] http://www.epa.gov/oppefed1/models/water/input_parameter_guidance.htm#Scigrow

Where:

CB_{DT50}: Upper 90th percentile confidence bound on the mean DT_{50}

AM_{DT50}: Arithmetic mean of DT_{50}

$t_{90,n-1}$: One-sided Student's t value at α=0.1 (e.g. n – 1 = 1: 3.078, n – 1 = 2: 1.886)

SD_{DT50}: Standard deviation of DT_{50}

n: Number of DT_{50} values

For FBZ as an example Equation 4-2 becomes:

$$CB_{DT50}[d] = 9.5\ d + \left[\frac{(1.886 \times 1.9\ d)}{3^{1/2}}\right] = 11.5\ d$$

The calculation of upper 90th percentile confidence bound on the mean DT_{50} is summarized in Table 4-10:

Table 4-10: Upper 90th percentile confidence bound on the mean DT_{50} based on results of CAKE evaluations (Table 4-9)

		FBZ	OXF	FBZ-SO_2
DT_{50}	d	9.4	319.4	
	d	11.4	705.7	186.5
	d	7.6	123.1	221.3
Arithmetic mean	d	9.5	382.7	203.9
Standard deviation	d	1.9	296.4	24.6
Number of values	n	3	3	2
One-sided Student's t value at α=0.1	n	1.886	1.886	3.078
Upper 90th percentile confidence bound on the mean	d	11.5	705.5	257.5

The upper 90th percentile confidence bound on the mean DT_{50} amount to 11.5 d, 705.5 d and 257.5 d for FBZ, OXF, and FBZ-SO_2.

4.3.2.1 Additional information

Kreuzig et al. (2007) investigated the fate of [^{14}C]-FBZ in two soils (silty clay and silty sand) at an initial concentration of 0.2 mg/kg. The soils were incubated in the dark at 20 ± 1°C under aerobic conditions for up to 102 d. In the silty clay soil, FBZ decreased over time, accounting to 91% applied radioactivity at the start of the study and to 44% applied radioactivity at 102 d. In the same period, the amount of OXF increased to 20% applied radioactivity. For the silty sand, degradation was slower, with FBZ accounting to 56% applied radioactivity at 102 d and OXF to 15% applied radioactivity. In the study of Kreuzig et al. (2007), the degradation of FBZ was slower than in the study of Mackie and Ayliffe (2000). The differences in DT_{50} values are likely the result of the varying prevalence of microbial biomass in the different soil types and other soil-specific factors such as surface area and moisture content of the soil and/or adsorption of FBZ to the soil.

Kreuzig et al. (2007) investigated degradation of FBZ in soil also in the presence of pig manure. Thereby, presence of manure accelerated the degradation of FBZ in both soils (silty clay and silty sand). In the silty clay soil, FBZ decreased from 61% applied radioactivity at the start of the study to 12% applied radioactivity at 9 d. Within 14 d, the amount of OXF increased to 43% applied radioactivity and decreased to 22% applied radioactivity at 102 d. Subsequently, FBZ-SO_2 was formed from OXF. For the silty sand, presence of manure also accelerated degradation of FBZ. The results thus indicate that FBZ degrades faster in the presence of pig manure.

Finally Kreuzig et al. (2007) investigated the degradation of FBZ under field conditions in presence of pig manure. Thereby, in a test conducted in April, FBZ concentrations decreased from 348 µg/kg to 151 µg/kg within 165 d. In a second test conducted in September, FBZ concentrations decreased from 466 µg/kg to 122 µg/kg within 50 d. As temperatures in this second test were higher than in the first one, the results indicate that FBZ degrades faster under higher temperatures..

Following the use of Safe-Guard Aquasol in pigs, excreted compounds will enter the environment only in the presence of pig manure (e.g. following the spreading of manure to agricultural land). Accordingly, the results determined by Mackie and Ayliffe (2000) in pure soil may be conservative as may be the DT_{50}/DT_{90} estimations with CAKE.

4.3.3 *Degradation in Slurry/Manure*

Internal studies were not performed to investigate the fate of FBZ in slurry/manure. Based on published information (Kreuzig et al., 2007), the storage of pig manure before land application will not result in a marked decrease of FBZ amounts entering the environment.

Kreuzig et al. (2007) investigated the fate of FBZ in porcine manure. [^{14}C]-FBZ was amended to manure at an initial concentration of 200 µg/kg and manure was incubated in the dark at 20 ± 1°C under anaerobic conditions for 102 d. FBZ decreased slowly, accounting to 94% applied radioactivity at the start of the study and to 80% applied radioactivity at 101 d. OXF was identified with a maximum of 4% applied radioactivity at 14 d. Considering these findings, the storage of pig manure before land application will not result in a marked decrease of FBZ amounts entering the environment.

4.3.4 *Photolysis and Hydrolysis*

4.3.4.1 Photolysis

4.3.4.1.1 Fenbendazole

A photodegradation study was conducted by Connor and Deetz (1995) in accordance with GLP and FDA TAD 3.10. The effect of simulated sunlight on the photolytic degradation of aqueous solutions of [^{14}C]-FBZ was tested at pH 5, 7 and 9. The test concentration was 19.5 µg/L.

Sampling and analysis for [^{14}C]-FBZ consisted of an extraction method where 4-5 separate tubes for the light-exposed and dark control solutions were separately combined, to provide triplicate solid phase extraction (SPE). Eluent from the solid phase columns were analyzed utilizing HPLC and subsequently LSC. Radiochromatograms (histograms) were conducted to quantify the concentration of FBZ present and to determine its degradation rate.

The temperature range during testing was 17.9 to 39.6 °C. The recovery ranged from 89.2% to 103% of applied radioactivity and demonstrated that there was no volatilization of FBZ or potential photodegradates during the study. During exposure, numerous small polar peaks were formed, with no single photodegradate representing >10% of the applied radioactivity. The presence of many small photodegradates could result from indirect photolysis mechanisms, e.g., complex radical formations, and may have been an important mechanism in the photolytic breakdown of FBZ.

FBZ was found to photolyze rapidly under simulated light conditions at pH 5, 7, and 9 with experimental photolytic DT_{50} values of <15 h. The environmentally relevant DT_{50} which were corrected for surface water geometry are similar (Table 4-11). Accordingly, FBZ is expected to be rapidly photolyzed in natural bodies of water.

Table 4-11: Photolytic degradation of FBZ calculated under winter conditions

pH	DT_{50}		Reference
	Experimental	Environmental	
5	14.5 h (0.60 d)	17.0 h (0.71 d)	Connor and Deetz (1995)
7	11.3 h (0.47 d)	12.7 h (0.53 d)	Connor and Deetz (1995)
9	10.1 h (0.42 d)	11.3 h (0.47 d)	Connor and Deetz (1995)

The overall light intensity achieved under simulated sunlight was less intense than conditions outside the Wareham laboratory on a typical winter day. Estimated values for the day-averaged rate constant, based on correction to the light conditions inside, correspond most closely to conditions at 50 °North latitude in winter based on a comparison of light intensity measurements under the simulated light source and outside the laboratory. Therefore, the photolytic DT_{50} of FBZ is theoretically expected to be even shorter under higher intensity sunlight conditions as would be available across the continental U.S. and during spring, summer, and autumn. However, in surface waters impacted by agricultural runoff, degradation will likely be slower due to the presence of suspended and dissolved particles and materials. In addition, depth may also limit light penetration which will impact the photolysis DT_{50}.

Connor and Deetz (1995) investigated the unmodified form of FBZ. However, because the physical-chemical properties are similar for modified and unmodified FBZ (Chapter 4.2) and the unmodified FBZ was tested in the soluble form (according to FDA TAD 3.10), it is expected that the modified FBZ acts similarly. Therefore, the photodegradation study conducted with unmodified FBZ can be used to assess the environmental fate of its modified form.

4.3.4.1.2 Oxfendazole

A photodegradation study was conducted by Nielsen et al. (1989) in accordance with FDA TAD 3.10. The effect of simulated sunlight on the photolytic degradation of aqueous solutions of OXF was tested at pH 5, 7 and 9.

OXF was found to photolyze rapidly under simulated light conditions at pH 5, 7, and 9 with experimental photolytic DT_{50} values of <1.4 h. The environmentally relevant DT_{50} (which were calculated for clear sky conditions at 40 °North latitude as a function of season and solution pH using the data obtained from the preceding measurements performed during the recent summer season) ranged from 1.7 h for summer season to 24.4 for winter season (Table 4-12). Accordingly, OXF is expected to be rapidly photolyzed in natural bodies of water at sunlight conditions as would be available across the continental U.S. and during winter, spring, summer, and autumn.

Table 4-12: Photolytic degradation of OXF

pH	Season	DT_{50}		Reference
		Experimental	Environmental	
5	n.r.	1.38 h (0.06 d)	n.r.	Nielsen et al. (1989)
7	n.r.	0.8 h (0.03 d)	n.r.	Nielsen et al. (1989)
9	n.r.	0.51 h (0.02 d)	n.r.	Nielsen et al. (1989)
5	Spring	n.r.	3.6 h (0.15 d)	Nielsen et al. (1989)
7	Spring	n.r.	4.6 h (0.19 d)	Nielsen et al. (1989)
9	Spring	n.r.	2.6 h (0.11 d)	Nielsen et al. (1989)
5	Summer	n.r.	2.4 h (0.10 d)	Nielsen et al. (1989)
7	Summer	n.r.	2.9 h (0.12 d)	Nielsen et al. (1989)
9	Summer	n.r.	1.7 h (0.07 d)	Nielsen et al. (1989)
5	Fall	n.r.	7.0 h (0.29 d)	Nielsen et al. (1989)
7	Fall	n.r.	8.6 h (0.36 d)	Nielsen et al. (1989)
9	Fall	n.r.	5.3 h (0.22 d)	Nielsen et al. (1989)
5	Winter	n.r.	19.9 h (0.83 d)	Nielsen et al. (1989)
7	Winter	n.r.	24.4 h (1.02 d)	Nielsen et al. (1989)
9	Winter	n.r.	14.6 h (0.61 d)	Nielsen et al. (1989)

n.r. – not relevant

4.3.4.2 Hydrolysis

4.3.4.2.1 Fenbendazole

A hydrolysis study was conducted by Adamovics (1980) to determine the hydrolysis rate of FBZ in selected aqueous systems. Three aqueous reaction mixtures of FBZ were stored at 25 °C in the dark at pH of 5, 7 and 9. At specified time intervals through 28 d, aliquots of the reaction mixtures were extracted with dichloromethane and analyzed by HPLC. The levels of FBZ found by HPLC were unchanged throughout the time period. At selected intervals, the dichloro-methane extract from the sample aliquots were also assayed by thin layer chromatography (TLC) which show one spot attributable to parent FBZ upon visualization by ultraviolet light

(UV). After 28 d, no significant hydrolysis of FBZ was indicated by HPLC or TLC. Therefore it is concluded that FBZ is not hydrolyzed in the tested range of conditions.

4.3.4.2.2 Oxfendazole

A hydrolysis study was conducted by Hussain and Ryan (1986) in accordance with FDA TAD 10.09. The purpose of this study was to determine the rate constant, DT_{50} and degradation products for hydrolysis of OXF in three buffer solutions of pH 5, 7, and 9 at 25 ± 1 °C. Buffer solutions of [^{14}C]-OXF were sampled at 1- to 3-d intervals for a 28-d period and analyzed for total radioactivity. Samples taken at d 0, 5, 8, 12, 16, 19, 23 and 28 were analyzed for degradation by TLC and autoradiography. Oxfendazole was found to hydrolyze at pH 9 with a DT_{50} of 17.6 d. At pH 7 and pH 5, less than 5% of the OXF was hydrolyzed and the DT_{50} could not be calculated. Incubation in pH 9 buffer resulted in the formation of a polar compound(s) as the major hydrolysis product. This degradate remained at the origin on the TLC plate. The concentration of the degradate increased steadily with the period of incubation to 66.5% of the recovered radioactivity at the end of 28 d.

5. Exposure Assessment

In the environment FBZ will degrade to OXF which will further degrade to FBZ-SO_2. Although FBZ is not considered to be persistent (DT_{90} <1 year), OXF has the potential to accumulate in soil and has greater potential for run-off and leaching. Therefore, separate exposure assessments are conducted for FBZ and its metabolites/degradates. Because OXF further degrades to FBZ-SO_2, and PC and fate data for FBZ-SO_2 have not been determined (with the exception of the DT_{50}), it is assumed that FBZ-SO_2 acts similarly to OXF, and a total residue approach is implemented for the two metabolites/degradates (see Figure 1-1). This procedure does not follow the total residue approach initially proposed by VICH for consideration of metabolites/degradates. However, due to significant differences in essential PC and fate properties (e.g. water solubility, soil adsorption and degradation) between FBZ and its metabolites/degradates this more complex approach is selected.

5.1 Predicted Environmental Concentration in Soil (PEC_{soil})

5.1.1 *Initial PEC_{soil}*

5.1.1.1 Fenbendazole

With respect to the environmental exposure scenarios considered to be relevant for the use of Safe-Guard Aquasol (Chapter 3), the initial PEC_{soil} is calculated for intensively reared pigs (held in enclosed buildings) only. FBZ residues will enter the environment via spreading of manure/slurry on agricultural land. The initial PEC_{soil} is calculated as follows:

Equation 5-1

$$Initial\ PEC_{soil}\ [\mu g/kg] = \frac{ID\ [mg/kg] \times BW\ [kg]\ \times T\ [n] \times F_H\ [n] \times MSL\ [kg/acre] \times 1000}{MP\ [d] \times MD\ [kg/d] \times W_{soil}\ [kg/acre]}$$

Where:

ID: Individual dose = 2.2 mg/kg (Chapter 1)

BW: Body weight = 50 kg (value traditionally used in environmental impact assessments of veterinary products)

T: Number of treatments = 3 (Chapter 1)

F_H: Fraction of herd treated = 1 (100% for anthelmintic therapeutics)

M_{SL}: Manure spreading limit = 22700 kg/acre x year (value traditionally used in environmental impact assessments of veterinary products)

1000: Conversion factor mg to µg

M_P: Manure production period = 60 d (value traditionally used in environmental impact assessments of veterinary products)

M_D: Manure production daily = 4.1 kg/d (value traditionally used in environmental impact assessments of veterinary products)

W_{soil}: Weight of soil = 910500 kg/acre (value traditionally used in environmental impact assessments of veterinary products)

For the use of Safe-Guard Aquasol for Swine, Equation 5-1 becomes:

$$Initial\ PEC_{soil}\ [\mu g/kg] = \frac{22\ mg/kg \times 50\ kg \times 3 \times 1 \times 22700 kg/acre \times 1000}{60\ d \times 4.1\ kg/d \times 910500 kg/acre} = 33.44\ \mu g/kg$$

The initial PEC_{soil} of 33.44µg/kg is the maximum initial environmental concentration in soil following the use of Safe-Guard Aquasol, as it is assumed that 100% of the administered FBZ is excreted and subsequently land applied unmetabolized. Accordingly, the initial PEC_{soil} is the maximum PEC_{soil} for FBZ.

5.1.1.2 Oxfendazole and Fenbendazole sulfone

In soil, FBZ will degrade to OXF which will further degrade to FBZ-SO_2 (Chapter 4.3.2). Thereby, according to the study conducted in three different soils, 100% FBZ will degrade to a maximum of 64.2% OXF. For FBZ-SO_2 the maximum fraction cannot be derived from Mackie and Ayliffe (2000) as the peak of the FBZ-SO_2 formation was not reached at the end of the study. Therefore it is conservatively assumed that the maximum fraction of FBZ-SO_2 is identical to the maximum fraction of OXF (64.2%). Accordingly, the initial PEC_{soil} for OXF and FBZ-SO_2 are calculated by multiplying the initial PEC_{soil} for FBZ with a factor of 0.642. The initial PEC_{soil} for OXF and FBZ-SO_2 is thus 21.47 µg/kg (33.44 µg/kg x 0.642).

5.1.2 *Plateau PEC_{soil}*

With a DT_{90} >1 year, OXF and FBZ-SO_2 are classified as persistent in soil (Chapter 4.3.2). Accordingly, a plateau PEC_{soil} is calculated to account for the possibility of accumulation in the environment as the application of manure in several successive years could lead to elevated concentrations of the compounds in soil. The plateau PEC_{soil} is calculated considering the respective initial PEC_{soil} based on the European GL in support of the VICH GLs (European Medicines Agency, 2005) according to Equation 5-2 to Equation 5-4:

Equation 5-2

$$PEC_{soil\ 1\ year}[\mu g/kg] = initial PEC_{soil}\ [\mu g/kg] \times e^{\left[\frac{(-\ln 2 \times 365)}{DT_{50}\ [d]}\right]}$$

Equation 5-3

$$Fs\ [n] = \frac{initial\ PEC_{soil}\ [\mu g/kg] - PEC_{soil\ 1\ year}\ [\mu g/kg]}{initial\ PEC_{soil}\ [\mu g/kg]}$$

Equation 5-4

$$Plateau\ PEC_{soil}\ [\mu g/kg] = \frac{initial\ PEC_{soil}\ [\mu g/kg]}{Fs}$$

Where:

$PEC_{soil\ 1\ year}$: PEC_{soil} one year after spreading

initial PEC_{soil}: PEC_{soil} immediately after spreading = 21.47 µg/kg for OXF and FBZ-SO_2 (Chapter 5.1.1.2)

DT_{50}: Disappearance half-life soil = 705.5 d for OXF and 257.5 d for FBZ-SO_2 (Chapter 4.3.2)

F_S: Fraction remaining in soil one year after application

plateau PEC_{soil}: PEC_{soil} at plateau concentration

For OXF as an example, Equation 5-2 becomes:

$$PEC_{soil\ 1\ year}[\mu g/kg] = 21.47\ \mu g/kg \times e^{\left[\frac{(-\ln 2 \times 365)}{705.5\ d}\right]} = 15.00\ \mu g/kg$$

Equation 5-3 becomes:

$$Fs = \frac{21.47\ \mu g/kg - 15.00\ \mu g/kg}{21.47\ \mu g/kg} = 0.30$$

Equation 5-4 becomes:

$$Plateau\ PEC_{soil}\ [\mu g/kg] = \frac{21.47\ \mu g/kg}{0.30} = 71.25\ \mu g/kg$$

The calculation of the plateau PEC_{soil} for OXF and FBZ-SO_2 is summarized in Table 5-1:

Table 5-1: Plateau PEC_{soil} for OXF and FBZ-SO_2

		OXF	FBZ-SO_2
initial PEC_{soil}	µg/kg	21.47	21.47
DT_{50}	d	705.5	257.5
$PEC_{soil\ 1\ year}$	µg/kg	15.00	8.04
F_S	n	0.30	0.63
plateau PEC_{soil}	µg/kg	71.25	34.32
		OXF/FBZ-SO_2	
plateau $PEC_{soil\ combined}$	µg/kg	105.57	

The plateau PEC_{soil} for OXF and FBZ-SO_2 amount to 71.25 µg/kg and 34.32 µg/kg, respectively, the plateau PEC_{soil} for both compounds combined to 105.57 µg/kg.

5.2 Predicted Environmental Concentration in Surface Water ($PEC_{surfacewater}$)

5.2.1 *Initial $PEC_{surfacewater}$*

The initial $PEC_{surfacewater}$ for FBZ and OXF/FBZ-SO_2 is calculated assuming that 1% of the total compound per acre applied to 10 acres of soil moves into a 1 acre (4047 m²) pond which is 2 m deep. This approach is traditionally used in environmental impact assessments of veterinary products. Combing these parameters, the initial $PEC_{surfacewater}$ is calculated for FBZ and OXF/FBZ-SO_2 according to Equation 5-5, considering the initial PEC_{soil} and plateau $PEC_{soil\ combined}$ respectively:

Equation 5-5

$$InititalPEC_{surfacewater}\ [\mu g/L] = PEC_{soil}\ [\mu g/kg] \times 0.011$$

Where:

PEC_{soil}: Initial PEC_{soil} for FBZ = 33.44 µg/kg for FBZ (Chapter 5.1.1.1)

Plateau $PEC_{soil\ combined}$ for OXF/FBZ-SO_2 = 105.57 µg/kg (Chapter 5.1.2)

For FBZ as example, Equation 5-5 becomes:

$$InititalPEC_{surfacewater}\ [\mu g/L] = 33.44\ \mu g/kg \times 0.011 = 0.37 \mu g/L$$

The calculation of the initial PEC_{sw} for FBZ and OXF/FBZ-SO_2 is summarized in Table 5-2:

Table 5-2: Initial PEC_{sw} for FBZ and OXF/FBZ-SO_2

		FBZ	OXF/FBZ-SO_2
PEC_{soil}	µg/kg	33.44	105.57
initial PEC_{sw}	µg/L	0.37	1.16

The initial PEC_{sw} for FBZ and OXF/FBZ-SO_2 amount to 0.37 µg/L and 1.16 µg/L, respectively.

5.2.2 *Refined $PEC_{surfacewater}$*

The refined $PEC_{surfacewater}$ for FBZ and OXF/FBZ-SO_2 is calculated by taking into account the effects of adsorption of the compounds onto the sediment assuming that sediment contains 5% OM and mixing of the compounds in water with the top 5 cm of sediment. This approach is traditionally used in environmental impact assessments of veterinary products. Combing these parameters, the refined $PEC_{surfacewater}$ is calculated for FBZ and OXF/FBZ-SO_2 according to Equation 5-6, considering the initial PEC_{soil} and plateau $PEC_{soil\ combined}$ respectively:

Equation 5-6

$$RefinedPEC_{surfacewater}\ [\mu g/L] = \frac{9.1 \times 10^4 \times PEC_{soil}\ [\mu g/kg]}{8.1 \times 10^6 + (3.0 \times 10^5 \times K_d[L\ /\ kg])}$$

Where:

PEC_{soil}: Initial PEC_{soil} for FBZ = 33.44 µg/kg for FBZ (Chapter 5.1.1.1)

Plateau $PEC_{soil\ combined}$ for OXF/FBZ-SO_2 = 105.57 µg/kg (Chapter 5.1.2)

K_d: Distribution coefficient = 236 L/kg for FBZ and 9 L/kg for OXF (Chapter 4.3.1.4)

For FBZ as example, Equation 5-6 becomes:

$$RefinedPEC_{surfacewater}\ [\mu g/L] = \frac{9.1 \times 10^4 \times 33.44\ [\mu g/kg]}{8.1 \times 10^6 + (3.0 \times 10^5 \times 236[L\ /\ kg])} = 0.039\ \mu g/L$$

The calculation of the refined PEC_{sw} for FBZ and OXF/FBZ-SO_2 is summarized in Table 5-3:

Table 5-3: Refined PEC_{sw} for FBZ and OXF/FBZ-SO_2

		FBZ	OXF/FBZ-SO_2
PEC_{soil}	µg/kg	33.44	105.57
K_d	L/kg	236	9
refined PEC_{sw}	µg/L	0.039	0.89

The refined PEC_{sw} for FBZ and OXF/FBZ-SO_2 amounts to 0.039 µg/L and 0.89 µg/L, respectively.

5.3 Predicted Environmental Concentration in Sediment ($PEC_{sediment}$)

The PEC in sediment ($PEC_{sediment}$) is calculated considering the refined $PEC_{surfacewater}$ according to the European GL in support of the VICH GLs (European Medicines Agency, 2005). Although the refinement of the $PEC_{surfacewater}$ considers adsorption onto the sediment, the use of the refined $PEC_{surfacewater,}$ is justified. According to VICH for compounds with a log K_{OW} <5, exposure of sediment organisms will take place via water rather than sediment (e.g. exposure via ingestion of sediment needs not to be considered if the log K_{OW} is <5). The $PEC_{sediment}$ is calculated according to Equation 5-7 to Equation 5-11:

Equation 5-7

$$Kp_{sed}\ [dm^3/kg] = Foc_{sed}\ [kg/kg] \times K_{OC}[dm^3/kg]$$

Equation 5-8

$$K_{sed-water}\ [m^3/m^3] = F_{water-sed}[m^3/m^3] + F_{solid-sed}[m^3/m^3] \times \frac{Kp_{sed}[dm^3/kg]}{1000} \times RHO_{solid}[kg/m^3]$$

Equation 5-9

$$CONV_{sed}[\mu g/kg] = \frac{RHO_{sed}[kg/m^3]}{F_{solid-sed}[m^3/m^3] \times RHO_{solid}[kg/m^3]}$$

Equation 5-10

$$PEC_{sediment}\ [mg/kg] = \frac{K_{sed-water}[m^3/m^3]}{RHO_{sed}[kg/m^3]} \times refined\ PEC_{surfacewater}\ [mg/L] \times CONV_{sed}[\mu g/kg] \times 1000$$

Equation 5-11

$$PEC_{sediment}\ [\mu g/kg] = PEC_{sediment}\ [mg/kg] \times 1000$$

Where:

F_{OCsed}:	Weight fraction organic carbon sediment= 0.05 kg/kg
K_{OC}:	Adsorption/desorption partition co-efficient normalized to the organic carbon content of soil = 14459 dm³/kg for FBZ and 887 dm³/kg for OXF (Chapter 4.3.1.4)
Kp_{sed}:	Partitioning co-efficient solids/water in sediment
$F_{water-sed}$:	Fraction water in sediment = 0.8 m³/m³
$F_{solid-sed}$:	Fraction solids in sediment = 0.2 m³/m³
RHO_{solid}:	Bulk density solids = 2500 kg/m³
$K_{sed-water}$:	Partitioning co-efficient sediment/water
RHO_{sed}:	Bulk density sediment = 1300 kg/m³
$CONV_{sed}$:	Conversion factor sediment
refined $PEC_{surfacewater}$:	$PEC_{surfacewater}$ after refinement = 0.000039 mg/L for FBZ and 0.00089 mg/L for OXF/FBZ-SO_2 (Chapter 5.2.2)

For FBZ as example, Equation 5-7 becomes:

$$Kp_{sed} = 0.05\ kg/kg \times 14459\ dm^3/kg = 723\ dm^3/kg$$

Equation 5-8 becomes:

$$K_{sed-water} = 0.8\ m^3/m^3 + 0.2\ m^3/m^3 \times \frac{723\ dm^3/kg}{1000} \times 2500\ kg/m^3 = 362\ m^3/m^3$$

Equation 5-9 becomes:

$$CONV_{sed} = \frac{1300\ kg/m^3}{0.2\ m^3/m^3 \times 2500\ kg/m^3} = 2.6\ \mu g/kg$$

Equation 5-10 becomes:

$$PEC_{sediment} = \frac{362\ m^3/m^3}{1300\ kg/m^3} \times 0.000039\ mg/L \times 2.6\ \mu g/kg \times 1000 = 0.02795\ mg/kg$$

Equation 5-11 becomes:

$$PEC_{sediment} = 0.02795\ mg/kg \times 1000 = 27.95\ \mu g/kg$$

The calculation of the $PEC_{sediment}$ for FBZ and OXF/FBZ-SO_2 is summarized in Table 5-4:

Table 5-4: $PEC_{sediment}$ for FBZ and OXF/FBZ-SO_2

		FBZ	OXF/FBZ-SO_2
F_{OCsed}	kg/kg	0.05	0.05
K_{OC}	dm^3/kg	14459	887 [a]
Kp_{sed}	dm^3/kg	723	44
$F_{water-sed}$	m^3/m^3	0.8	0.8
$F_{solid-sed}$	m^3/m^3	0.2	0.2
RHO_{solid}	kg/m^3	2500	2500
$K_{sed-water}$	m^3/m^3	362	23
RHO_{sed}	kg/m^3	1300	1300
$CONV_{sed}$	µg/kg	2.6	2.6
refined $PEC_{surfacewater}$	mg/L	0.000039	0.00089
$PEC_{sediment}$	mg/kg	0.02795	0.04101
$PEC_{sediment}$	µg/kg	27.95	41.01

[a] – Value is for OXF.

The $PEC_{sediment}$ for FBZ and OXF/FBZ-SO_2 amount to 27.95 µg/kg and 41.01 µg/kg, respectively.

5.4 Predicted Environmental Concentration in Groundwater ($PEC_{groundwater}$)

The $PEC_{groundwater}$ is calculated using US EPAs SCI-GROW modeling software (version 2.3). SCI-GROW is a screening model to estimate pesticide concentrations in vulnerable groundwater. The concentrations estimated by SCI-GROW represent conservative or high-end exposure values because the model is based on groundwater monitoring studies which were

conducted by applying pesticides at maximum allowed rates and frequency to vulnerable sites (i.e., shallow aquifers, sandy-permeable soils, and substantial rainfall and/or irrigation to maximize leaching). In most cases, a large majority of the use areas will have groundwater that is less vulnerable to contamination than the areas used to derive the SCI-GROW estimate. For this reason, SCI-GROW provides conservative estimates.

Next to compound specific environmental fate properties (soil K_{OC} and DT_{50}), the application rate needs to be provided for calculations. The application rate for FBZ and OXF/FBZ-SO_2 is calculated according to Equation 5-12, considering the initial PEC_{soil} and plateau $PEC_{soil\ combined}$, respectively:

Equation 5-12

$$AR[lb/acre] = \frac{PEC_{soil}[\mu g/kg] \times W_{soil}[kg/acre]}{10^9 \times CF[n]}$$

Where:

PEC_{soil}: Initial PEC_{soil} for FBZ = 33.44 µg/kg for FBZ (Chapter 5.1.1.1)

Plateau $PEC_{soil\ combined}$ for OXF/FBZ-SO_2 = 105.57 µg/kg (Chapter 5.1.2)

W_{soil}: Weight of soil = 910500 kg/acre (value traditionally used in environmental impact assessments of veterinary products)

CF: Conversion factor kg – lb = 0.45359237

For FBZ as example, Equation 5-12 becomes:

$$AR = \frac{33.44\,\mu g/kg \times 910500\,kg/acre}{10^9 \times 0.45359237} = 0.067\,lb/acre$$

The calculation of the application rate for FBZ and OXF/FBZ-SO_2 is summarized in Table 5-5:

Table 5-5: Application rate for FBZ and OXF/FBZ-SO_2

		FBZ	OXF/FBZ-SO_2
CF	n	0.45359237	0.45359237
W_{soil}	kg/acre	910500	910500
PEC_{soil}	µg/kg	33.44	105.57
AR	lb/acre	0.067	0.212

The application rates for FBZ and OXF/FBZ-SO_2 amount to 0.067 lb/acre and 0.212 lb/acre, respectively.

For the SCI-GROW calculations it is conservatively assumed that the complete amount of manure allowed for annual land application is applied in a single event (number of applications

is thus 1). In case manure is land applied at two or more occasions, the resulting $PEC_{groundwater}$ will be lower.

The SCI-GROW input parameters and resulting $PEC_{groundwater}$ are summarized in Table 5-6. The SCI-GROW calculations are presented in Appendix 14.4.

Table 5-6: SCI-GROW input parameters and estimated ground water concentrations for FBZ and OXF/FBZ-SO_2

		FBZ		OXF/FBZ-SO_2	
Application rate	lb/acre	0.067	(Table 5-5)	0.212	(Table 5-5)
Number of applications	n	1		1	
Soil K_{OC} (median)	L/kg	16136	(Table 4-6)	546 [a]	(Table 4-6)
Aerobic soil DT_{50} (mean)	d	9.5	(Table 4-10)	382.7 [a]	(Table 4-10)
$PEC_{groundwater}$	µg/L	0.0004	(Appendix 14.4)	0.116	(Appendix 14.4)

[a] – Values are for OXF

The $PEC_{groundwater}$ for FBZ and OXF/FBZ-SO_2 amount to 0.0004 µg/L and 0.116 µg/L, respectively.

As the $PEC_{groundwater}$ is lower than the refined $PEC_{surfacewater}$ (Chapter 5.2.2), the $PEC_{groundwater}$ will not be considered in the environmental impact assessment.

5.5 Summary of predicted environmental concentrations

PEC values for different compartments for FBZ and OXF/FBZ-SO_2 used in the risk characterization are summarized in Table 5-7.

Table 5-7 PEC values for different compartments for FBZ and OXF/FBZ-SO_2

		FBZ	Source	OXF/FBZ-SO_2	Source
initial PEC_{soil}	µg/kg	33.44	Equation 5-1	21.47 [a]	Chapter 5.1.1.2
plateau $PEC_{soil\ combined}$	µg/kg	n.d.	-	105.57	Table 5-1
initial PEC_{sw}	µg/L	0.37	Table 5-2	1.16	Table 5-2
refined PEC_{sw}	µg/L	0.039	Table 5-3	0.89	Table 5-3
$PEC_{sediment}$	µg/kg	27.95	Table 5-4	41.01	Table 5-4
$PEC_{groundwater}$	µg/L	0.0004	Table 5-6	0.116	Table 5-6

n.d. – not determined
[a] – for both OXF and FBZ-SO_2 the initial PEC_{soil} amounts to 21.47 µg/kg.

6. Effects Assessment

Safe-Guard Aquasol is another formulation of the approved to Safe-Guard 20%. Accordingly, for FBZ the toxicology to non-target organisms was principally assessed based on existing information including publications (partly included in the EA for Safe-Guard 20%). In case toxicity was considered to be investigated according to acceptable standards, new studies were not initiated. In contrast, the primary transformation product in soil, OXF, was not considered in the EA for Safe-Guard 20%. Accordingly, a set of acute and chronic effect studies according to OECD GLs was conducted to assess the toxicity of OXF to non-target organisms, except for fish. For fish available information were considered in order to avoid testing of a vertebrate species. FBZ-SO_2 is considered to be a secondary metabolite of FBZ. As such, FBZ-SO_2 is considered to be as toxic as OXF (the primary metabolite of FBZ) and not as toxic as FBZ. Accordingly, effects data were not collected or generated for FBZ-SO_2 as they were for OXF and FBZ (see Figure 1-1).

Internal studies are generally rated valid if they are conducted according to OECD protocols as requested in VICH GL 38. Studies are also rated as valid when they were not performed according to OECD GL if they were completed before implementation of VICH GL 38 and if they were conducted according to other test GL in place at this time, such as FDA TAD and EPA GLs.

6.1 Toxicity to Terrestrial Organisms

6.1.1 *Soil microorganisms*

6.1.1.1 Fenbendazole

In an early development phase an antimicrobial screening study was performed (Anonymous, 1976). The antimicrobial activity of FBZ against a number of microorganisms was investigated including gram positive aerobic bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus faecium*), gram negative aerobic bacteria (*Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*) and mycoplasma (*Mycoplasma gallisepticum*). Anaerobic bacteria were also tested as follows: Several strains of *Bacteroides fragilis*, *Bacteroides ovatus*, *Bacteroides thetajotaornicron*, *Sphaerophorus varius*, *Sphaerophorus freundii*, *Peptococcus anaerobius*, *Peptococcus variabilis*, *Peptostreptococcus anaerobius*, *Peptostreptococcus variabilis*, *Propionibacterium acnes* as well as several clostridia strains including *Clostridium perfringens* and *Clostridium septicum*. The test method was a bacteriostatic (growth inhibition) test. The minimum inhibitory concentration was determined after an incubation of 18 h at 37 °C. The highest tested concentration of FBZ was 100 µg/mL (100 mg/L) agar. No antibacterial effect could be found against any of the tested bacteria. Because FBZ is not intended to be used as an antimicrobial, a study to determine the effects of FBZ on soil microorganisms was not conducted; however, the results of the MIC study suggest that minimal impacts are expected on exposed soil microorganisms.

6.1.1.2 Oxfendazole

For OXF, effects on the nitrogen transformation activity of soil microorganisms were investigated by Twilley and Schaefer (2013) according to OECD GL 216 and GLP. Test systems were dosed with OXF at calculated mean concentrations of 0, 101 and 1000 mg/kg dry soil, and incubated at approximately 20 ºC for 28 d. Soil samples were collected on d 0, 7, 14 and 28, and analyzed to determine nitrate concentrations. After 28 d, soils treated with OXF at 101 mg/kg dry soil showed an inhibition of nitrate formation of 18.6% and soils treated at 1000 mg/kg dry soil showed an inhibition of nitrate formation of 21.4%, compared with untreated controls. As these values are <25% there were no long-term adverse effects caused by OXF on the nitrogen transformation activity of microorganisms in soil, per OECD 216.

6.1.2 ***Terrestrial Plants***

6.1.2.1 Fenbendazole

For FBZ, effects on terrestrial plants were investigated by Hoberg and Deetz (1995a, 1995b). The effects on seed germination and root elongation were investigated according to FDA TAD 4.06 and GLP (Hoberg and Deetz, 1995a). Six plant species, three Dicotyledonae and three Monocotyledonae, were investigated as follows: *Zea mays* (corn), *Triticum aestivum* (wheat), and *Lolium perenne* (ryegrass) as monocotyledon species and *Glycine max* (soybean), *Cucumis sativus* (cucumber), and *Lycopersicon esculentum* (tomato) as dicotyledon species. Plants were exposed to FBZ of measured concentrations of 61, 110, 240, 480, and 970 µg/L for corn, cucumber and perennial ryegrass, of 0.36, 3.6, 36, 61, 150, 310, 530 and 1000 µg/L for soybean and tomato, and of 61, 150, 310, 530 and 1000 mg/L for wheat. The exposure period was 5 d for corn, cucumber, perennial ryegrass and wheat and 6 d for tomato and soybean. Temperatures ranged from 21-24 °C during the testing with corn, cucumber and perennial ryegrass, and from 21-23 °C for the tests with soybean, tomato and wheat. Based on the lack of observed effects for all species, it was determined that percent germination and root elongation were unaffected by the exposure to FBZ at a measured concentrations as high as 970 mg/L for corn, cucumber and perennial ryegrass and 1000 mg/L for soybean, tomato and wheat.

The effects on survival, shoot length, shoot weight and root weight were investigated according to FDA TAD 4.07 and GLP (Hoberg and Deetz, 1995b). Six plant species, three Dicotyledonae and three Monocotyledonae, were investigated as follows: *Zea mays* (corn), *Triticum aestivum* (wheat), and *Lolium perenne* (ryegrass) as monocotyledon species and *Glycine max* (soybean), *Cucumis sativus* (cucumber), and *Lycopersicon esculentum* (tomato) as dicotyledon species. Plants were exposed to FBZ of measured concentrations of 36, 64, 150, 360, 810 and 1600 mg/kg for 21 d. Temperatures ranged from 22-26 °C during the testing with call species. Based on the endpoints monitored during this study (e.g., survival, shoot length, shoot and root weight) for the six plant species tested, only tomato demonstrated sensitivity to FBZ. Tomato survival was not affected over the range of concentrations tested, while shoot length was significantly reduced at the highest concentration tested, 1600 mg/kg. Tomato shoot weight was significantly reduced at the two highest concentrations tested, 810 and 1600 mg/kg. Tomato root weight was determined to be the most sensitive parameter for this species and within this study. The NOEC value established for tomato root weight was 36 mg/kg. The respective EC_{50} can be given with >36 mg/kg.

Hoberg and Deetz (1995a, 1995b) investigated the unmodified form of FBZ. However, because the PC properties are similar for modified and unmodified FBZ (Chapter 4.2) and the unmodified FBZ was tested in the soluble form, it is expected that the modified FBZ acts similarly. Therefore, the studies conducted with unmodified FBZ can be used to assess the effects on terrestrial plants of its modified form.

The results of the studies from Hoberg and Deetz (1995a, 1995b) are summarized in Table 6-1:

Table 6-1: Effects of FBZ on terrestrial plants

Species	Endpoint	Test duration	NOEC	Reference
Zea mays (corn)	Germination	5 d	970 mg/L	Hoberg and Deetz (1995a)
	Root elongation	5 d	970 mg/L	Hoberg and Deetz (1995a)
	Survival	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot length	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot weight	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Root weight	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
Triticum aestivum (wheat)	Germination	5 d	1000 mg/L	Hoberg and Deetz (1995a)
	Root elongation	5 d	1000 mg/L	Hoberg and Deetz (1995a)
	Survival	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot length	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot weight	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Root weight	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
Lolium perenne (ryegrass)	Germination	5 d	970 mg/L	Hoberg and Deetz (1995a)
	Root elongation	5 d	970 mg/L	Hoberg and Deetz (1995a)
	Survival	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot length	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot weight	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Root weight	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
Glycine max (soybean)	Germination	6 d	1000 mg/L	Hoberg and Deetz (1995a)
	Root elongation	6 d	1000 mg/L	Hoberg and Deetz (1995a)
	Survival	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot length	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot weight	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Root weight	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
Cucumis sativus (cucumber)	Germination	5 d	970 mg/L	Hoberg and Deetz (1995a)
	Root elongation	5 d	970 mg/L	Hoberg and Deetz (1995a)
	Survival	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot length	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot weight	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Root weight	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
Lycopersicon esculentum (tomato)	Germination	6 d	1000 mg/L	Hoberg and Deetz (1995a)
	Root elongation	6 d	1000 mg/L	Hoberg and Deetz (1995a)
	Survival	21 d	1600 mg/kg	Hoberg and Deetz (1995b)
	Shoot length	21 d	810 mg/kg	Hoberg and Deetz (1995b)
	Shoot weight	21 d	360 mg/kg	Hoberg and Deetz (1995b)
	Root weight	21 d	36 mg/kg	Hoberg and Deetz (1995b)

6.1.2.2 Oxfendazole

For OXF, effects on terrestrial plants were investigated by Sindermann et al. (2013a) according to OECD GL 208 and GLP. Six plant species, four Dicotyledonae and two Monocotyledonae, encompassing six families were investigated as follows: *Allium cepa* (onion) and *Lolium perenne* (ryegrass) as monocotyledon species and *Brassica rapa* (turnip), *Cucumis sativus* (cucumber), *Lactuca sativa* (lettuce), and *Lycopersicon esculentum* (tomato) as dicotyledon species. Plants were exposed to OXF of nominal concentrations of 0 (negative and solvent controls), 0.51, 1.3, 3.2, 8.0 and 20 mg/kg dry soil. Soil incorporation of OXF at a concentration of up to 20 mg/kg resulted in no adverse effects on seedling emergence, survival, height and dry weight for the six species tested. The NOEC and LOEC for all endpoints were determined to be 20 mg/kg and >20 mg/kg, respectively. No treatment group reductions in emergence, survival, height and dry weight of 50% or greater relative to control means were observed in any species; therefore, the EC_{50} was >20 mg/kg, the highest concentration tested. The results of the study are summarized in Table 6-2:

Table 6-2: Effects of OXF on terrestrial plants (Sindermann et al., 2013a)

Species	Endpoint	EC_{50}	NOEC
Allium cepa (onion)	Survival	>20 mg/kg	20 mg/kg
	Seedling emergence	>20 mg/kg	20 mg/kg
	Height	>20 mg/kg	20 mg/kg
	Dry weight	>20 mg/kg	20 mg/kg
Lolium perenne (ryegrass)	Survival	>20 mg/kg	20 mg/kg
	Seedling emergence	>20 mg/kg	20 mg/kg
	Height	>20 mg/kg	20 mg/kg
	Dry weight	>20 mg/kg	20 mg/kg
Brassica rapa (turnip)	Survival	>20 mg/kg	20 mg/kg
	Seedling emergence	>20 mg/kg	20 mg/kg
	Height	>20 mg/kg	20 mg/kg
	Dry weight	>20 mg/kg	20 mg/kg
Cucumis sativus (cucumber)	Survival	>20 mg/kg	20 mg/kg
	Seedling emergence	>20 mg/kg	20 mg/kg
	Height	>20 mg/kg	20 mg/kg
	Dry weight	>20 mg/kg	20 mg/kg
Lactuca sativa (lettuce)	Survival	>20 mg/kg	20 mg/kg
	Seedling emergence	>20 mg/kg	20 mg/kg
	Height	>20 mg/kg	20 mg/kg
	Dry weight	>20 mg/kg	20 mg/kg
Lycopersicon esculentum (tomato)	Survival	>20 mg/kg	20 mg/kg
	Seedling emergence	>20 mg/kg	20 mg/kg
	Height	>20 mg/kg	20 mg/kg
	Dry weight	>20 mg/kg	20 mg/kg

6.1.3 *Earthworm*

6.1.3.1 Fenbendazole

For FBZ, effects on earthworm were investigated by Garvey and Deetz (1995) according to FDA TAD 4.12 and GLP. Adults of *Lumbricus terrestris* were exposed for 28 d to measured test concentrations of 56, 120, 240, 500 and 960 mg/kg and then removed to evaluate mortality and body weights. The 28 d LC_{50} based on measured concentrations was calculated to be 180 mg/kg. The LOEC was determined to be 120 mg/kg, based on survival at termination (d 28). The corresponding NOEC was 56 mg/kg. Garvey and Deetz (1995) investigated the unmodified form of FBZ. The results of the study are summarized in Table 6-3:

Table 6-3: Effects of OXF on earthworm (Garvey and Deetz, 1995)

Species	Endpoint	LC_{50}	NOEC
Lumbricus terrestris	Mortality	180 mg/kg	56 mg/kg

6.1.3.2 Oxfendazole

For OXF, effects on earthworm reproduction were investigated by Sindermann et al. (2013b) according to OECD GL 222 and GLP. Adults of *Eisenia fetida* were exposed for 28 d to nominal test concentrations of 0.094, 0.188, 0.375, 0.75, 1.5, 3, 6 and 12 mg/kg dry soil and then removed to evaluate mortality and body weights. The cocoons and the soil were returned to test chambers for another 28 d to evaluate effects upon reproductive output (number of juveniles at test termination). There was less than 50% mortality of adult earthworms exposed to OXF up to a nominal concentrations of 12 mg/kg dry soil, therefore the LC_{50} for adult mortality was determined to be greater than 12 mg/kg dry soil. When compared with the pooled control group, there were no statistically significant adverse effects upon earthworm weight in the treatment groups during the adult exposure period. Therefore, based on body weight data, the LOEC was determined to be >12 mg/kg and the NOEC 12 mg/kg. No treatment group mean number of juveniles was 50% reduced from the controls therefore the EC_{50} for reproduction was greater than 12 mg/kg, the highest test level. There were no statistically significant (p>0.05) effects on the numbers of juveniles produced the treatment groups, therefore, the NOEC for reproduction was determined to be 12 mg/kg dry soil. The results of the study are summarized in Table 6-4:

Table 6-4: Effects of OXF on earthworm (Sindermann et al., 2013b)

Species	Endpoint	LC_{50}, EC_{50}	NOEC
Eisenia fetida	Adult mortality	>12 mg/kg	-
	Body weight	-	12 mg/kg
	Reproduction	>12 mg/kg	12 mg/kg

6.1.4 *Dung Breeding Insects*

The exposure of dung breeding insects to FBZ residues in porcine feces is very unlikely as swine are typically held in enclosed buildings (not pasture) (U.S. EPA, 2012a), swine manure is typically held in a liquid form (slurry) (U.S. EPA, 2012b), and slurry is typically injected into the ground while application to agricultural land (U.S. EPA, 2012b). Accordingly, land applied porcine excreta will not provide a suitable breeding ground for dung breeding insects. This is in line with VICH, which requests to assess effects to dung breeding insects only for those parasiticides which are used in pasture reared animals.

6.1.5 *Summary Toxicity to Terrestrial Organisms*

The results of toxicity tests conducted with terrestrial organisms are summarized in Table 6-5. For simplicity, the most conservative (lowest) NOEC, LC_{50} or EC_{50} values are presented only. If available, values are presented based on measured test concentrations.

Table 6-5: Toxicity tests conducted with terrestrial organisms

Test system	Compound	Endpoint	Reference
Terrestrial plants	FBZ [1]	EC_{50}: >36 mg/kg	Hoberg and Deetz (1995b)
		NOEC: 36 mg/kg	Hoberg and Deetz (1995b)
	OXF [2]	EC_{50}: >20 mg/kg	Sindermann et al. (2013a)
		NOEC: 20 mg/kg	Sindermann et al. (2013a)
Earthworm	FBZ	LC_{50}: 180 mg/kg	Garvey and Deetz (1995)
		NOEC: 56 mg/kg	Garvey and Deetz (1995)
	OXF	EC_{50}: >12 mg/kg	Sindermann et al. (2013b)
		NOEC: 12 mg/kg	Sindermann et al. (2013b)

[1] - Representative, most sensitive species: Tomato.
[2] – All tested species (onion, ryegrass, turnip, cucumber, lettuce, and tomato) were equally insensitive.

For effects on soil microorganisms, according to VICH no endpoint values need to be derived for the risk assessment.

6.2 Toxicity to Aquatic Organisms

6.2.1 *Algal Growth Inhibition*

6.2.1.1 Fenbendazole

For FBZ, effects on algal growth were not investigated. As demonstrated by Connor and Detz (1995), FBZ is subject to rapid photolysis in natural bodies of water. The photolytic processes will take place in a wavelength range greater than 290 nm (as tested by the use of a filter that limits the UV radiation). Consequently, an algal toxicity study regarding OECD GL 201 would be impractical. Following OECD GL 201, the algae toxicity test should be performed with continuous, uniform fluorescent illumination in the photosynthetically effective wavelength range of 400 to 700 nm. Consequently, the photolytic degradation of FBZ during the test period of 72 h cannot be avoided. Considering the low toxicity of OXF to green algae (Chapter 6.2.1.2), and

the low toxicity of both, FBZ and OXF, to terrestrial plants (Chapter 6.1.2), toxicity of FBZ to green algae is unlikely.

6.2.1.2 Oxfendazole

For OXF, effects on algal growth were investigated by Arnie et al. (2012) according to OECD GL 201 and GLP. The green alga, *Pseudokirchneriella subcapitata*, was exposed to five test concentrations, negative control (culture medium) and a solvent control for 72 h. Nominal concentrations selected for use in this study were 0.10, 0.26, 0.64, 1.6 and 4.0 mg/L. Measured concentrations on Day 0 ranged from 97 to 104% of the target nominal concentrations. Measured concentrations declined slightly over the 72 h exposure period as the measured concentrations on Day 3 ranged from 77 to 89% of nominal. The results of the study are based on the measured test concentrations. Effects were evaluated based on cell density, yield and growth rate. The 72 h EC_{50} values were unable to be calculated due to lack of significant effects and were empirically estimated to be greater than the highest concentration tested (4.0 mg/L). The 72 h NOEC, based on effects on cell density, growth rate and yield was determined to be greater than or equal to 4.0 mg/L. Considering the mean measured concentrations, the respective endpoint values are >3.7 mg/L for EC_{50} and 3.7 mg/L for NOEC. The results of the study are summarized in Table 6-6:

Table 6-6: Effects of OXF on algal growth based on measured concentrations (Arnie et al., 2012)

Species	Endpoint	EC_{50}	NOEC
Pseudokirchneriella subcapitata	Cell density	>3.7 mg/L	3.7 mg/L
	Yield	>3.7 mg/L	3.7 mg/L
	Growth rate	>3.7 mg/L	3.7 mg/L

6.2.2 *Toxicity to Aquatic Invertebrate*

6.2.2.1 Fenbendazole

For FBZ, acute effects on aquatic invertebrates were investigated by Meller and Zenide (2003) according to OECD GL 202 and GLP. The cladoceran *D. magna* was exposed to five test concentrations and negative control (culture medium) for 48 h under static conditions. Nominal concentrations selected for use in this study were 3.5, 7.8, 17.1, 37.6, 82.6, 181.8 and 400 µg/L. The analytically determined concentrations revealed an overall recovery based on the results of the initial measured concentration of 6.08%. Since it could be demonstrated that the test item concentrations maintained within ± 20% of the measured initial concentrations throughout the test, the effect concentrations were based on the measured initial concentrations. Therefore the effect concentrations were corrected by the factor of 0.0608. The 48 h EC_{50} value was determined from the immobility data and was 8.8 µg/L.

Meller and Zenide (2003) investigated the unmodified form of FBZ. However, because the PC properties are similar for modified and unmodified FBZ (Chapter 4.2) and the unmodified FBZ was tested in the soluble form, it is expected that the modified FBZ acts similarly. Therefore, the study conducted with unmodified FBZ can be used to assess the effects on aquatic invertebrates of its modified form.

For FBZ, chronic effects on aquatic invertebrates were investigated by Egeler et al. (2013) according to OECD GL 211 and GLP, using modified FBZ. The cladoceran *D. magna* was exposed to five test concentrations and negative control (medium and solvent) for 21 d under static-renewal conditions. Nominal concentrations selected for use in this study were 0.19, 0.38, 0.75, 1.5 and 3.0 µg/L. Test solutions were renewed every 2-3 d. Time-weighted mean (TWM) measured test concentrations were determined from samples of test water collected from each treatment and control group at test initiation, at the beginning and end of each renewal cycle, and at test termination. For the three highest nominal test concentrations (0.75, 1.5 and 3.0 µg/L), the TWM measured test concentrations were 0.52, 1.13 and 2.21 µg/L, which represented 69.08, 75.57 and 73.64% of the nominal concentrations, respectively. The two lowest test concentrations (0.19 and 0.38 µg/L) were below nominal values. However, TWM measured test concentrations could not be calculated due to the absence of detectable FBZ in individual samples of the used solutions. The study results were based on TWM measured test concentrations. There were no statistically significant treatment-related effects on immobility of parent daphnids, age at first reproduction, and length of parental daphnids. The reproduction assessed as cumulative number of living offspring per parent animal alive at the end of the test was slightly inhibited at 2.21 µg/L (TWM measured test concentration) compared to the control animals (Table 6-7). Thus the overall NOEC, based on reproduction, was 1.13 µg/L and the LOEC was 2.21 µg/L.

Table 6-7: Mean cumulative number of living offspring per surviving adult *Daphnia magna* exposed to FBZ for 21 days

TWM measured test concentrations	Mean no. neonates per surviving adult ± standard deviation	Percent of control
Negative control	122.1 ± 16.3	96.5
Solvent control	126.5 ± 17.7	-
Tween-80 control	128.5 ± 11.3	101.6
0.19 [a]	120.7 ± 15.9	93.9
0.38 [a]	119.9 ± 20.3	99.3
0.52	119.9 ± 16.8	100.0
1.13	121.8 ± 23.1	101.6
2.21	105.9 ± 21.0 *	86.9

[a] – Time weighted means (TWMs) could not be calculated due to the absence of detectable FBZ in the used solutions. Therefore, nominal concentrations are reported.
* - Indicates a statistical difference when compared to solvent controls (Williams multiple sequential t-test, $p<0.05$).

The results of the FBZ studies are summarized in Table 6-8:

Table 6-8: Effects of FBZ on *Daphnia magna* based on measured concentrations

Endpoint	EC_{50}	LOEC	NOEC	Reference
Immobility	48 h: 8.8 µg/L	n.a.	n.a.	Meller and Zenide (2003)
Immobility adults	n.a.	21 d: >2.21 µg/L	21 d: 2.21 µg/L	Egeler et al. (2013)
Length adults	n.a.	21 d: >2.21 µg/L	21 d: 2.21 µg/L	Egeler et al. (2013)
Age 1st reproduction	n.a.	21 d: >2.21 µg/L	21 d: 2.21 µg/L	Egeler et al. (2013)
Reproduction [1]	n.a.	21 d: 2.21 µg/L	21 d: 1.13 µg/L	Egeler et al. (2013)

n.a. – Not applicable.
[1] - Assessed as cumulative number of living offspring per parent animal alive at the end of the test.

6.2.2.2 Oxfendazole

For OXF, acute effects on aquatic invertebrates were investigated by Brougher et al. (2013) according to OECD GL 202 and GLP. The cladoceran *D. magna* was exposed to five test concentrations and negative control (culture medium) for 48 h under static conditions. Nominal concentrations selected for use in this study were 0.047, 0.094, 0.19, 0.38 and 0.75 mg/L. Measured concentrations of the samples ranged from approximately 87 to 116% of nominal. The mean measured test concentrations for this study were 0.045, 0.10, 0.19, 0.35 and 0.70 mg/L. The results of the study are based on the nominal test concentrations. Daphnia in the negative control appeared normal throughout the test, with no immobility or overt signs of toxicity observed. Percent immobility at test termination in the 0.047, 0.094, 0.19, 0.38 and 0.75 mg/L treatment groups was 20, 95, 100, 100 and 100%, respectively. Signs of toxicity observed in all treatment groups included lethargy. The 48 h EC_{50} value was determined from the immobility data and was 0.059 mg/L. The NOEC was <0.047 mg/L.

For OXF, chronic effects on aquatic invertebrates were investigated by Minderhout et al. (2013) according to OECD GL 211 and GLP. The cladoceran *D. magna* was exposed to five test concentrations and negative control (dilution water) for 21 d under static-renewal conditions. Nominal concentrations selected for use in this study were 6.3, 13, 25, 50 and 100 µg/L. Test solutions were renewed every 2-3 d. TWM measured test concentrations were determined from samples of test water collected from each treatment and control group at test initiation, at the beginning and end of each renewal cycle each week (except the new solutions on d 3 of the test), and at test termination. TWM measured test concentrations were 5.9, 12, 23, 45 and 90 µg/L, which represented 93, 90, 90, 89 and 90% of the nominal concentrations, respectively. The study results were based on TWM measured test concentrations. There were no statistically significant treatment-related effects on survival or growth, measured as length and dry weight. Daphnids exposed to OXF at concentrations ≥45 µg/L had statistically significant reductions in reproduction in comparison to the negative control (Table 6-9). Consequently, the NOEC, based on reproduction, was 23 µg/L and the LOEC was 45 µg/L. The 21 d EC_{50} values for adult immobility and reproduction were both >90 µg/L, the highest concentration tested.

Table 6-9: Mean cumulative number of living offspring per surviving adult *Daphnia magna* exposed to OXF for 21 days

TWM measured test concentrations	Mean no. neonates per surviving adult ± standard deviation	Percent of control
Negative control	268 ±19	-
5.9	263 ± 20	97.8
12	256 ± 17	95.2
23	266 ± 13	97.4
45	242 ± 18 *	90.0
90	214 ± 31 *	79.6

* - Indicates a statistically significant decrease in reproduction in comparison to the negative control (Dunnett's one-tailed test, p≤0.05).

Based on the findings of the 48-h acute *D. magna* study, immobilities should have been expected in the 21-d chronic study at similar exposure concentrations with the resulting 21-d EC_{50} being lower than the 48-h EC_{50}. However, the 21-d EC_{50} reported from the chronic study was higher than the 48-h EC_{50}, likely due to the fact that daphnids were fed during the 21-d chronic study whereas they were not fed during the 48-h acute study. This relationship of increased survival in the presence of food was demonstrated in a non-GLP trial to compare the survival of neonate daphnids with and without feeding during exposure to OXF (Appendix 14.5). In addition, the chronic *D. magna* study conducted by Minderhout et al. (2013) deviated from OECD GL 211 in that *D. magna* were fed 0.6 mg C/daphnia/d. This feeding rate is more than three-times the recommended range of 0.1 to 0.2 mg C/daphnia/d and may have resulted in greater survival and reproductive output, potentially resulting in a higher reported NOEC. Although the control and treated *D. magna* in the 21-d study were fed at the same rate, the exact impact of the 3x feeding rate over the 1x feeding rate on reproductive output is not known.

The results of the OXF studies are summarized in Table 6-10:

Table 6-10: Effects of OXF on *Daphnia magna* based on measured concentrations

Endpoint	EC_{50}	NOEC	Reference
Immobility	48 h: 0.059 mg/L	48 h: <0.047 mg/L	Brougher et al. (2013)
	21 d: >90 µg/L	21 d: 90 µg/L	Minderhout et al. (2013)
Reproduction	21 d: >90 µg/L	21 d: 23 µg/L	Minderhout et al. (2013)

6.2.3 *Toxicity to Fish*

6.2.3.1 Fenbendazole

For FBZ, effects on fish were investigated by Wilson and LeBlanc (1981) according to internal procedures established in the conducting laboratory. This study followed in principle OECD GL 204 (Fish Prolonged Toxicity Test*),* which is not a chronic test but an acute one used in place of OECD GL 203 (Fish Acute Toxicity Test) (requested by VICH), when a longer observation period is considered useful for measurement of lethal and other observed effects in fish. Bluegill sunfish *(Lepomis macrochirus)* were exposed to five test concentrations and a negative control

and a solvent control for 21 d under flow-through conditions. Nominal concentrations selected for use in this study were 0.005, 0.01, 0.02, 0.04 and 0.08 mg/L. FBZ concentrations were measured on days 0, 7, 14 and 21. There were no effects on survival through d 7. Adverse effects were noted by d 8 in the 0.04 and 0.08 mg/L treatments, with 30 and 20% mortality, respectively. The highest mortality occurred between d 8 and 12, with relatively few deaths after d 12. By d 14, there was 70% and 100% mortality in the 0.04 and 0.08 mg/L treatments. By d 21, mortalities reached 95% and 100%, respectively. There were also fish in the 0.40 mg/L treatment that were light colored or had deteriorating caudal fins during on/after d 14. The 21 d LC_{50}, based on nominal concentration (estimated by the moving average method) was determined to be 0.028 mg/L. The results of this study suggest that it could take up to seven days for FBZ to come to equilibrium in the carcass tissue, resulting in a delay in toxic effects (i.e., latent toxicity). Due to the nature of FBZ (i.e., very low solubility), an exposure period greater than 96-h is needed to see acute effects and the prolonged acute toxicity test is more appropriate for determining potential toxicity of FBZ to fish.

FBZ concentrations were measured on d 0, 7, 14 and 21. Many measured concentrations were above the saturation point (0.01 mg/L) indicating that FBZ was present as dissolved, fine particulate (<0.45 µm), and coarse particulate (>0.45 µm). Samples from all time points were filtered through a 0.22 micron filter prior to analysis to remove particulate FBZ and accurately measure dissolved FBZ concentrations; however, several samples from days 0, 7, and 21 could not be analyzed because of insufficient sample volume. Therefore, only data from day 14 were used to report filtered water concentrations. Accordingly, the LC_{50} is based on concentrations measured at a single time point in the middle of the test only. The measured concentrations of the filtered samples are presented in Table 6-11:

Table 6-11: Measured FBZ concentrations in filtered d 14 water samples (Wilson and LeBlanc, 1981)

Nominal concentration	Measured concentration	Recovery	Average recovery
0.08 mg/L	0.021 mg/L	26%	32%
0.04 mg/L	0.01 mg/L	25%	
0.01 mg/L	0.0044 mg/L	44%	

The overall average recovery based on the results of the measured d 14 concentration was 32%. In order to reflect the toxicity of the soluble FBZ fraction the effect concentrations is corrected by the factor of 0.32. The corrected 21 d LC_{50} is thus 0.009 mg/L (0.028 mg/L x 0.32).

Wilson and LeBlanc (1981) investigated the unmodified form of FBZ. However, because the PC properties are similar for modified and unmodified FBZ (Chapter 4.2) and the corrected LC50 is based on the soluble fraction of the unmodified FBZ, it is expected that the modified FBZ acts similarly. Therefore, the study conducted with unmodified FBZ can be used to assess the effects on fish of its modified form.

6.2.3.2 Oxfendazole

For OXF, acute effects on fish were investigated by Bowman et al. (1986a) and Bowman et al. (1986b). Both studies were conducted according to US EPA GL EPA-660/3-75-009 and GLP.

Bowman et al. (1986a) investigated the acute toxicity of OXF to rainbow trout (*Oncorhynchus mykiss* formerly *Salmo gairdneri*), which were exposed to five test concentrations for 96 h under static conditions. The study was conducted at nominal test concentrations of 0.56, 1.0, 1.8, 3.2 and 5.6 mg/L. The actual concentrations were determined by HPLC at 0, 48 and 96 h. The average measured concentrations were 0.57, 0.95, 1.7, 2.1 and 2.5 mg/L, respectively. Overall, the mean measured concentrations averaged 80 (±24)% of nominal. The 2.1 and 2.5 mg/L measured concentrations were observed to have a surface film at 0 and 96 h. The mean measured values were used for the LC_{50} calculation. No mortality occurred during the 96 h test period, accordingly LC_{50} values were reported as >2.5 mg/L. No abnormal effects were observed in any concentration tested during the 96 h exposure period. Accordingly, the NOEC was 2.5 mg/L.

Bowman et al. (1986b) investigated the acute toxicity of OXF to bluegill sunfish (*Lepomis macrochirus*), which were exposed to five test concentrations for 96 h under static conditions. The study was conducted at nominal test concentrations of 0.56, 1.0, 1.8, 3.2 and 5.6 mg/L. The actual concentrations were determined by HPLC at 0, 48 and 96 h. The average measured concentrations were 0.54, 0.99, 1.8, 2.5 and 2.7 mg/L, respectively. Overall, the mean measured concentrations averaged 84 (±22)% of nominal. The 2.5 and 2.7 mg/L measured concentrations were observed to have a surface film at 0 and 96 h. The mean measured values were used for the LC_{50} calculation. No mortality occurred during the 96 h test period, accordingly LC_{50} values were reported as >2.7 mg/L. No abnormal effects were observed in any concentration tested during the 96 h exposure period. Accordingly, the NOEC was 2.7 mg/L.

The results of the OXF studies are summarized in Table 6-12:

Table 6-12: Acute effects of OXF to fish based on measured concentrations

Species	EC_{50}	NOEC	Reference
Rainbow trout	96 h: >2.5 mg/L	96 h: 2.5 mg/L	Bowman et al. (1986a)
Bluegill sunfish	96 h: >2.7 mg/L	96 h: 2.7 mg/L	Bowman et al. (1986b)

Like FBZ, also OXF might have the potential to cause a latent toxicity in fish (with effects occurring after the acute exposure period of 96 h only). Latent toxicity can generally occur when the water solubility is very low (and bioconcentration is slow) and thus an extended period of time is needed to reach a critical toxic body burden in fish. However, the water solubility of OXF is much greater than of FBZ (3.87 mg/L versus 0.08 mg/L, respectively; Table 4-1 and Table 4-2) while the log K_{OW} is much lower (1.95 versus 3.32/3.4, respectively; Table 4-1 and Table 4-2). Accordingly, the bioconcentration is expected to remain low whatever the exposure period is. Thus, an extension of the exposure period beyond 96 h would unlikely result toxic effects. In conclusion the results from the 96 h studies presented above are used for the risk characterization.

6.2.3.3 Supporting information

For FBZ, the findings of Wilson and LeBlanc (1981) are supported in a weight of evidence approach by Barrows and LeBlanc (1980). Bluegill sunfish *(Lepomis macrochirus)* were

exposed to five test concentrations of [^{14}C]-FBZ and a negative control for 21 d under flow-through conditions. Nominal concentrations selected for use in this study were 0.0041, 0.0074, 0.014, 0.029 and 0.061 mg/L. The 21 d LC_{50}, based on measured concentration was determined to be 0.019 mg/L. However, water samples were not filtered prior to analysis. Accordingly, the LC_{50} presented in this study does not reflect the toxicity of the soluble FBZ fraction. Because water samples were not filtered prior to analysis, it is likely that solubilized and particulate FBZ in the water were accounted for in determining exposure concentrations. Therefore, it is possible, that an LC_{50}, based solely on the soluble FBZ fraction, may be lower than 0.019 mg/L.

6.2.4 *Summary Toxicity to Aquatic Organisms*

The results of toxicity tests conducted with aquatic organisms are summarized in Table 6-13. For simplicity, the most conservative (lowest) NOEC or EC_{50} values are presented only. Values are presented based on measured test concentrations.

Table 6-13: Toxicity tests conducted with aquatic organisms

Test system	Compound	Endpoint	Reference
Algae	FBZ	n.a.	-
		n.a.	-
	OXF	EC_{50} (72 h): >3.7 mg/L	Arnie et al. (2012)
		NOEC (72 h): 3.7 mg/L	Arnie et al. (2012)
Invertebrate	FBZ	EC_{50} (48 h): 8.8 µg/L	Meller and Zenide (2003)
		NOEC (21 d): 1.13 µg/L	Egeler et al. (2013)
	OXF	EC_{50} (48 h): 0.059 mg/L	Brougher et al. (2013)
		NOEC (48 h): <0.047 mg/L	Brougher et al. (2013)
		EC_{50} (21 d): >90 µg/L	Minderhout et al. (2013)
		NOEC (21 d): 23 µg/L	Minderhout et al. (2013)
Fish	FBZ	LC_{50} (21 d): 0.009 mg/L	Wilson and LeBlanc (1981)
	OXF	LC_{50} (96 h): >2.5 mg/L	Bowman et al. (1986a)
		NOEC (96 h): >2.5 mg/L	Bowman et al. (1986a)

n.a. – Not applicable as an algal toxicity study regarding OECD GL 201 would be impractical due to expected the photolytic degradation of FBZ during the test.

6.3 Predicted No Effect Concentrations (PNECs)

6.3.1 *Tier A PNECs*

The Tier A Predicted No Effect Concentrations (PNECs) are presented in Table 6-12 for terrestrial key non-target organisms and in Table 6-13 for aquatic non-target organisms. All PNECs are presented with the unit of µg/kg (terrestrial) or µg/L (aquatic). The PNEC is the ratio of the toxicity value (EC_{50}, LC_{50}, and NOEC) divided by the assessment factor (AF) (Equation 6-1):

Equation 6-1

$$PNEC = \frac{Toxicity\ value(EC_{50}, LC_{50}, NOEC)}{AF[n]}$$

For the results of the FBZ acute Daphnia study as an example, Equation 6-1 becomes:

$$PNEC = \frac{8.8[\mu g/L]}{1000} = 0.0088[\mu g/L]$$

Toxicity values reported as “greater than” were conservatively treated as if they were “equal to.”

The PNECs for the terrestrial non-target organisms as required under VICH Phase II Tier A were determined based on effect studies using an AF of 100 for plants (considering the EC_{50} value) and 10 for earthworm (for OXF). Because the earthworm study for FBZ (Garvey and Deetz, 1995) does not cover reproductive endpoints, it is appropriate to use an assessment factor of 100 when calculating a PNEC, rather than the traditional AF of 10 applied to chronic toxicity tests (as requested by VICH). An AF of 10 is considered not to be conservative in this case. The Tier A PNECs for terrestrial non-target organisms are presented in Table 6-14:

Table 6-14: Tier A PNECs for terrestrial non-target organisms

Compound	Organism	Endpoint	Toxicity value [µg/kg]	Source	AF [n]	PNEC [µg/kg]
FBZ	Plants	EC_{50}	36000	Table 6-5	100	360
	Earthworm	LC_{50}	180000	Table 6-5	100	1800
OXF	Plants	EC_{50}	20000	Table 6-5	100	200
	Earthworm	NOEC	12000	Table 6-5	10	1200

For soil microorganisms, PNEC values need not to be calculated as the risk assessment principle is different compared to other non-target organisms (Chapter 7.1).

The PNECs for the aquatic non-target organisms as required under VICH Phase II Tier A were determined based on acute effect studies by considering the EC_{50} values using an AF of 100 for algae and 1000 for invertebrates and fish (for OXF). Because the fish acute study for FBZ (Wilson and LeBlanc, 1981) was conducted over 21 d, it is appropriate to use an assessment factor of 100 when calculating a PNEC, rather than the traditional AF of 1000 applied to 96 h acute toxicity tests (as requested by VICH). An AF of 1000 is considered overly conservative. The Tier A PNECs for aquatic non-target organisms are presented in Table 6-15:

Table 6-15: Tier A PNECs for aquatic non-target organisms

Compound	Organism	Endpoint	Toxicity value [µg/L]	Source	AF [n]	PNEC [µg/L]
FBZ	Algae	EC_{50}	n.a.	Table 6-13	100	n.a.
	Invertebrate	EC_{50}	8.8	Table 6-13	1000	0.0088
	Fish	LC_{50}	9	Table 6-13	100	0.09
OXF	Algae	EC_{50}	3700	Table 6-13	100	37
	Invertebrate	EC_{50}	59	Table 6-13	1000	0.059
	Fish	LC_{50}	2500	Table 6-13	1000	2.5

n.a. – Not applicable as an algal toxicity study regarding OECD GL 201 would be impractical due to expected the photolytic degradation of FBZ during the test.

6.3.2 *Tier B PNECs*

The Tier B risk characterization considers the effects determined in long-term exposures, typically regarded as chronic effects, upon the aquatic and terrestrial non-target organisms. Adequate studies were conducted for aquatic non-target organisms only, namely algae (Arnie et al., 2012) and invertebrates (Egeler et al., 2013 and Minderhout et al., 2013). The Tier B PNECs are presented in Table 6-16 for aquatic non-target organisms. All PNECs are presented with the unit of µg/L. The PNECs are calculated as described in Equation 6-1. The standard AF of 10 requested by VICH is used except for the invertebrate for OXF. In the study of Minderhout et al. (2013) daphnids were fed with 3-times the feeding rate recommended in OECD GL 211. The impact of the 3-times feeding rate over the 1-time feeding rate on the reproductive output is not known and it is unclear whether the NOEC would be lower if the feeding rate followed OECD GL 211. Therefore, for reason of conservativeness, an additional AF of 2 is included when calculating the PNEC. Accordingly the AF for invertebrate for OXF is 20. For algae, the same study and species is considered as in Tier A but the NOEC (instead of EC_{50}) is used in Tier B.

Table 6-16: Tier B PNECs for aquatic non-target organisms

Compound	Organism	Endpoint	Toxicity value [µg/L]	Source	AF [n]	PNEC [µg/L]
FBZ	Algae	NOEC	n.a.	-	10	n.a.
	Invertebrate	NOEC	1.13	Table 6-13	10	0.113
OXF	Algae	NOEC	3700	Table 6-13	10	370
	Invertebrate	NOEC	23	Table 6-13	20	1.15

n.a. – Not applicable as an algal toxicity study regarding OECD GL 201 would be impractical due to expected the photolytic degradation of FBZ during the test.

The PNEC in sediment is calculated using equilibrium partitioning. This method uses the PNEC for the aquatic invertebrate and the sediment/water partitioning coefficient as input. The sediment PNEC is calculated considering the European GL in support of the VICH GLs (European Medicines Agency, 2005) according to Equation 6-2 to Equation 6-5:

Equation 6-2

$$Kp_{sed}\ [dm^3/kg] = Foc_{sed}\ [kg/kg] \times K_{OC}[dm^3/kg]$$

Equation 6-3

$$K_{sed-water}\ [m^3/m^3] = F_{water-sed}[m^3/m^3] + F_{solid-sed}[m^3/m^3] \times \frac{Kp_{sed}[dm^3/kg]}{1000} \times RHO_{solid}[kg/m^3]$$

Equation 6-4

$$CONV_{sed}[\mu g/kg] = \frac{RHO_{sed}[kg/m^3]}{F_{solid-sed}[m^3/m^3] \times RHO_{solid}[kg/m^3]}$$

Equation 6-5

$$PNEC_{sediment}\ [\mu g/kg] = \frac{K_{sed-water}[m^3/m^3]}{RHO_{sed}[kg/m^3]} \times PNEC_{surfacewater}\ [\mu g/L] \times CONV_{sed}[\mu g/kg] \times 1000$$

Where:

F_{OCsed}:	Weight fraction organic carbon sediment= 0.05 kg/kg
K_{OC}:	Adsorption/desorption partition co-efficient normalized to the organic carbon content of soil = 14459 dm³/kg for FBZ and 887 dm³/kg for OXF (Chapter 4.3.1.4)
Kp_{sed}:	Partitioning co-efficient solids/water in sediment
$F_{water-sed}$:	Fraction water in sediment = 0.8 m³/m³
$F_{solid-sed}$:	Fraction solids in sediment = 0.2 m³/m³
RHO_{solid}:	Bulk density solids = 2500 kg/m³
$K_{sed-water}$:	Partitioning co-efficient sediment/water
RHO_{sed}:	Bulk density sediment = 1300 kg/m³
$CONV_{sed}$:	Conversion factor sediment
$PNEC_{surfacewater}$:	$PNEC_{surfacewater}$ for the aquatic invertebrate = 0.0088 and 0.113 µg/L for FBZ, and 0.059 and 1.15 µg/L for OXF for acute and chronic exposure respectively (Chapter 6.3.1 and 6.3.2)

For FBZ as example, Equation 6-2 becomes:

$$Kp_{sed} = 0.05\ kg/kg \times 14459\ dm^3/kg = 723\ dm^3/kg$$

Equation 6-3 becomes:

$$K_{sed-water} = 0.8\ m^3/m^3 + 0.2\ m^3/m^3 \times \frac{723\ dm^3/kg}{1000} \times 2500\ kg/m^3 = 362\ m^3/m^3$$

Equation 6-4 becomes:

$$CONV_{sed} = \frac{1300\ kg/m^3}{0.2\ m^3/m^3 \times 2500\ kg/m^3} = 2.6\ \mu g/kg$$

Equation 6-5 becomes:

$$PNEC_{sediment} = \frac{362\ m^3/m^3}{1300\ kg/m^3} \times 0.0088\ \mu g/L \times 2.6\ \mu g/kg \times 1000 = 6.38\ \mu g/kg$$

The calculation of the sediment PNEC for FBZ and OXF/FBZ-SO_2 is conducted considering acute and chronic PNECs for the aquatic invertebrate (Table 6-17):

Table 6-17: Sediment PNEC for FBZ and OXF/FBZ-SO_2

		FBZ		OXF/FBZ-SO_2	
		Acute PNEC	Chronic PNEC	Acute PNEC	Chronic PNEC
F_{OCsed}	kg/kg	0.05	0.05	0.05	0.05
K_{OC}	dm^3/kg	14459	14459	887 [a]	887 [a]
Kp_{sed}	dm^3/kg	723	723	44	44
$F_{water-sed}$	m^3/m^3	0.8	0.8	0.8	0.8
$F_{solid-sed}$	m^3/m^3	0.2	0.2	0.2	0.2
RHO_{solid}	kg/m^3	2500	2500	2500	2500
$K_{sed-water}$	m^3/m^3	362	362	23	23
RHO_{sed}	kg/m^3	1300	1300	1300	1300
$CONV_{sed}$	kg/kg	2.6	2.6	2.6	2.6
$PNEC_{sw}$	µg/L	0.0088	0.113	0.059	1.15
PNEC	µg/kg	6.38	81.87	2.71	52.82

[a] – Values are for OXF

The sediment PNEC for FBZ amounts to 6.38 µg/kg if based on the acute PNEC for the aquatic invertebrate and to 81.87 µg/kg if based on the chronic PNEC. For OXF/FBZ-SO_2 the sediment PNECs amount to 2.71 µg/kg and 52.82 µg/kg, respectively.

7. Risk Characterization

For the risk characterization, in a first step PEC/PNEC ratios are calculated for FBZ and OXF/FBZ-SO_2 independently. In a second step, individual PEC/PNEC ratios are summed up and compared to the trigger value of 1. In case the combined PEC/PNEC ratio is <1, risk is per definition absent for the parallel exposure of non-target organisms to FBZ and OXF/FBZ-SO_2.

7.1 Tier A Risk Characterization

The initial Tier A risk characterization is principally presented as the ratio of the initial PEC values to the Tier A (acute) PNEC values for representative surrogate species. However, for the terrestrial compartment for OXF/FBZ-SO_2 the Tier A risk characterization is based on the plateau PEC_{soil}. In case the PEC/PNEC ratio for the initial Tier A risk characterization is ≥1, the ratio of the refined PEC values to the Tier A PNEC values is calculated.

The Tier A PEC/PNEC ratios for terrestrial non-target organisms for the initial/plateau PEC_{soil} are presented in Table 7-1:

Table 7-1: Tier A individual and combined PEC/PNEC ratios for terrestrial non-target organisms

Compound			Plants		Earthworm	
			Value	Source	Value	Source
FBZ	PEC	µg/kg	33.44	Table 5-7	33.44	Table 5-7
	PNEC	µg/kg	360	Table 6-12	1800	Table 6-12
	PEC/PNEC	n	0.093		0.019	
OXF/FBZ-SO_2	PEC	µg/kg	105.57	Table 5-7	105.57	Table 5-7
	PNEC	µg/kg	200	Table 6-12	1200	Table 6-12
	PEC/PNEC	n	0.528		0.088	
Combined	PEC/PNEC	n	0.621		0.107	

The combined PEC/PNEC ratios based on initial/plateau PEC_{soil}, reflecting parallel exposure to FBZ and OXF/FBZ-SO_2, do not exceed the trigger value of 1 for terrestrial plants and earthworm.

Effects in soil microorganism are also assessed in Tier A, however the PEC/PNEC approach is not applicable. For soil microorganisms the rate of nitrate formation between the treatment is compared with the control. No long-term effects are expected if the difference in rate of nitrogen transformation of the treated soil is equal or less than 25% of the untreated soil on day 28. Over the period of 28 d nitrogen transformation in soil is not affected by OXF when added in concentrations of 101 and 1000 mg/kg dry soil (Twilley and Schaefer, 2013). Considering the plateau PEC_{soil} for OXF/FBZ-SO_2 of 72.31 µg/kg, risk is excluded for OXF/FBZ-SO_2. For FBZ no antibacterial effect could be found against any of the tested bacteria. Considering the absence of toxicity for both, FBZ and OXF/FBZ-SO_2, risk to soil microorganism is also excluded for the parallel exposure to both compounds.

The Tier A PEC/PNEC ratios for aquatic non-target organisms for initial and refined $PEC_{surfacewater}$ are presented in Table 7-2:

Table 7-2: Tier A individual and combined PEC/PNEC ratios for aquatic non-target organisms

Compound			Initial $PEC_{surfacewater}$ [1]			Refined $PEC_{surfacewater}$ [2]		
			Algae	Invert.	Fish	Algae	Invert.	Fish
FBZ	PEC	µg/L	0.37	0.37	0.37	0.039	0.039	0.039
	PNEC	µg/L	n.a.	0.009	0.09	n.a.	0.009	0.09
	PEC/PNEC	n	n.a.	41.80	4.09	n.a.	4.38	0.43
OXF/FBZ-SO_2	PEC	µg/L	1.16	1.16	1.16	0.89	0.89	0.89
	PNEC	µg/L	37	0.059	2.5	37	0.059	2.5
	PEC/PNEC	n	0.031	19.58	0.46	0.024	15.13	0.36
Combined	PEC/PNEC	n	0.031	61.48	4.55	0.024	19.52	0.79

n.a. – Not applicable as an algal toxicity study regarding OECD GL 201 would be impractical due to expected photolytic degradation of FBZ during the test.
[1] – Source PEC values: Table 5-7; source PNEC values: Table 6-13.
[2] – Source PEC values: Table 5-7; source PNEC values: Table 6-13.

The combined PEC/PNEC ratios based on initial $PEC_{surfacewater}$, reflecting parallel exposure to FBZ and OXF/FBZ-SO_2, do not exceed the trigger value of 1 for algae. Considering the refined $PEC_{surfacewater}$, the PEC/PNEC ratio is <1 also for fish. As the trigger value is exceeded for aquatic invertebrate for refined $PEC_{surfacewater}$, a Tier B risk characterization is conducted accordingly.

7.2 Tier B Risk Characterization

Tier B risk characterization is only performed for those surrogate species for which the Tier A PEC/PNEC ratio of refined PEC values is ≥1. This is applicable for aquatic invertebrate only. Also, the risk characterization for sediment is conducted at this level.

The Tier B PEC/PNEC ratios for aquatic invertebrate for initial and refined $PEC_{surfacewater}$ are presented in Table 7-3:

Table 7-3: Tier B individual and combined PEC/PNEC ratios for aquatic invertebrates

Compound			Initial $PEC_{surfacewater}$		Refined $PEC_{surfacewater}$	
			Value	Source	Value	Source
FBZ	PEC	µg/L	0.37	Table 5-7	0.039	Table 5-7
	PNEC	µg/L	0.113	Table 6-14	0.113	Table 6-14
	PEC/PNEC	n	3.26		0.34	
OXF/FBZ-SO_2	PEC	µg/L	1.16	Table 5-7	0.89	Table 5-7
	PNEC	µg/L	1.15	Table 6-14	1.15	Table 6-14
	PEC/PNEC	n	1.01		0.78	
Combined	PEC/PNEC	n	4.26		1.12	

The combined PEC/PNEC ratios based on initial $PEC_{surfacewater}$, reflecting parallel exposure to FBZ and OXF/FBZ-SO_2, does exceed the trigger value of 1 for aquatic invertebrate. Considering the refined $PEC_{surfacewater}$, the PEC/PNEC ratio slightly exceeds the trigger value of 1.

The sediment PEC/PNEC ratios based on acute and chronic effect data for aquatic invertebrate are presented in Table 7-4:

Table 7-4: Sediment PEC/PNEC ratios based on acute and chronic effect data for aquatic invertebrate

Compound			Acute effect data		Chronic effect data	
			Value	Source	Value	Source
FBZ	PEC	µg/kg	27.95	Table 5-7	27.95	Table 5-7
	PNEC	µg/kg	6.38	Table 6-15	81.87	Table 6-15
	PEC/PNEC	n	4.38		0.34	
OXF/FBZ-SO_2	PEC	µg/kg	41.01	Table 5-7	41.01	Table 5-7
	PNEC	µg/kg	2.71	Table 6-15	52.82	Table 6-15
	PEC/PNEC	n	15.13		0.78	
Combined	PEC/PNEC	n	19.52		1.12	

The combined sediment PEC/PNEC ratio, reflecting parallel exposure to FBZ and OXF/FBZ-SO_2, does exceed the trigger value of 1 when acute effect data for aquatic invertebrate are considered. Considering chronic effect data for aquatic invertebrate the sediment the PEC/PNEC ratio slightly exceeds the trigger value of 1.

7.3 Summary of Risk Characterization

PEC/PNEC ratios for terrestrial plants, earthworms, algae, and fish were below one in Tier A indicating acceptable risk and no need for further analysis. But the PEC/PNEC ratio for aquatic invertebrates (i.e., *D. magna*) exceeded one (PEC/PNEC = 19.52) and therefore, a 21-day reproduction study was conducted on *D. magna* as part of a Tier B analysis. In addition, the PEC/PNEC ratio was estimated for sediment invertebrates. The conclusions for the most sensitive receptors (i.e., aquatic invertebrates) are based on the results of the Tier B analysis. The risk assessment indicates that FBZ and OXF/FBZ-SO_2, individually, do not present significant environmental risk because the PEC/PNEC ratios were below one for aquatic invertebrates (0.34 and 0.78 for FBZ and OXF/FBZ-SO_2, respectively). The combined PEC/PNEC ratio for FBZ and OXF/FBZ-SO_2 was greater than one (1.12), suggesting the potential need for further analysis. However, because the risk assessment methodology used herein included a number of conservative approaches and assumptions to ensure that non-target organisms would be protected, it is concluded that there will be no significant environmental risk. Conservative approaches and assumptions used include the following:

- The PEC_{soil} was calculated using a conservative manure production period of 60 d even though more current swine manure management practices allow for at least 90 to 180 d of storage, particularly in storage pits beneath the slotted floors (U.S. EPA, 2012b).

- The calculation of the soil K_d values for FBZ are conducted by excluding data from the highest test concentration. Inclusion of these data would have resulted in a higher K_d value and thus lower refined $PEC_{surfacewater}$ and higher sediment PNEC.
- The calculation of soil DT_{50} values is conservative as a SFO kinetic is applied. The use of more complex kinetics would have resulted in a better fit and lower DT_{50} values, which finally would have resulted in lower $PEC_{surfacewater}$ and $PEC_{sediment}$.
- The calculation if the initial PEC_{soil} for FBZ-SO_2 is conservative as it is assumed that the maximum fraction of FBZ-SO_2 present in soil following degradation of FBZ is identical to the maximum fraction of OXF. Realistically this fraction will be lower, which in turn will result in a lower $PEC_{surfacewater}$.
- The calculation of the PNEC for aquatic invertebrates for OXF is conservative as an AF of 20 (instead of 10 as described in VICH GL 38) is applied. A reduced AF would have resulted in a higher PNEC for aquatic invertebrates and sediment.
- The $PEC_{surfacewater}$ is calculated assuming that all compound present on agricultural land moves to surface water in a single run-off event. Accordingly, the $PEC_{surfacewater}$ represents the maximum single peak environmental concentration. For the risk characterization these PEC values are compared to PNEC values for aquatic invertebrate and sediment that are derived from a 21-d NOEC value. This approach is conservative as the use of a 21-d moving average $PEC_{surfacewater}$ would have resulted in lower PEC values for surface water and sediment.

Considering the high degree of conservatism throughout the assessment, the final conclusion drawn at beginning of this section t is thus valid. This is also supported by the fact that negative impacts to soil microorganisms can be excluded based on available data.

8. Summary and Conclusions

The use of Safe-Guard Aquasol in swine could result in the introduction of FBZ and its metabolites into the environment via runoff from the land application of manure containing FBZ, OXF, and FBZ-SO_2. This EA prepared for Safe-Guard Aquasol addresses concerns regarding the fate and effects of these compounds in the environment. While fate data were generated for FBZ, OXF, and FBZ-SO_2, effects data were determined for only FBZ and OXF. Therefore, a total residue approach was used for the metabolites and it was assumed that FBZ-SO_2 is as toxic as OXF. Because FBZ has a short half-life while OXF and FBZ-SO_2 have the potential to persist and accumulate in the environment (DT_{90} >1 year), a complex approach was used in the risk assessment to determine the PEC values whereby traditional calculations were used to estimate the PEC_{soil} for FBZ and a plateau PEC_{soil} was calculated for OXF and FBX-SO_2. PEC/PNEC ratios were then calculated separately for FBZ and OXF/FBZ-SO_2, and ultimately, a combined total PEC/PNEC ratio was calculated to account for the presence of all three compounds simultaneously (see conceptual model in Figure 1-1). Based on the Tier A assessment, PEC/PNEC ratios for terrestrial plants, earthworms, algae, and fish were below a value of one indicating acceptable risk and no need for further analysis. Because the PEC/PNEC ratios of *D. magna* exceeded a value of one in Tier A, they were the most sensitive species and were assessed further under Tier B. The PEC/PNEC ratios for aquatic and sediment invertebrates slightly exceeded one (1.12) under Tier B, but taking into account all the available information and conservative assumptions made in this EA, this risk assessment supports the conclusion that significant environmental impacts are not expected from the use of the Safe-Guard Aquasol.

9. Mitigation Measures

Because the use of Safe-Guard Aquasol in accordance with label directions poses no unacceptable short-term or long-term risks to aquatic or terrestrial ecosystems, no mitigation measures are required.

10. Alternatives to the Proposed Action

The proposed action would not be expected to have any substantial adverse effects on human health or the environment. Therefore, alternatives to the proposed action do not need to be considered.

11. List of Preparers

This document was prepared by Dr. Gregor Scheef (MSD Animal Health Innovation GmbH).

12. Certification

The undersigned official certifies that the information presented in this EA is true, accurate, and complete to the best of their knowledge.

Gregor Scheef 15-Jan-2015

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Date

15-Jan-2015

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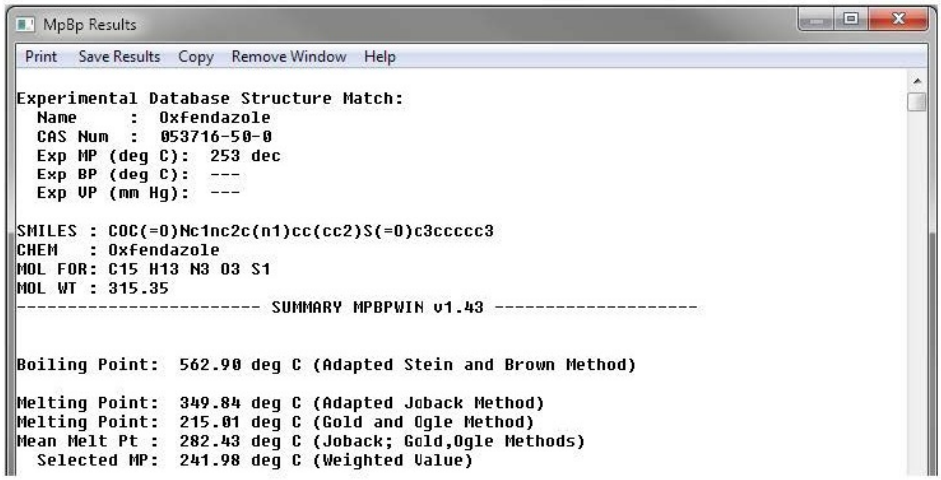
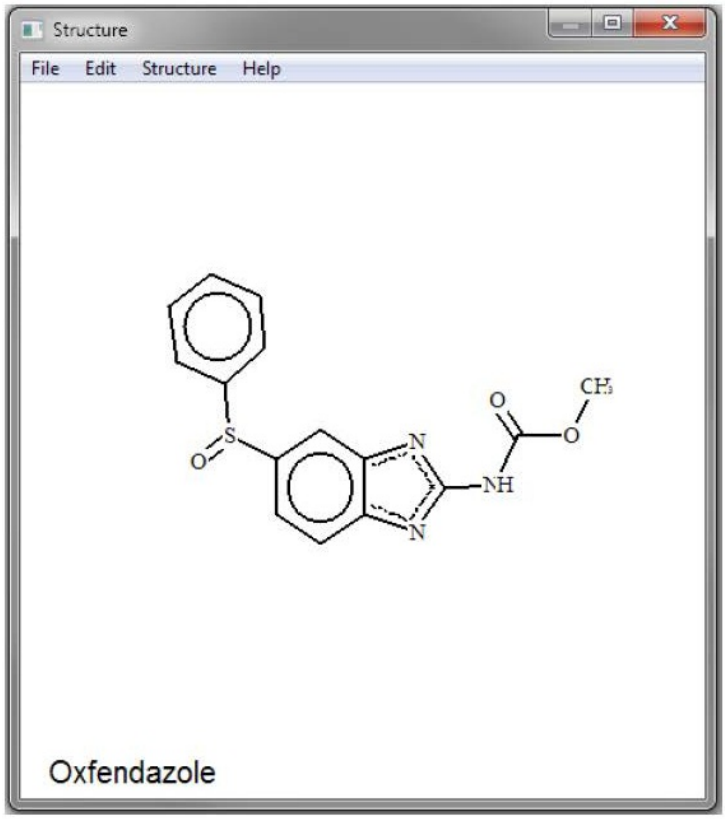
14. Appendices

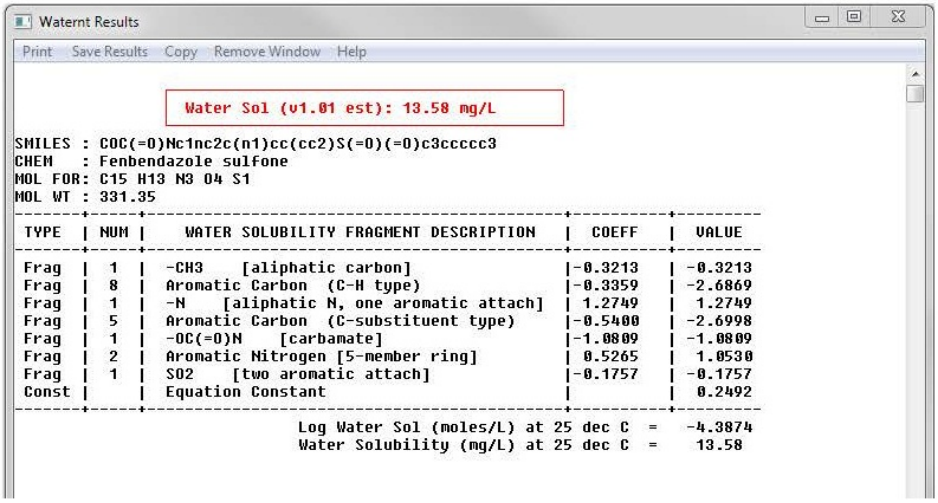
14.1 Estimation Program Interface (EPI) Suite calculations

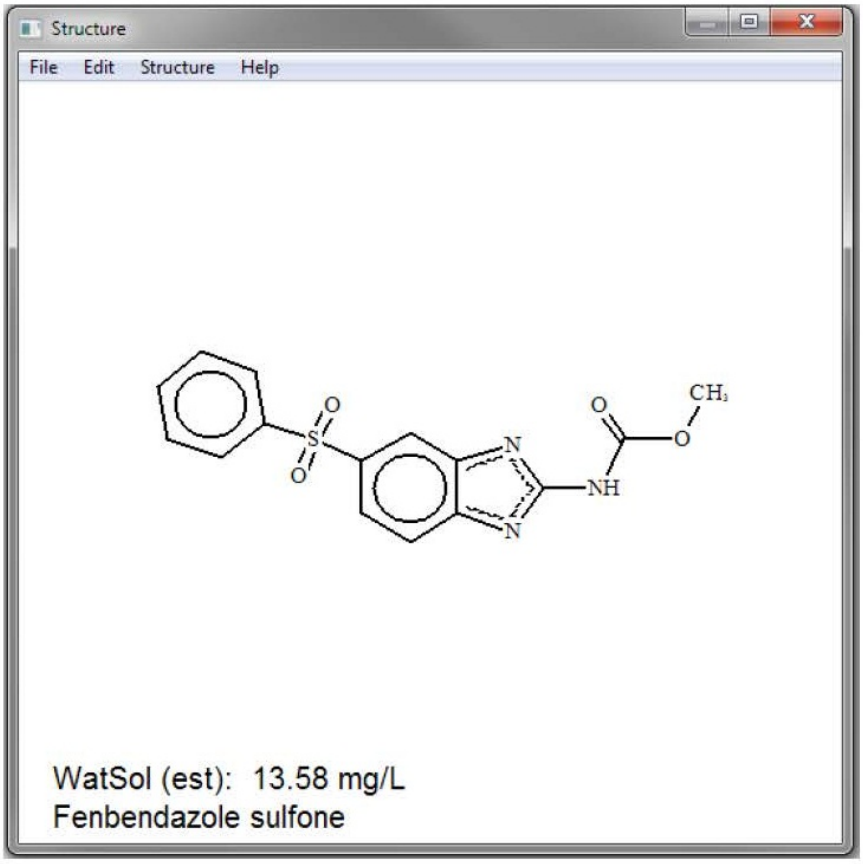
14.1.1 *MPBPWIN estimation for OXF – melting point*





14.1.2 *WATERNT estimation for FBZ-SO_2 – water solubility*



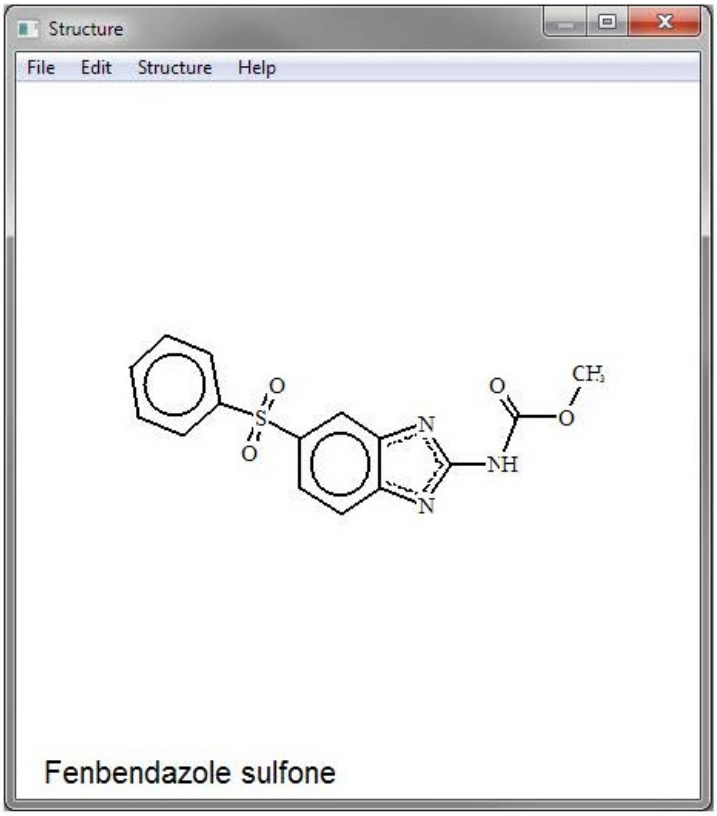


14.1.3 *MPBPWIN estimation for FBZ-SO$_2$ – melting point and vapor pressure*

MpBp Results

Print Save Results Copy Remove Window Help

```
Experimental Database Structure Match:  no data

SMILES : COC(=O)Nc1nc2c(n1)cc(cc2)S(=O)(=O)c3ccccc3
CHEM   : Fenbendazole sulfone
MOL FOR: C15 H13 N3 O4 S1
MOL WT : 331.35
------------------------ SUMMARY MPBPWIN v1.43 ---------------------


Boiling Point:  571.08 deg C (Adapted Stein and Brown Method)

Melting Point:  349.84 deg C (Adapted Joback Method)
Melting Point:  219.79 deg C (Gold and Ogle Method)
Mean Melt Pt :  284.82 deg C (Joback; Gold,Ogle Methods)
  Selected MP:  245.80 deg C (Weighted Value)

Vapor Pressure Estimations (25 deg C):
  (Using BP: 571.08 deg C (estimated))
  (Using MP: 245.80 deg C (estimated))
    VP:  1.18E-015 mm Hg (Antoine Method)
       :  1.58E-013 Pa  (Antoine Method)
    VP:  1.68E-012 mm Hg (Modified Grain Method)
       :  2.23E-010 Pa  (Modified Grain Method)
    VP:  5.79E-012 mm Hg (Mackay Method)
       :  7.72E-010 Pa  (Mackay Method)
  Selected VP:  1.68E-012 mm Hg (Modified Grain Method)
              :  2.23E-010 Pa (Modified Grain Method)
  Subcooled liquid VP:  4.16E-010 mm Hg (25 deg C, Mod-Grain method)
                      :  5.55E-008 Pa  (25 deg C, Mod-Grain method)

-------+-----+--------------------+----------+---------
 TYPE  | NUM |  BOIL DESCRIPTION  |  COEFF   |  VALUE
-------+-----+--------------------+----------+---------
 Group |  1  |  -CH3              |   21.98  |   21.98
 Group |  1  |  -COO- (ester)     |   78.85  |   78.85
 Group |  1  |  >NH (nonring)     |   45.28  |   45.28
 Group |  8  |  CH (aromatic)     |   28.53  |  228.24
 Group |  3  |  -C (aromatic)     |   30.76  |   92.28
 Group |  2  |  C (3a aromatic)   |   45.46  |   90.92
 Group |  2  |  N (aromatic)      |   39.88  |   79.76
 Group |  1  |  >S(=O)(=O)        |  171.58  |  171.58
 Corr  |  1  |  Imidazole [NH]    |  165.00  |  165.00
   *   |     |  Equation Constant |          |  198.18
=============+====================+==========+=========
RESULT-uncorr|  BOILING POINT in deg Kelvin  | 1172.07
RESULT- corr |  BOILING POINT in deg Kelvin  |  844.24
             |  BOILING POINT in deg C       |  571.08
-------------------------------------------------------


-------+-----+--------------------+----------+---------
 TYPE  | NUM |  MELT DESCRIPTION  |  COEFF   |  VALUE
-------+-----+--------------------+----------+---------
 Group |  1  |  -CH3              |   -5.10  |   -5.10
 Group |  1  |  -COO- (ester)     |   53.60  |   53.60
 Group |  1  |  >NH (nonring)     |   52.66  |   52.66
 Group |  8  |  CH (aromatic)     |    8.13  |   65.04
 Group |  3  |  -C (aromatic)     |   37.02  |  111.06
 Group |  2  |  C (3a aromatic)   |   37.02  |   74.04
 Group |  2  |  N (aromatic)      |   68.40  |  136.80
 Group |  1  |  >S(=O)(=O)        |  150.00  |  150.00
   *   |     |  Equation Constant |          |  122.50
=============+====================+==========+=========
   RESULT    |  MELTING POINT in deg Kelvin  |  760.60
 RESULT-limit|  MELTING POINT in deg Kelvin  |  623.00
             |  MELTING POINT in deg C       |  349.84
-------------------------------------------------------
```


Structure
File Edit Structure Help
O
S
O
N
N
NH
O
O
CH3
Fenbendazole sulfone

14.1.4 *KOWWIN estimation for FBZ-SO_2 – Log K_{OW}*

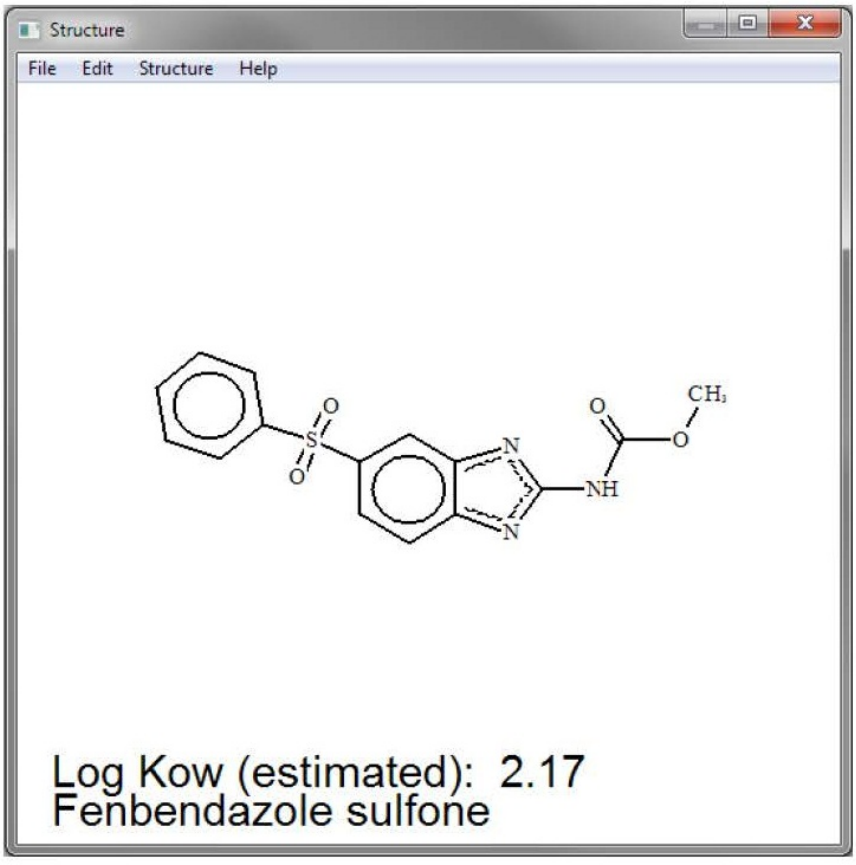
Kowwin Results

Print Save Results Copy Remove Window Help

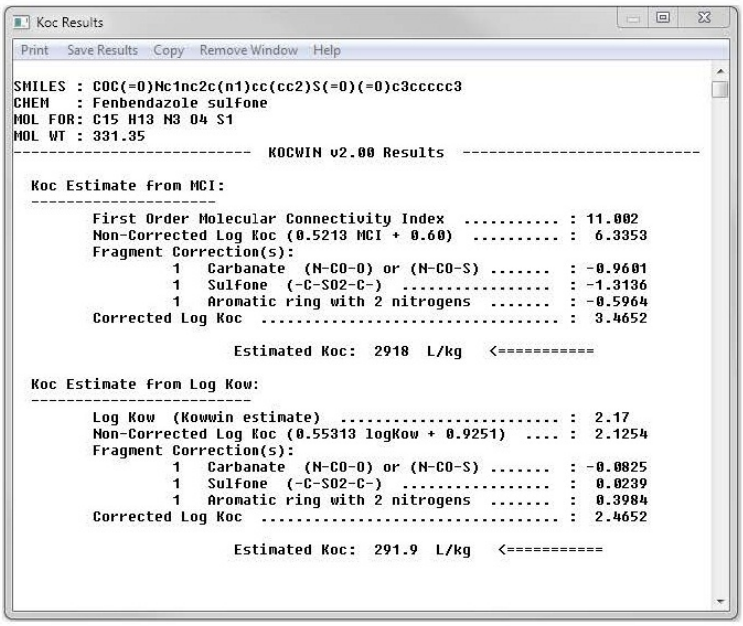
Log Kow(version 1.68 estimate): 2.17

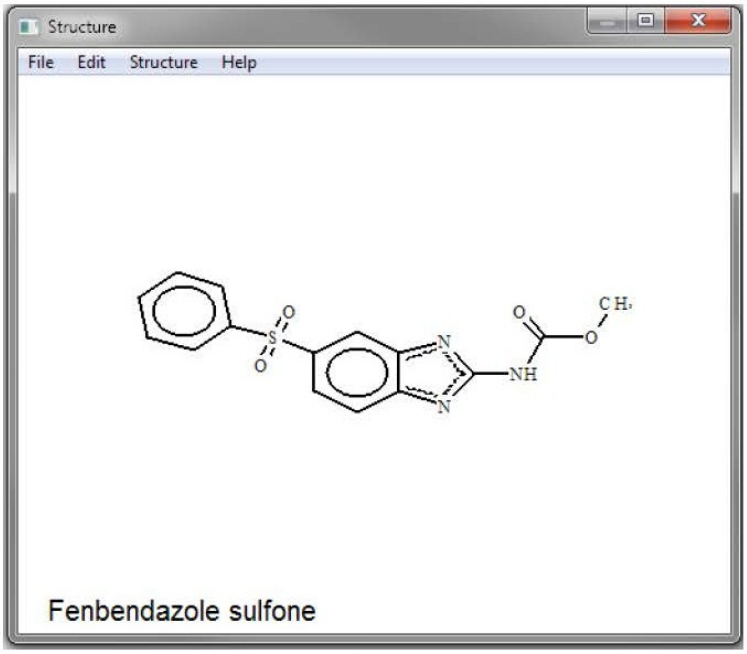
SMILES : COC(=O)Nc1nc2c(n1)cc(cc2)S(=O)(=O)c3ccccc3
CHEM : Fenbendazole sulfone
MOL FOR: C15 H13 N3 O4 S1
MOL WT : 331.35

TYPE	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	-CH3 [aliphatic carbon]	0.5473	0.5473
Frag	13	Aromatic Carbon	0.2940	3.8220
Frag	1	-N [aliphatic N, one aromatic attach]	-0.9170	-0.9170
Frag	1	-OC(-O)N [carbamate]	0.1283	0.1283
Frag	2	Aromatic Nitrogen [5-member ring]	-0.5262	-1.0524
Frag	1	SO2 [two aromatic attach]	-1.1500	-1.1500
Factor	1	Imidazole type -> 2-amino type correction	0.5596	0.5596
Const		Equation Constant		0.2290
			Log Kow =	2.1668



14.1.5 *KOCWIN estimation for FBZ-SO_2 – soil K_{OC}*





14.2 **Calculations of average Koc values for FBZ**

14.2.1 ***Clay loam***

Table 14-1: Adsorption isotherm for [^{14}C]-FBZ in clay loam (from Mackie and Ayliffe, 1999; Table 3)

Initial concentration [µg/kg]	Replicate	Solution concentration [µg/kg]	Soil concentration [µg/kg]
40	A	1.664	172
	B	1.617	176
	C	1.534	180
190	A	3.482	840
	B	3.715	849
	C	3.864	854
991	A	16.397	4375
	B	15.481	4098
	C	15.841	3991
4970	A	36.901	24240
	B	36.183	26901
	C	34.534	25718

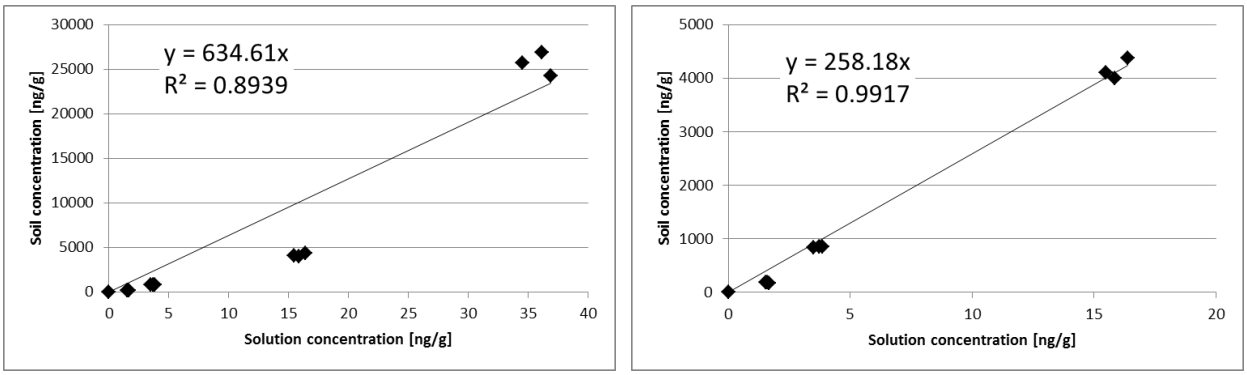


Figure 14-1: Best fit of the adsorbed concentration versus the solution concentration data pairs for FBZ in clay loam, resulting in average K_d values of 634.61 L/kg (based on all test concentrations) and 258.18 L/kg (without highest test concentration).

14.2.2 *Loamy sand*

Table 14-2: Adsorption isotherm for [^{14}C]-FBZ in loamy sand (from Mackie and Ayliffe, 1999; Table 4)

Initial concentration [µg/kg]	Replicate	Solution concentration [µg/kg]	Soil concentration [µg/kg]
40	A	1.163	176
	B	1.403	179
	C	1.092	166
190	A	3.899	821
	B	4.285	829
	C	4.036	795
991	A	33.294	3925
	B	30.110	4012
	C	30.033	4000
4970	A	62.615	24664
	B	63.027	25707
	C	60.533	24506

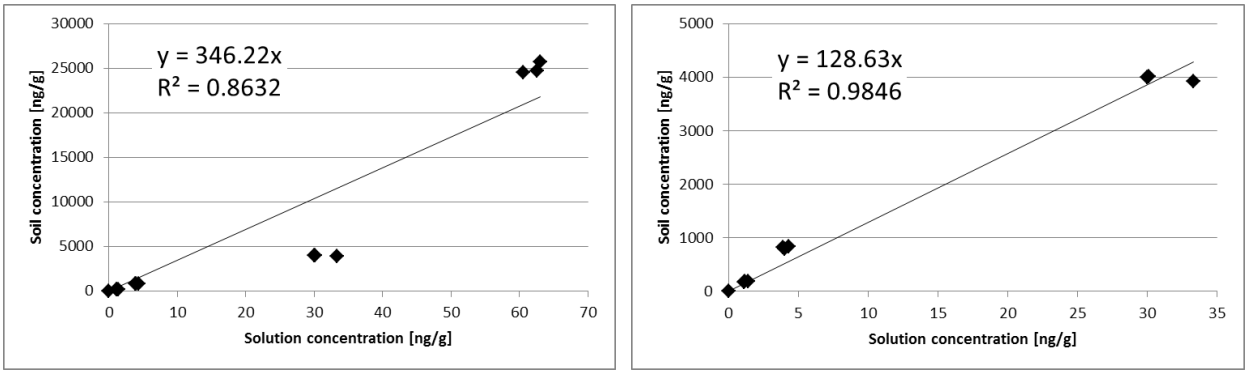


Figure 14-2: Best fit of the adsorbed concentration versus the solution concentration data pairs for FBZ in loamy sand, resulting in average K_d values of 346.22 L/kg (based on all test concentrations) and 128.63 L/kg (without highest test concentration).

14.2.3 *Sandy loam*

Table 14-3: Adsorption isotherm for [^{14}C]-FBZ in sandy loam (from Mackie and Ayliffe, 1999; Table 5)

Initial concentration [µg/kg]	Replicate	Solution concentration [µg/kg]	Soil concentration [µg/kg]
40	A	1.204	197
	B	1.113	186
	C	0.930	177
190	A	2.749	872
	B	2.579	833
	C	2.853	866
991	A	13.762	4210
	B	13.144	4419
	C	13.460	4410
4970	A	36.181	25375
	B	37.380	25390
	C	34.824	25134

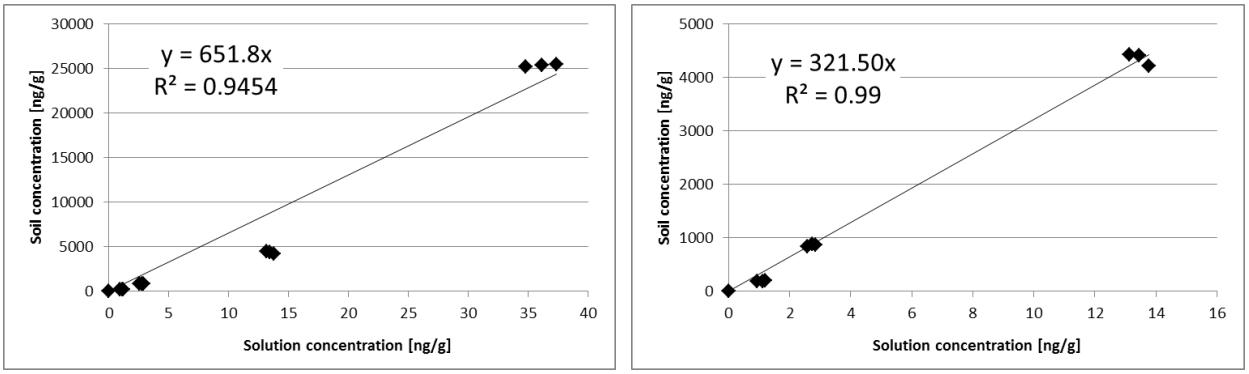


Figure 14-3: Best fit of the adsorbed concentration versus the solution concentration data pairs for FBZ in sandy loam, resulting in average K_d values of 651.8 L/kg (based on all test concentrations) and 321.50 L/kg (without highest test concentration).

14.3 **CAKE evaluations**

14.3.1 ***Data used for estimation of DT_{50}/DT_{90} values for FBZ, OXF, and FBZ-SO_2***

Table 14-4: Data used for estimation of DT_{50}/DT_{90} values for FBZ, OXF, and FBZ-SO_2 (from Mackie and Ayliffe, 2000; Tables 4 to 6)

Soil type	Sampling interval [days]	Compound [% applied radioactivity]		
		FBZ	OXF	FBZ-SO_2
Sandy loam	0	97.90	n.d.	n.d.
	4	62.21	27.00	n.d.
	8	46.29	40.07	7.25
	16	24.57	39.90	7.96
	32	28.09	47.19	3.60
	64	16.09	58.37	4.82
	100	15.65	55.41	6.00
	120	6.97	61.93	6.42
	180	9.00	57.13	5.49
	365	n.d.	22.67	37.84
Loamy sand	0	99.33	n.d.	n.d.
	4	71.30	19.59	n.d.
	8	67.27	24.39	n.d.
	16	45.77	44.55	n.d.
	32	30.16	53.45	1.39
	64	19.56	64.24	2.83
	100	20.18	60.47	2.51
	120	10.13	62.52	7.02
	180	6.21	55.85	8.22
	365	n.d.	50.22	10.29
Clay loam	0	94.28	n.d.	n.d.
	4	60.78	26.04	n.d.
	8	35.78	43.33	6.08
	16	31.67	35.77	7.33
	32	19.01	51.80	4.11
	64	8.28	51.42	8.05
	100	2.60	28.38	25.81
	120	6.63	37.99	21.57
	180	1.13	24.61	13.99
	365	1.39	12.86	24.40

n.d. – not detected

14.3.2 *Sandy loam – FBZ / OXF – SFO*

CAKE Kinetic Evaluation Report

Study: New Study

Study date: 06-May-13
Report generated: 12-Jun-14

Sandy loam (SFO)

Model Setup:

Topology: Parent, A1, A2
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)

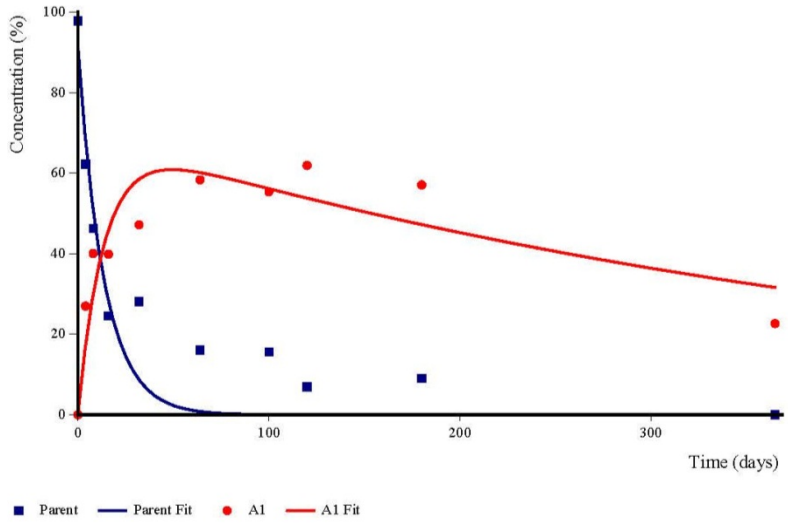
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No
f_Parent_to_A1	0.5	0 to 1	No
k_A1	0.1	0 to (unbounded)	No
f_A1_to_A2	0.5	0 to 1	No
k_A2	0.1	0 to (unbounded)	No

Fit step: Parent+A1

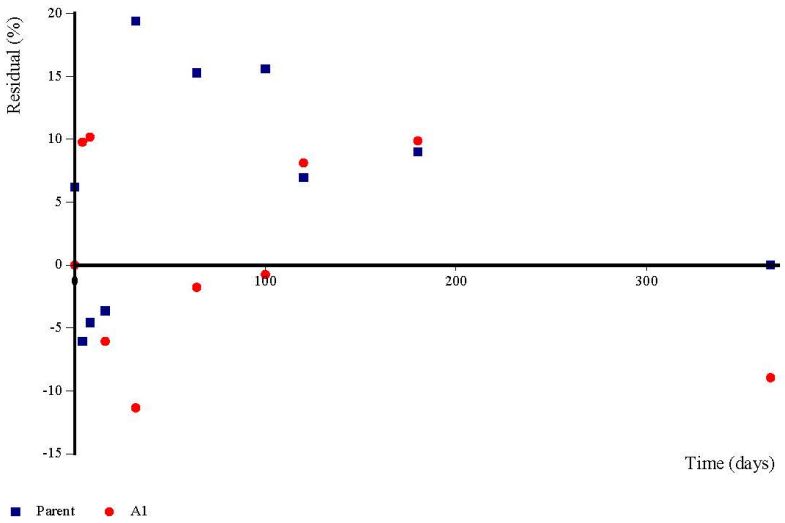
Graphical Summary:

Observations and Fitted Model:



Internal Use Only

Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	89.72	0 to (unbounded)	No
k_Parent	0.06725	0 to (unbounded)	No
f_Parent_to_A1	0.7733	0 to 1	No
k_A1	0.00231	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	91.7	9.518	2.301E-08	71.52	111.9
k_Parent	0.07368	0.01521	8.952E-05	0.04145	0.106
f_Parent_to_A1	0.7391	0.1226	8.743E-06	0.4793	0.999
k_A1	0.00217	0.0008618	0.01141	0.0003434	0.004

χ^2

Parameter	Error %	Degrees of Freedom
All data	22.47	16
Parent	27.35	8
A1	14.68	9

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	9.407	31.25
A1	319.4	1061

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9047	0.8603
Parent	0.9328	0.867
A1	0.836	0.8231

Parameter Correlation:

	Parent_0	k_Parent	f_Parent_to_A1	k_A1
Parent_0	1	0.4833	-0.7901	-0.1997
k_Parent	0.4833	1	-0.6422	-0.4133
f_Parent_to_A1	-0.7901	-0.6422	1	0.6128
k_A1	-0.1997	-0.4133	0.6128	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	97.9	91.7	6.199
4	62.21	68.29	-6.083
8	46.29	50.86	-4.569
16	24.57	28.21	-3.638
32	28.09	8.677	19.41
64	16.09	0.821	15.27
100	15.65	0.05785	15.59
120	6.97	0.01325	6.957
180	9	0.0001595	9
365	0	2.614E-06	-2.614E-06

Compartment A1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
4	27	17.22	9.777
8	40.07	29.9	10.17
16	39.9	45.97	-6.069
32	47.19	58.54	-11.35
64	58.37	60.15	-1.782
100	55.41	56.17	-0.755
120	61.93	53.81	8.119
180	57.13	47.25	9.88
365	22.67	31.63	-8.955

Sequence Creation Information:
Fit generated by CAKE version 1.4 (Release)
running on R version 2.12.2 (2011-02-25)

Report Information:
Report generated by CAKE version 1.4 (Release)
CAKE developed by Tessella Plc, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 2.0.50727.5477

14.3.3 *Loamy sand – FBZ / OXF / FBZ-SO_2 – SFO*

CAKE Kinetic Evaluation Report

Study: New Study

Study date: 06-May-13
Report generated: 12-Jun-14

Loamy sand (SFO)

Model Setup:

Topology: Parent, A1, A2
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)

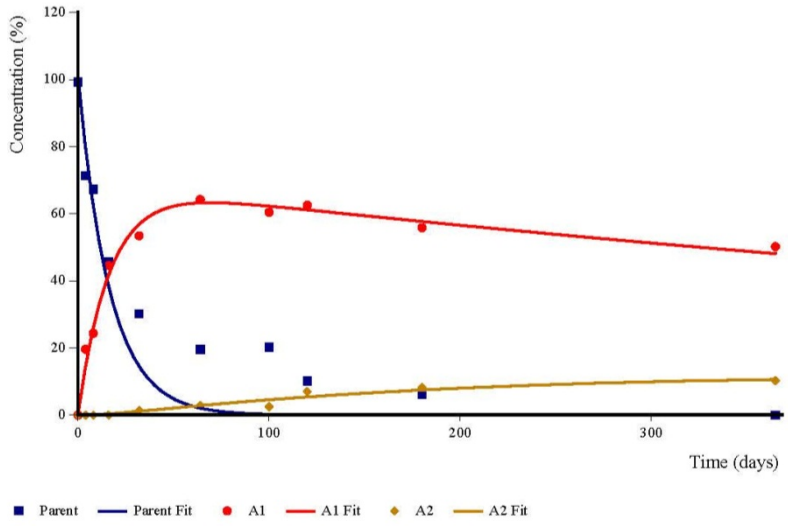
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No
f_Parent_to_A1	0.5	0 to 1	No
k_A1	0.1	0 to (unbounded)	No
f_A1_to_A2	0.5	0 to 1	No
k_A2	0.1	0 to (unbounded)	No

Fit step: Final

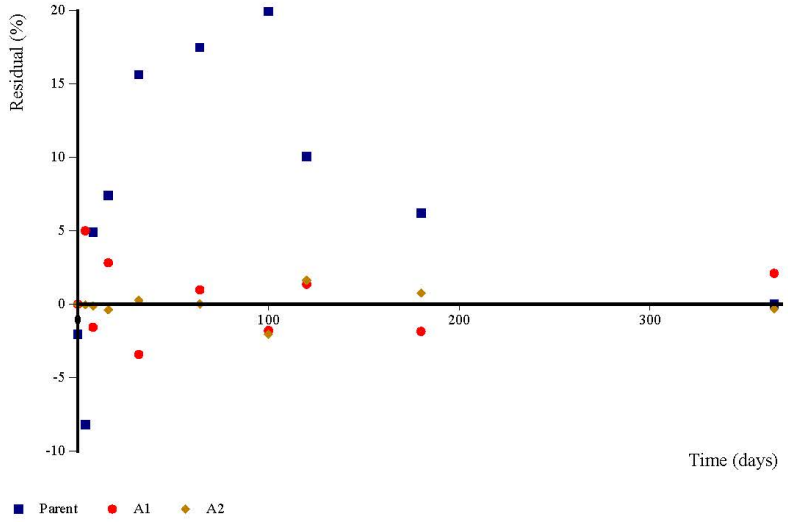
Graphical Summary:

Observations and Fitted Model:

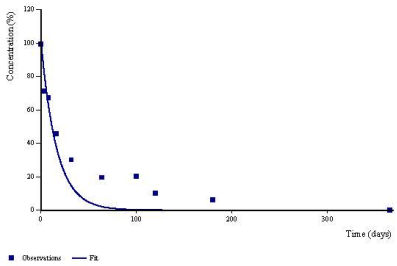


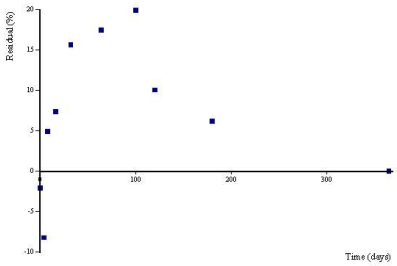
Internal Use Only

Residuals:



Compartment Parent:





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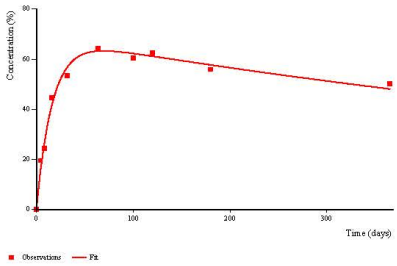
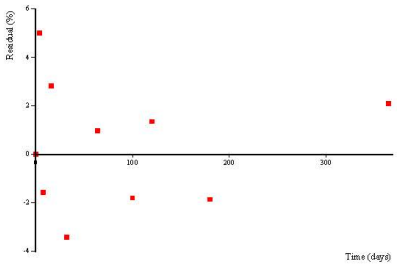
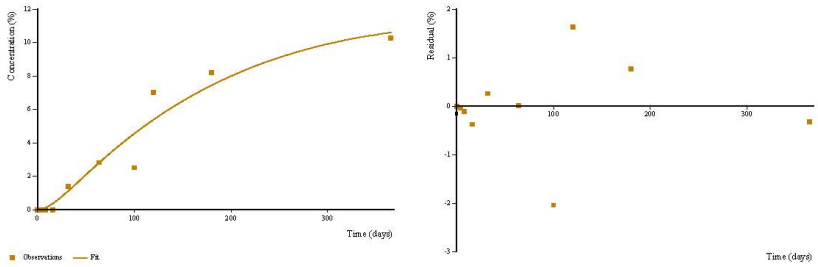




Compartment A2:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	102.2	0 to (unbounded)	No
k_Parent	0.06321	0 to (unbounded)	No
f_Parent_to_A1	0.6501	0 to 1	No
k_A1	0.0008425	0 to (unbounded)	No
f_A1_to_A2	1	0 to 1	No
k_A2	0.0026	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	101.4	8.751	1.292E-11	83.31	119.4
k_Parent	0.06073	0.005529	3.817E-11	0.04932	0.072
f_Parent_to_A1	0.6683	0.06592	1.878E-10	0.5323	0.804
k_A1	0.0009823	0.0002245	0.0001019	0.0005188	0.001
f_A1_to_A2	1	0.2986	0.001337	0.3837	1.616
k_A2	0.003716	0.001452	0.008616	0.0007187	0.007

χ^2

Parameter	Error %	Degrees of Freedom
All data	21.46	24
Parent	24.16	8
A1	4.365	9
A2	20.95	9

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	11.41	37.91
A1	705.7	2344
A2	186.5	619.6

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9582	0.9442
Parent	0.9603	0.8727
A1	0.9874	0.9855
A2	0.9432	0.9427

Parameter Correlation:

	Parent_0	k_Parent	f_Parent_to_A1	k_A1	f_A1_to_A2	k_A2
Parent_0	1	0.2512	-0.9351	-0.133	0.1067	0.007836
k_Parent	0.2512	1	-0.4585	-0.5293	0.4248	0.0312
f_Parent_to_A1	-0.9351	-0.4585	1	0.4086	-0.3483	-0.05298
k_A1	-0.133	-0.5293	0.4086	1	-0.842	-0.1843
f_A1_to_A2	0.1067	0.4248	-0.3483	-0.842	1	0.6361
k_A2	0.007836	0.0312	-0.05298	-0.1843	0.6361	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	99.33	101.4	-2.039
4	71.3	79.51	-8.207
8	67.27	62.36	4.911
16	45.77	38.36	7.408
32	30.16	14.52	15.64
64	19.56	2.079	17.48
100	20.18	0.2335	19.95
120	10.13	0.06932	10.06
180	6.21	0.001813	6.208
365	0	1.516E-06	-1.516E-06

Compartment A1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
4	19.59	14.58	5.008
8	24.39	25.96	-1.571
16	44.55	41.73	2.821
32	53.45	56.87	-3.42
64	64.24	63.25	0.9853
100	60.47	62.26	-1.792
120	62.52	61.16	1.361
180	55.85	57.7	-1.852
365	50.22	48.11	2.105

Compartment A2

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
4	0	0.02968	-0.02968
8	0	0.1093	-0.1093
16	0	0.3735	-0.3735
32	1.39	1.125	0.265
64	2.83	2.819	0.0114
100	2.51	4.549	-2.039
120	7.02	5.392	1.628
180	8.22	7.45	0.7699
365	10.29	10.61	-0.3187

Sequence Creation Information:
Fit generated by CAKE version 1.4 (Release)
running on R version 2.12.2 (2011-02-25)

Report Information:
Report generated by CAKE version 1.4 (Release)
CAKE developed by Tessella Plc, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 2.0.50727.5477

14.3.4 *Clay loam – FBZ / OXF / FBZ-SO_2 – SFO*

CAKE Kinetic Evaluation Report

Study: New Study

Study date: 06-May-13
Report generated: 12-Jun-14

Clay loam (SFO)

Model Setup:

Topology: Parent, A1, A2
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)

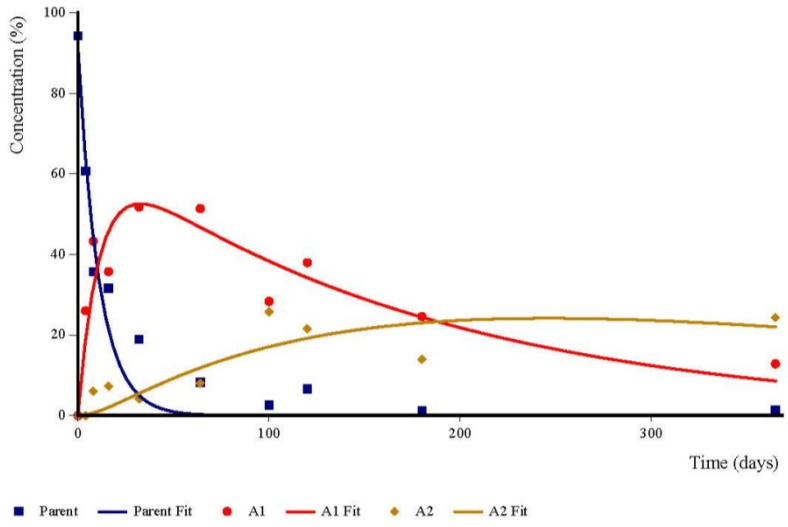
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No
f_Parent_to_A1	0.5	0 to 1	No
k_A1	0.1	0 to (unbounded)	No
f_A1_to_A2	0.5	0 to 1	No
k_A2	0.1	0 to (unbounded)	No

Fit step: Final

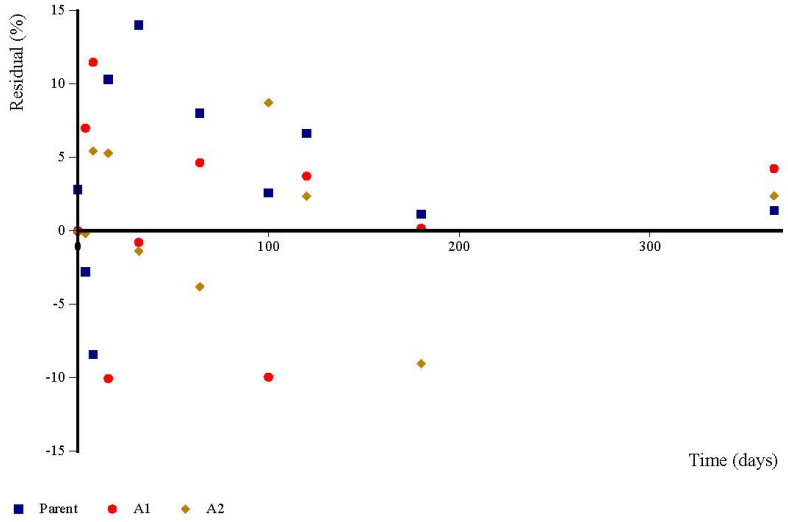
Graphical Summary:

Observations and Fitted Model:

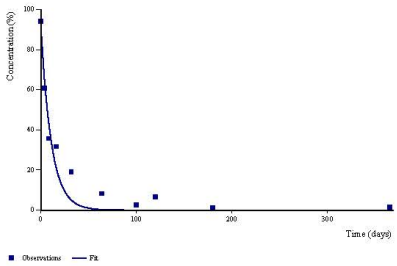


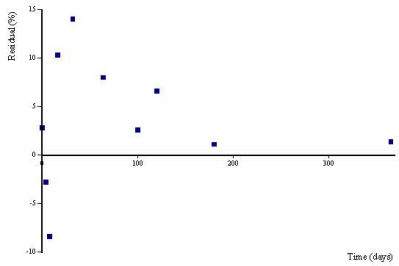
Internal Use Only

Residuals:

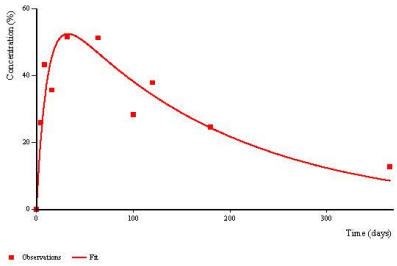


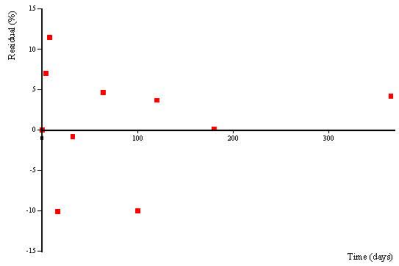
Compartment Parent:



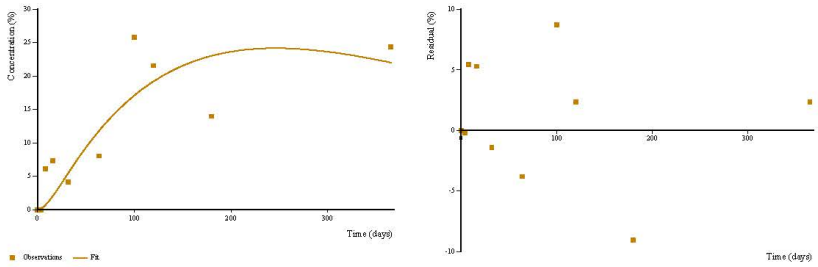


Compartment A1:





Compartment A2:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	91.28	0 to (unbounded)	No
k_Parent	0.09012	0 to (unbounded)	No
f_Parent_to_A1	0.6923	0 to 1	No
k_A1	0.005606	0 to (unbounded)	No
f_A1_to_A2	0.8033	0 to 1	No
k_A2	0.00316	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	91.47	6.996	1.038E-12	77.03	105.9
k_Parent	0.09092	0.01477	1.158E-06	0.06045	0.121
f_Parent_to_A1	0.6909	0.1041	3.658E-07	0.476	0.906
k_A1	0.005633	0.001473	0.0004106	0.002593	0.009
f_A1_to_A2	0.7988	0.3224	0.01033	0.1333	1.464
k_A2	0.003133	0.002218	0.0853	-0.001444	0.008

χ^2

Parameter	Error %	Degrees of Freedom
All data	24.99	24
Parent	21.87	8
A1	16.25	9
A2	34.05	9

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	7.623	25.32
A1	123.1	408.8
A2	221.3	735

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.929	0.9152
Parent	0.9631	0.9403
A1	0.8411	0.8212
A2	0.7409	0.7157

Parameter Correlation:

	Parent_0	k_Parent	f_Parent_to_A1	k_A1	f_A1_to_A2	k_A2
Parent_0	1	0.5188	-0.7019	-0.2063	0.1697	0.06403
k_Parent	0.5188	1	-0.6381	-0.3977	0.327	0.1234
f_Parent_to_A1	-0.7019	-0.6381	1	0.6647	-0.6077	-0.2678
k_A1	-0.2063	-0.3977	0.6647	1	-0.7913	-0.4363
f_A1_to_A2	0.1697	0.327	-0.6077	-0.7913	1	0.7886
k_A2	0.06403	0.1234	-0.2678	-0.4363	0.7886	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	94.28	91.47	2.813
4	60.78	63.58	-2.799
8	35.78	44.19	-8.414
16	31.67	21.35	10.32
32	19.01	4.985	14.03
64	8.28	0.2717	8.008
100	2.6	0.01029	2.59
120	6.63	0.00167	6.628
180	1.13	6.975E-06	1.13
365	1.39	2.674E-07	1.39

Compartment A1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
4	26.04	19.04	7.001
8	43.33	31.85	11.48
16	35.77	45.83	-10.06
32	51.8	52.58	-0.7827
64	51.42	46.78	4.645
100	28.38	38.35	-9.966
120	37.99	34.27	3.724
180	24.61	24.44	0.1705
365	12.86	8.62	4.24

Compartment A2

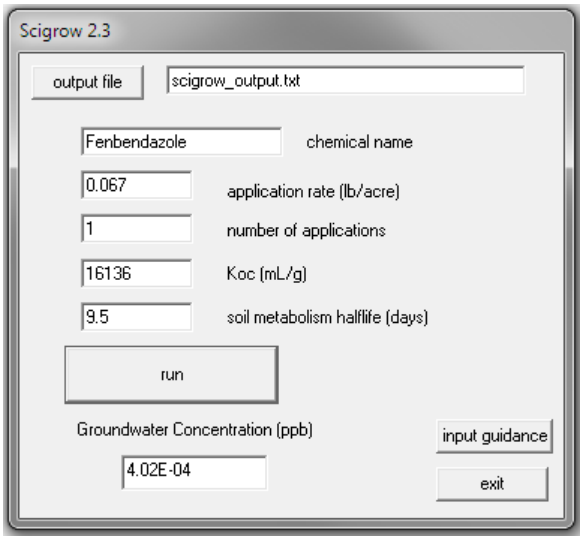
Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
4	0	0.1817	-0.1817
8	6.08	0.6424	5.438
16	7.33	2.044	5.286
32	4.11	5.497	-1.387
64	8.05	11.85	-3.805
100	25.81	17.08	8.728
120	21.57	19.21	2.364
180	13.99	23.03	-9.044
365	24.4	22.03	2.374

Sequence Creation Information:
Fit generated by CAKE version 1.4 (Release)
running on R version 2.12.2 (2011-02-25)

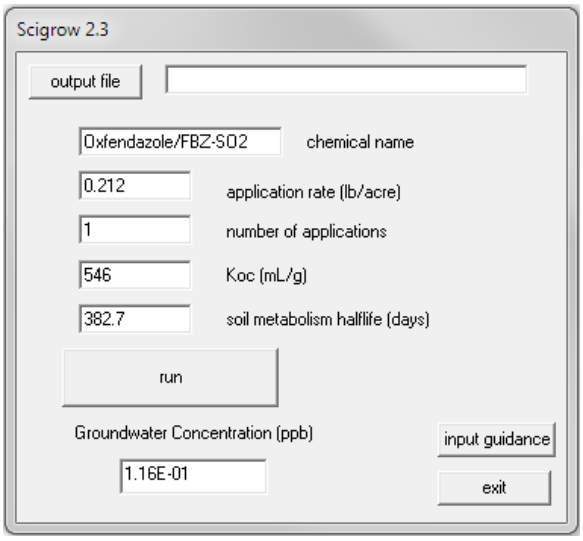
Report Information:
Report generated by CAKE version 1.4 (Release)
CAKE developed by Tessella Plc, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 2.0.50727.5477

14.4 **SCI-GROW calculations**

14.4.1 ***FBZ***



14.4.2 ***OXF/FBZ-SO$_2$***



14.5 **Survival of neonate daphnids with/without feeding during exposure to OXF**

The results of a non-GLP trial to compare the survival of neonate daphnids with and without feeding during exposure to OXF are presented below. The trial was conducted at concentrations of 50 and 100 µg/L under static conditions for 48 h. Two replicate test chambers were prepared for each treatment concentration. Each replicate contained 5 neonate daphnids <24 h old at initiation of the exposure. One replicate in each treatment group received feed (0.5 mL of YCT supplemented with 1.0 mL of green algae, *Pseudokirchneriella subcapitata*, and 0.40 mL of combine vitamin stock solution) once daily, while the other replicate received no feed. The feeding was equivalent to 0.12 mg C/daphnid/day, which is within the rate recommended by OECD GL 211. At the end of the trial, no immobility was observed in the treatment groups receiving feed during the exposure period. However there were 40 and 100% immobility noted in the 50 and 100 µg/L treatment groups, respectively for the replicates receiving no feed during the exposure. The results of the trial for Daphnia not receiving feed are comparable to the results of the acute study of Brougher et al. (2013) (percent immobility at 45 and 100 µg/L was 20 and 95%, respectively).

OXFENDAZOLE

RESULTS OF A STATIC RANGEFINDING TEST WITH *Daphnia magna*

STUDY: Oxfendazole: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)
SPONSOR: Merck & Co., Ltd.
PROJECT NO.: 105A-205

Nominal Concentration [1] (µg a.i./L)	Number Immobile in 24-Hour Period / Cumulative Number Immobile / Number Originally Exposed (Observations [3])		Cumulative Percent Immobility
	24 Hours	48 Hours	
Fed during Test			
50	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0
100	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0
Not Fed During Test			
50	0 / 0 / 5 (5 AN)	2 / 2 / 10 (3 AN)	40
100	0 / 0 / 5 (5 AN)	5 / 5 / 5	100

[1] Test solution appearance: All clear and colorless at test initiation and termination.
[2] Observations: AN = appear normal.