Document Title

Tier 2 Summary

of Metabolism and Residues Data

for the active substance Fenhexamid (KBR 2738)

(Specification no.: 1020000006806)

Substance(s)

FENHEXAMID

(Annex I renewal)

Data Requirements

Regulation EC/1141/2010

on the renewal of the inclusion of AIR2 active substances

in conjunction with

Directive 91/414/EEC and **Regulation EC/1107/2009**

According to OECD format guidance for industry data submissions

(SANCO/10387/2010 rev. 8 - on the renewal of active substances included in Annex I)

Annex II

Document M

Section 4, Point 6

Date

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Bayer CropScience

OWNERSHIP STATEMENT

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IIA 6 Metabolism and Residues Data

This document is a revision of the metabolism and residue chapter evaluated in the EU listing process (Annex I) of Fenhexamid and was prepared with the purpose of supporting the Annex I renewal.

Plant and animal metabolism studies were submitted with the original EU dossier and these have concluded that the parent active substance is the main residue since no metabolite exceeded 10% of the total radioactive residue in any study performed. Additional plant metabolism studies were conducted later in lettuce and field pea to potentially support additional crops and they are included in this updated EU dossier together with a confined rotational crop study to support uses where a succeeding crop scenario could occur. From all available plant studies it could be concluded that the metabolism pattern was consistent across all tested plant species.

A goat metabolism study, not reported in the original dossier because the intended uses were on crops not relevant as feed items, was conducted and evaluated with the JMPR review in 2005. The study is included in this AIR dossier for the sake of completeness (the RMS may decide if this study should be evaluated or not).

Residue studies in/on stone fruit (nectarines, peaches, cherries, plums), berries and small fruit (grapes, strawberries, raspberries), kiwi and fruiting vegetables (tomatoes) were included in the initial dossier. The representative uses chosen for the Annex I renewal are: grapes, strawberries and tomatoes and the GAPs supported for the inclusion renewal are the same as those evaluated in the first inclusion.

During the EU review further grape trials conducted in the EU were submitted and subsequently evaluated (ECCO Peer Review Meetings, 'Full Report on Fenhexamid' ECCO Team at BBA, Braunschweig of 28 February.).
As a registration on grapes was granted in the USA during the Peer Review process, Bayer AG recommended reconsidering the grape MRL proposal given in the draft assessment report (2 mg/kg) by submitting the US data so that the MRL would also cover imports (ECCO Peer Review Meetings, 'Full Report on Fenhexamid' ECCO Team at BBA, Braunschweig of 28 February 2000, pages 155 – 186).
The data submitted were considered sufficient to derive processing factors, but one open point was the recalculation of material balances where the necessary data are available. Two processing studies on grapes are submitted with this AIR dossier providing information on mass balances (preparation of wine and raisins).

Relative to the metabolism and residue section all further data requirements addressed in the 'Full Report on Fenhexamid' (ECCO Peer Review Meetings, ECCO Team at BBA, Braunschweig of 28 February) were fulfilled.

Further residue trials on tomatoes, cucumber, peppers, lettuce, green bean and onions were submitted on national level and MRLs were set for these additional uses.

In the process of the MRL review program under Article 12/2 of the MRL Reg. 396/2005, Tier I Summaries from all trials (trials from the original dossier, additional European grape trials and US data on grapes) were provided to the RMS (CRD) so that all necessary data are already available. Therefore, no field residue data will be included in the amended Annex II dossier.

The *confined rotational crop* study showed relatively low transfer of soil residues to rotational crops, especially when they were sown approximately 130 days after the application onto bare soil. Since the crops supported during the 1st inclusion are not considered relevant to be grown in rotations, establishing MRLs in rotational crop commodities is not required.

Livestock feeding studies were not conducted since the simulation of the feed-to-food transfer was regarded as not relevant due to the crops to be applied.

The TMDI for a 60-kg adult is 5.4% of the ADI, based on the FAO/WHO European diet (Final review report for the active substance fenhexamid, European Commission, 6497/VI/99-rev. 2 of 19 October 2000). The total NEDIs (UK diet) for adults, children and infants were max. 4% of the ADI (ECCO Peer Review Meetings, 'Full Report on Fenhexamid' ECCO Team at BBA, Braunschweig of 28 February 2000).
An ARfD was not derived and therefore an acute exposure does not have to be calculated.
The chronic dietary risk assessment is updated applying the EFSA PRIMo model (version 2) for estimation on the dietary intake of pesticide residues.

IIA 6.1 Stability of residues

IIA 6.1.1 Stability of residues during storage of samples

The stability of residues during storage of samples was demonstrated during the EU evaluation process by means of all supervised residue trials (including further grape trials subsequently submitted and evaluated). The stability of fenhexamid derived residues upon deep frozen storage was investigated in various matrices and the maximum storage period estimated. Further details on residue stability during samples storage can be found in the EU Monograph and the 'Full Report on Fenhexamid' (ECCO Peer Review Meetings, 2000).

IIA 6.1.2 Stability of residues in sample extracts

The storage stability of pesticide residues in sample extracts is generally checked during the development of the applicable analytical residue methods.

Additionally, during residue analyses on regular sample sets, the analytical performance of the methods must be checked with concurrent recoveries on each sample set. Therefore the relevant information on the stability in the final or any intermediate step can be derived from the fortification experiments performed during method validation. If the recoveries in the fortified samples are within the acceptable range, stability is sufficiently proven.

IIA 6.2 Metabolism, distribution and expression of residues

Plant metabolism
In the original EU dossier three plant metabolism studies were conducted with [phenyl-UL-

^{14}C]fenhexamid in grapes, tomatoes and apples. They were all considered appropriate in the initial evaluation. The following conclusions were drawn for the metabolism of fenhexamid in plants:
Parent active substance is the main residue. No metabolite exceeded 10% of the total radioactive residue in any study. Metabolites were formed by hydroxylation and by conjugation of the active substance. Metabolites arising from cleavage of the parent molecule were not found.
The metabolism of fenhexamid in plants is well characterized and the active substance was defined as the only component of the residue in food.
Additional plant metabolism studies were conducted later in lettuce (1999, Doc no. **M-005762-01-1**) and field pea (1999, Doc. no. **M-016814-01-1**) to potentially support additional crops. These two target crop studies are included in the present dossier to allow a common overview on a wide range of crops. Furthermore, a confined rotational crop study (1997, Doc. no. M-003800-01-1) was prepared to be able to support uses where a succeeding crop scenario could occur. This confined rotational crop study is also included in the present EU dossier. The five plant metabolism studies and the confined rotational crop study were also included in the JMPR dossier (2005). It can be concluded from all available plant studies that the metabolism pattern was consistent across all tested plant species.
Structures, report names and further information of parent compound and metabolites are given in the list of metabolites presented in Document N.

The mg/kg-values or ppm values of fenhexamid (KBR 2738) and of metabolites in tables and text are expressed as parent compound equivalents (mg a.s. equivalents/kg), if not otherwise stated.

IIA 6.2.1 In plants, at least three crops from three different crop categories

Report:	**KIIA 6.2.1 /01; [redacted] 1999**
Title:	Metabolism of KBR 2738 in Lettuce
Report No & Document No	MR-860/98 **M-005762-01-1**
Guidelines:	US EPA Residue Chemistry Test Guideline OPPTS 860.1300 Nature of the Residue – Plants
GLP	yes

Executive Summary

Lettuce plants were treated twice with [phenyl-UL-^{14}C]KBR 2738 (formulated as WP 50) in a greenhouse study. Applications were conducted using a computer controlled track sprayer with a flat fan nozzle and corresponded to a field application rate of 0.843 kg a.s./ha each. The first application was conducted approx. 5 weeks before harvest, followed by a second application approx. 4 weeks later (day 0), 7 days before harvest. A total of 92.8 mg a.s. (2 x 46.4 mg a.s.) was applied to the test area (approx. 0.55 m^2, ten plants) corresponding to a field application rate of 1.687 kg a.s./ha.
The total radioactive residue (TRR) in lettuce (day 7) amounted to 19.83 mg/kg parent compound equivalents as determined by summation of the radioactivity in the combined methanol/water extracts and the solids. The majority (98.1% of TRR, 19.44 mg/kg) was readily extracted by homogenisation with methanol and methanol/water. Following extraction, 92.2% (18.28 mg a.s. equiv./kg) partitioned into the dichloromethane phase, 5.9% (1.16 mg a.s. equiv./kg) remained in the aqueous phase, and 1.9% (0.39 mg a.s. equiv./kg) was not extracted.
The results of the chromatographic analyses at day 7 are given in Table 6.2.1-2. In total, 93.6% of the TRR in lettuce was identified, and further 4.5% was characterised.

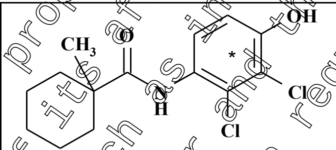
The major radioactive component identified was unchanged parent compound, which amounted to approximately 91% (18 mg/kg). The main metabolites were the glucoside of KBR 2738 (M01) with 0.3% (0.06 mg a.s. equiv./kg) and the malonyl glucoside of KBR 2738 (M02) with 2.6% of TRR (0.51 mg a.s. equiv./kg). At least 9 metabolites were characterised, not exceeding 1.9% of TRR, each. It was shown by TLC analysis with a solvent system which is especially suitable for the investigation of 2,3-dichloro-4-hydroxyaniline (DCHA) that DCHA was not a metabolite in lettuce.

The proposed metabolic pathway of KBR 2738 in lettuce was the direct conjugation of the aromatic hydroxyl group with glucose or glucose and malonic acid. The presence of small amounts of 2-hydroxy-KBR 2738 glucoside and 4-hydroxy-KBR 2738 glucoside were additionally derived from hydrolysis experiments.

I. Material and Methods

A. Materials

1. Test Material

Chemical structure		* position of the radiolabel
Radiolabelled test material	[phenyl-UL-^{14}C]KBR 2738	
Specific radioactivity	1.70 MBq/mg (45.9 µCi/mg)	
Radiochemical purity	> 99% (HPLC and TLC)	
Application rate	Two spray applications each at 0.843 kg a.s./ha	
Preparation of application solution	The WG 50 formulation was simulated by homogenising the active ingredient with the blank formulation of the WP 50. The application solution was prepared by dissolving the formulation in 100 ml of water.	

2. Soil: [redacted]3 (Germany), sandy loam soil, 1.98% organic carbon, pH 6.3 ($CaCl_2$), cation exchange capacity (CEC) 10 [meq/100 g]

3. Plant: Lettuce, variety Victoria King,
representative for crop group: leafy vegetables

B. Study Design

Experimental conditions:

Growth:

Lettuce was sown and plants were transplanted into small pots after 7 days. After 13 days they were transplanted into a 1 m² planting container which was filled with a sandy loam soil. Plants were grown in a greenhouse (see table).

Growth of lettuce in the greenhouse	Temp. (° C)	Day	Temp. (° C)	Night
	20	6.00 am - 8.00 pm	14	8.00 pm - 6.00 am

Application:

The application conditions simulated the practice conditions of two spray applications to lettuce, each

at 750 g a.s./ha in a spray volume of 1000 l/ha. The target rates corresponded to the anticipated maximum application rates in agricultural practice. The first application was conducted immediately after transplantation of the lettuce plants at the 5 leaves stage (growth stage 15 of the BBCH code) ca. 5 weeks before harvest using a planting container. The second application (day 0) was conducted 7 days before harvest according to the intended use in practice when ca. 50% of the final size was reached. As a result, a total of 92.8 mg a.i. was applied (46.4 mg a.s. x 2) to ten lettuce plants grown on a test area of 0.55 m² corresponding to a field rate of 1687 g a.s./ha.

Sampling:

The ten lettuce plants were harvested 7 days after the second application. The ten plants were combined, weighed (1359.2 g harvest weight) and homogenised in liquid nitrogen. The samples were stored in aliquots of 50 g to ca. 400 g at -20°C or below.

C. Analytical Procedures

Extraction:

An aliquot (200.0 g) of the homogenised lettuce was successively macerated with methanol (2x ca. 300 ml) and methanol/water 1:1 (v/v, ca. 300 ml) using a Polytron homogeniser. The suspension was filtered by suction yielding the methanol/water extract (combined filtrates) and the solids (non-extractable residue). The methanol/water extract was evaporated to the aqueous remainder at ca. 40°C using a rotary evaporator. The aqueous remainder was extracted with dichloromethane (3x ca. 300 ml) leaving the aqueous phase (167 ml). The dichloromethane solution was concentrated yielding the dichloromethane phase (100 ml). For the combustion of aliquots, the solids were air-dried.
Enzymatic hydrolyses (β-glucosidase, cellulase) and separate chemical hydrolysis with 1 N hydrochloric acid by heating under reflux were additionally conducted with the aqueous phase to evaluate the significance of hydrolysis products (aglycons). The obtained hydrolysis products were extracted with ethyl acetate (for the chemical hydrolysis only after neutralisation) and analysed by TLC.

Quantitation:

Parent compound and metabolites in the extracts (phases) were quantified by TLC.

Identification and characterisation:

Parent compound and metabolites were identified by TLC co-chromatography using reference compounds, ^{14}C-reference compounds from the apple metabolism study, and mass spectroscopy. HPLC was used for the fractionation of phases.

Storage stability:

The early extraction after harvest (starting on the same day) and the comparison of metabolite structures with other studies assured that the reported pattern of parent compound and metabolites adequately reflected the residue components at harvest.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]KBR 2738 was investigated in lettuce following two spray applications. A very high portion of radioactivity was extracted by conventional extraction (98.1% of the TRR) as shown in Table 6.2.1-1.

Table 6.2.1-1: Extraction of lettuce (day 7) following two spray applications of [phenyl-UL-^{14}C]KBR 2738 at a total field rate of 1.687 kg a.s./ha

	% TRR	ppm
TRR	100.0	19.83
methanol/water extracts	[98.1]	[19.44]
dichloromethane phase	92.2	18.28
aqueous phase	5.9	1.16
Total extracted	98.1	19.44
Unextractable (post extraction solids, PES)	1.9	0.39

Table 6.2.1-2: Residues in lettuce (day 7) following two spray applications of [phenyl-UL-^{14}C]KBR 2738 at a total field rate of 1.687 kg a.s./ha

Compounds and ^{14}C-Fractions	**% TRR**	**ppm**
TRR	100.0	**19.83**
KBR 2738, parent compound	**90.7**	**17.99**
M01 (glucoside of KBR 2738)	0.3	0.06
M37 (malonyl glucoside of KBR 2738) [a]	2.6	0.51
Total identified	**93.6**	**18.56**
U10 (aqueous phase)	0.7	0.13
U8, U11 (aqueous phase) each ≤0.4 %, ≤0.09 mg/kg	0.8	0.16
U7 (aqueous phase)	0.3	0.05
U4, U5, U6, (aqueous phase) each ≤0.2 %, ≤0.05 mg/kg	0.6	0.12
U1, U9 (aqueous phase), each ≤0.1 %, ≤0.02 mg/kg	0.2	0.04
TLC-origin (dichloromethane phase + aqueous phase)	1.9	0.37
Total characterised [b]	**4.5**	**0.89**
Total extractable	**98.1**	**19.44**
Unextractable (post extraction solids, PES)	1.9	0.39
Accountability	100.0	19.83

a) M37 was a completely identified member of the general group M02 (conjugate of KBR 2738)
b) unidentified metabolites were characterised by extraction and chromatographic behaviour

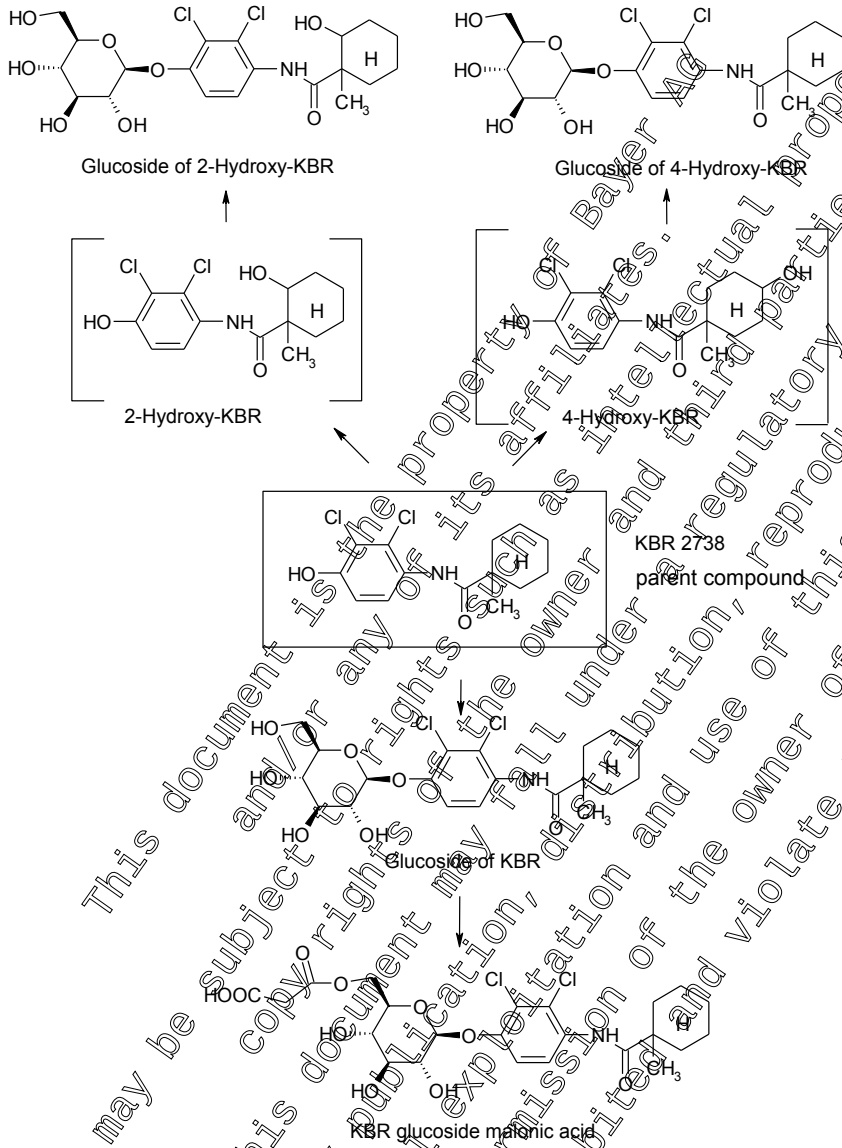
The chromatographic analyses of the radioactive residues in the extracts are shown in Table 6.2.1-2. KBR 2738 was the main residue accounting for 90.7% of the TRR in lettuce and two conjugated metabolites were identified. M01 (KBR-glucoside) amounted to 0.3% TRR. The second metabolite (2.6%) can be described with the general structure "M02" but the chemical structure was completely identified as M37 (KBR-glucose-malonic acid). This was proven by enzymatic and spectroscopic investigations. Hydrolysis experiments with the aqueous phase using enzymatic and chemical procedures revealed that 2-hydroxy-KBR 2738 and 4-hydroxy-KBR 2738 were present as metabolites but only in small quantities and only conjugated as glucosides which were not further quantified in detail. Special TLC analyses with an unpolar solvent system were conducted to investigate the presence of DCHA (M34) but this compound was not found as a metabolite.
The proposed metabolic pathway is shown in Figure 6.2.1-1. Two intermediate metabolites are shown in brackets.

III. Conclusions

The metabolism of the fungicide KBR 2738 was investigated in lettuce following spray application of [phenyl-UL-^{14}C] KBR 2738. Unchanged parent compound was the main residue. Two metabolites were identified and quantified individually. They were formed from KBR 2738 by conjugation of the aromatic hydroxyl group with glucose (resulting in M01) or glucose and malonic acid (resulting in

M37). The glucosides of 2-hydroxy-KBR 2738 and 4-hydroxy-KBR 2738 were found as metabolites in small quantities by enzymatic methods.

Figure 6.2.1-1: Proposed metabolic pathway of [phenyl-UL-^{14}C]KBR 2738 in lettuce



Report:	**KIIA 6.2.1 /02; [redacted] 1999**
Title:	Metabolism of KBR 2738 in field pea
Report No & Document No	MR-130/99 **M-016814-01-1**
Guidelines:	US EPA Residue Chemistry Test Guideline OPPTS 860.1300 Nature of the Residue – Plants
GLP	yes

Executive Summary

In a greenhouse study [phenyl-UL-^{14}C]fenhexamid (formulated as WP 50 / ingredients of a WG 50) was applied twice to field peas simulating practical spray application conditions. The first application was conducted at the beginning of flowering (growth stage 61) and the second application (day 0) when full flowering (growth stage 65) was reached according to the projected treatments in practice. The field peas were grown in a 1 m^2 planting container. A computer controlled track sprayer with a flat fan nozzle was used for application. The total application rate of the active substance amounted to 168.6 mg, which corresponded to a seasonal field rate of 1.686 kg a.s./ha. The field peas were harvested in four fractions and analysed in the metabolism study: hay (day 9), vines (day 21), pods incl. seeds (day 21), and dry seeds (day 77).

The TRR in separate field pea fractions was determined by summation of the radioactivity of the combined methanol/water extracts and in the solids after this solvent extraction, calculated in active substance equivalents. The TRR in hay of field peas was 24.02 mg a.s. equiv./kg, the TRR in vines was 14.32 mg a.s. equiv./kg and in pods was 0.23 mg a.s. equiv./kg. Finally, the TRR of dry seeds amounted to 0.20 mg a.s. equiv./kg.

The majority (93.5%) of the TRR in field pea hay (day 9) was readily extracted by homogenisation with methanol and methanol/water. Following extraction, 88.0% partitioned into the dichloromethane phase 1 and 5.4% remained in the aqueous phase 1. The solids of the first extraction step (6.5%) were exhaustively extracted with dioxane/2N HCl. A smaller amount of 1.0% partitioned from the extract into the dichloromethane phase 2 and 3.6% remained in the aqueous phase 2. A total of 2.0% (0.49 mg a.s. equiv./kg) remained unextracted (solids).

The distribution of TRR in vines and pods was similar to those in hay. In vines, a total of 1.5% (0.22 mg a.s. equiv./kg) was unextracted. In pods, the final solids amounted to 3.5% (≤ 0.01 mg a.s. equiv./kg).

The distribution of TRR in dry seeds differed from those of the other fractions. Only a relatively low portion of the radioactivity (31.0% of the TRR) was extracted by homogenisation with methanol/water. Following extraction, 17.0% (0.03 mg a.s. equiv./kg) partitioned into the dichloromethane phase 1, and 14.0% (0.03 mg a.s. equiv./kg) remained in the aqueous phase 1. The solids from the first extraction step were not only hydrolysed with dioxane/HCl but the resulting solids were additionally extracted with 1N KOH. From the hydrolysis extract, 14.2% (0.03 mg a.s. equiv./kg) partitioned into the dichloromethane phase 2, and the main portion of 28.0% (0.06 mg a.s. equiv./kg) remained in the aqueous phase 2. After hydrolysis, a relatively high amount of the TRR was still unextracted. The subsequent KOH extract (17.2%, 0.03 mg a.s. equiv./kg) was not further investigated due to the high matrix load. A total of 9.6% (0.02 mg a.s. equiv./kg) remained unextracted in the solids of dry seeds after both exhaustive extraction steps.

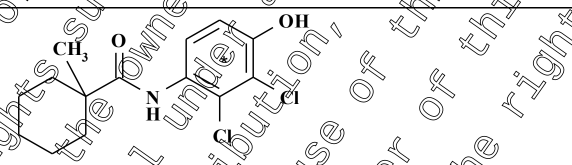
The major amount of the TRR of hay, vines, and pods was readily extracted using methanol/water and was mainly due to unchanged parent compound accounting for approximately 80% of the TRR. Further portions of 0.4% of the parent compound were identified in hay and vines, 3.7% in pods and

11.4% in dry seeds after exhaustive extraction using dioxane/2N HCl. The aqueous phases 1 (obtained after extraction with methanol/water) were further characterised by total hydrolysis using acidic (1N HCl) and enzymatic (β-glucosidase, cellulase) methods, followed by partition of the hydrolysis products (aglycons) with ethyl acetate and TLC analysis. This procedure allowed the identification of further amounts of parent compound (1.0 to 1.5%), as well as of low amounts of the two metabolites 2 hydroxy-KBR 2738 (M03) and 4-hydroxy-KBR 2738 (M06) obtained after hydrolysis of the respective conjugates. A couple of further unknown components were detected in low amounts and characterised by their TLC behaviour. Unconjugated hydroxylated derivatives of the parent compound were not identified in the field pea. The total amount of KBR 2738 obtained from all extracts and the quantitation of identified aglycons are given in Table 6.2.1-5 and Table 6.2.1-6.
In dry seeds only the parent compound was identified. However, the extraction of radioactive residues was more difficult and two exhaustive extraction steps were needed after methanol/water extraction (dioxane/2N HCl followed by 1N KOH, see above).
From these results it can be concluded that only the parent compound is relevant for the residue definition. Special care was taken for the investigation of DCHA (2,3-dichloro-4-hydroxyaniline = M34) as a possible aglycon following hydrolysis but it was not found in any analysed sample.

I. Material and Methods

A. Materials

1. Test Material

Chemical structure		* position of the radiolabel
Radiolabelled test material	[phenyl-UL-^{14}C]KBR 2738	
Specific radioactivity	1.70 MBq/mg (45.9 µCi/mg)	
Radiochemical purity	> 98% (HPLC and TLC)	
Application rate	Two spray applications each at 0.843 kg a.s./ha	
Preparation of application solution	The WG 50 formulation was simulated by homogenising the active ingredient with the blank formulation of the WP 50. The application solution was prepared by dissolving the formulation in 100 ml of water.	

2. Soil: [redacted]3 (Germany), sandy loam soil, 1.98% organic carbon, pH 6.3 ($CaCl_2$), cation exchange capacity (CEC) 10 [meq/100 g]

3. Plant: Field pea, variety Edula,
representative for crop group: pulses

B. Study Design

Experimental conditions:

Growth:

Field peas were sown in four rows into a 1 m² planting container which was filled with a sandy loam soil. Plants were grown in a greenhouse (see table).

Growth of field peas in the greenhouse	Temp. (° C)	Day	Temp. (° C)	Night
	19-20	6.00 am - 8.00 pm	13-14	8.00 pm - 6.00 am

Application:
The application conditions simulated the practice conditions of two spray applications to field peas, each at 750 g a.s./ha in a spray volume of 1000 l/ha. The target rates corresponded to the anticipated maximum application rates in agricultural practice. The first application was conducted at the beginning of flowering (growth stage 61 of the BBCH code). The second application (day 0) was conducted when full flowering was reached (growth stage 65 of the BBCH code). As a result, a total of 168.6 mg a.s. was applied (84.3 mg a.s. x 2) on peas on a test area of 1 m² corresponding to a field rate of 1686 g a.s./ha.

Sampling:
The first sample was taken as a hay fraction 9 days after the second application after full flowering through pod formation. A half row of pea plants was cut above the soil surface. The plant material (207.5 g) was cut in pieces and homogenised in liquid nitrogen. An aliquot (100.0 g) was used for immediate extraction and the rest was stored frozen (-20°C or below).
The second sample was taken when the pods and peas were in a succulent stage 21 days after the second application. One row of pea plants was cut above the soil surface and the plants were separated into pods and vines. Some of the pods were opened to check the size of the peas. As the peas were relatively small (ca. 2-4 mm diameter) the pods were not subdivided but analysed as one pod fraction. The pods (302.1 g) were cut in pieces and homogenised in liquid nitrogen. The vines were cut in pieces, weighed (516.4 g) and homogenised in liquid nitrogen. An aliquot (100.0 g) of pods and vines was used for immediate extraction and the rest was stored frozen.
The last sample was taken at maturity 77 days after the second application. The plants were cut above the soil surface. The dry seeds were removed from the pods and weighed (125.1 g). They were stored without homogenisation and 50.0 g were used for immediate extraction. The remaining parts of the pods were combined with the straw, weighed (211.3 g) and stored directly uncut. The straw was kept in reserve but was not used for the study.

C. Analytical Procedures
Extraction:
Conventional extraction of hay:
An aliquot (100.0 g) of the homogenised hay was successively macerated with methanol (2x ca. 200 ml) and methanol/water 1:1 (v/v, ca. 200 ml) using a Polytron homogeniser. The suspension was filtered by suction yielding the methanol/water extract (combined filtrates) and the solids 1. The methanol/water extract was evaporated to the aqueous remainder at ca. 40°C using a rotary evaporator. The aqueous remainder was extracted with dichloromethane (3x ca. 200 ml) leaving the aqueous phase 1 (73.5 ml). The dichloromethane solution was concentrated yielding the dichloromethane phase 1 (50 ml). For the combustion of aliquots, the solids 1 were airdried.

Exhaustive extraction of hay:
An aliquot (0.5 g of 8.87 g) of solids 1 of hay was hydrolysed with dioxane/2N HCI 9:1 (20 ml) for 1 hour in a closed vial at 100°C using a microwave. The suspension was filtered by suction and washed with small amounts of dioxane/2 N HCI yielding the dioxane/HCI extract (26 ml) and the solids (non-extractable residue). An aliquot (5 ml of 26 ml) of the dioxane/HCI extract was used for partitioning. Water was added (20 ml) and the extract evaporated to the aqueous remainder at ca. 40°C using a rotary evaporator. The aqueous remainder was extracted with dichloromethane (3x ca. 20 ml) leaving the aqueous phase 2 (17.5 ml). The dichloromethane solution was concentrated yielding the dichloromethane phase 2 (10 ml). Vines were extracted analogously to hay using the same amount of plant material (100.0 g) and solvent volumes as described above.

Conventional and exhaustive extraction of pods:
Pods were extracted analogously to hay using the same amount of plant material (100.0 g) and solvent volumes as described above. However, due to the low amount of radioactivity in the dioxane/HCI extract the partitioning procedure was not conducted.

Conventional and exhaustive extraction of dry seeds:
The conventional extraction of dry seeds was conducted analogously to hay, however, due to the dryness of the plant material and a lower amount available only 50.0 g were extracted using the same solvent volumes. An aliquot (2.0 g of 35.3 g,) of solids 1 was hydrolysed with dioxane/2N HCI 9:1 (20 ml) for 1 hour in a closed vial at 100°C using a microwave. The suspension was filtered by suction and washed with small amounts of dioxane/2 N HCI yielding the dioxane/HCI extract (29 ml) and the solids 2. An aliquot (10 ml of 29 ml) of the dioxane/HCI extract was used for partitioning. Water was added (30 ml) and the extract evaporated to the aqueous remainder at ca. 40°C using a rotary evaporator. The aqueous remainder was extracted with dichloromethane (3x ca. 20 ml) leaving the aqueous phase 2 (31 ml). The dichloromethane solution was concentrated yielding the dichloromethane phase 2 (10 ml).
The exhaustive extraction for dry seeds was completed by treatment of solids 2 with 1N KOH at room temperature for one hour yielding the KOH extract and the solids (nonextractable residue).

Quantitation:
Parent compound and metabolites in the extracts (phases) were quantified by TLC.

Identification and characterisation:
Parent compound and metabolites were identified by TLC co-chromatography using reference compounds, ^{14}C-reference compounds from the apple metabolism study, and mass spectroscopy. HPLC was used for the fractionation of phases.

Storage stability:
The early extraction of all samples after sampling or harvest (starting on the same day) and the comparison of metabolite structures with other studies assured that the pattern of parent compound and metabolites reflected the residue components at harvest.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]KBR 2738 was investigated in field pea following two spray applications. A very high portion of radioactivity was extracted by conventional extraction (92.8-93.5% of the TRR) for hay, vines and pods, however less (31.0%) for dry seeds as shown in Table 6.2.1-3 and Table 6.2.1-4.
The chromatographic analyses of the extracted radioactive residues are shown in Table 6.2.1-5 and Table 6.2.1-6. KBR 2738 was the main residue accounting for more than 80% of the TRR with the exception of dry seeds (only 20.9%). Unconjugated 2-hydroxy-KBR 2738 and 4-hydroxy-KBR 2738 were not present.

Hydrolysis experiments with the aqueous phase 1 using enzymatic (β-glucosidase, cellulase) and acidic methods (1 N HCl) revealed that 2-hydroxy-KBR 2738 and 4-hydroxy-KBR 2738 were present as glucosides but only in small quantities. For analysis, the hydrolysis products (aglycons) were partitioned with ethyl acetate and investigated by TLC.

Special TLC analyses with an unpolar solvent system were conducted to investigate the presence of DCHA (M34) but this compound was not found as a metabolite. This compound was also not found following exhaustive extraction.
The proposed metabolic pathway is shown in Figure 6.2.1-2.

Table 6.2.1-3 Extraction of field pea hay and vines following two spray applications of [phenyl-UL-^{14}C]KBR 2738 at a total field rate of 1.686 kg a.s./ha

	Hay (day 9)		Vines (day 21)	
	% TRR	ppm	% TRR	ppm
TRR	100.0	24.02	100.0	14.32
methanol/water extracts	[93.5]	[22.45]	[92.8]	[13.29]
dichloromethane phase 1	88.0	21.15	86.8	12.45
aqueous phase 1	5.4	1.30	6.0	0.86
dioxane/HCl extract *	[4.6]	[1.08]	[5.7]	[0.82]
dichloromethane phase 2	1.0	0.23	1.1	0.16
aqueous phase 2	3.6	0.85	4.6	0.66
Total extracted	98.0	23.53	98.5	14.10
Unextractable (post extraction solids, PES)	2.0	0.49	1.5	0.22

* extract was neutralised and partitioned into dichloromethane phase 2 and aqueous phase 2

Table 6.2.1-4 Extraction of field pea pods and dry seeds following two spray applications of [phenyl-UL-^{14}C]KBR 2738 at a total field rate of 1.686 kg a.s./ha

	Pods (day 21)		Dry seeds (day 77)	
	% TRR	ppm	% TRR	ppm
TRR	100.0	0.23	100.0	0.20
methanol/water extracts	[93.5]	[0.22]	[31.0]	[0.06]
dichloromethane phase 1	79.3	0.18	17.0	0.03
aqueous phase 1	14.2	0.03	14.0	0.03
dioxane/HCl extract	3.0	≤0.01	[42.2] *	[0.09] *
dichloromethane phase 2	-	-	14.2	0.03
aqueous phase 2	-	-	28.0	0.06
KOH extract	-	-	17.2	0.03
Total extracted	96.5	0.22	90.4	0.18
Unextractable (post extraction solids, PES)	3.5	≤0.01	9.6	0.02

* extract was neutralised and partitioned into dichloromethane phase 2 and aqueous phase 2

Table 6.2.1-5: Residues in field pea samples following two spray applications of [phenyl-UL-^{14}C]KBR 2738 at a total field rate of 1.686 kg a.s./ha

	Hay (day 9)		Vines (day 21)	
Compounds and ^{14}C-Fractions	**% TRR**	**ppm**	**% TRR**	**ppm**
TRR	100.0	24.02	100.0	14.32
KBR 2738, parent compound (sum of all extracts)	**87.1**	**20.94**	**86.4**	**12.38**
- KBR 2738, parent compound (dichloromethane phase 1)	85.7	20.60	84.5	12.10
- KBR 2738, parent compound (dichloromethane phase 2)	0.4	0.10	0.4	0.06
- KBR 2738, parent compound (from hydrolysed aqueous phase dissolved in ethyl acetate)	1.0	0.24	1.5	0.22
M01 (glucoside of KBR 2738)	-	-	-	-
M02 (conjugate of KBR 2738)	-	-	-	-
M37 (malonyl glucoside of KBR 2738)	-	-	-	-
M03 (2-hydroxy-KBR 2738)	0.3	0.06	0.4	0.05
M04 (glucoside of 2-hydroxy-KBR 2738)	-	-	-	-
M06 (4-hydroxy-KBR 2738)	0.3	0.06	0.3	0.04
M07 (glucoside of 4-hydroxy-KBR 2738)	-	-	-	-
M08 (conjugate of 4-hydroxy-KBR 2738)	-	-	-	-
M08 (conjugate of 4-hydroxy-KBR 2738)+other hydroxy-KBR 2738 metabolites)	-	-	-	-
Total identified	**87.7**	**21.06**	**87.1**	**12.47**
sum of hydrolysis products from aqueous phase 1 dissolved in ethyl acetate	0.3	0.07	0.3	0.05
hydrolysis products remaining in aqueous phase 2	1.0	0.24	1.4	0.19
TLC-origin (dichloromethane phase 1)	2.3	0.55	2.3	0.33
TLC-origin (dichloromethane phase 2)	0.6	0.13	0.7	0.10
TLC-origin (ethyl acetate phase of hydrolysed aqueous phase 1)	0.6	0.14	0.4	0.06
TLC-origin (aqueous phase 2)	2.6	0.61	3.3	0.47
radioactivity partitioned into aqueous phase after acidic hydrolysis of aqueous phase 1	3.0	0.73	3.1	0.44
Total characterised [b)]	**10.4**	**2.47**	**11.5**	**1.64**
Total extractable	**98.0**	**23.53**	**98.5**	**14.10**
Unextractable (post extraction solids, PES)	2.0	0.49	1.5	0.22
Accountability	100.0	24.02	100.0	14.32

Table 6.2.1-6: Residues in field pea samples following two spray applications of [phenyl-UL-^{14}C]KBR 2738 at a total field rate of 1.686 kg a.s./ha

	Pods (day 21)		Dry seeds (day 77)	
Compounds and ^{14}C-Fractions	**% TRR**	**ppm**	**% TRR**	**ppm**
TRR	100.0	0.23	100.0	0.20
KBR 2738, parent compound (sum of all extracts)	**81.2**	**0.19**	**20.9**	**0.04**
- KBR 2738, parent compound (dichloromethane phase 1)	77.5	0.18	9.5	0.02
- KBR 2738, parent compound (dichloromethane phase 2)	3.7	≤0.01	11.4	0.02
- KBR 2738, parent compound (from hydrolysed aqueous phase dissolved in ethyl acetate)	-	-	n.d.	n.d.
M01 (glucoside of KBR 2738)	-	-	-	-
M02 (conjugate of KBR 2738)	-	-	-	-
M37 (malonyl glucoside of KBR 2738)	-	-	-	-
M03 (2-hydroxy-KBR 2738)	n.d.	n.d.	n.d.	n.d.
M04 (glucoside of 2-hydroxy-KBR 2738)	-	-	-	-
M06 (4-hydroxy-KBR 2738)	0.4	<0.01	n.d.	n.d.
M07 (glucoside of 4-hydroxy-KBR 2738)	-	-	-	-
M08 (conjugate of 4-hydroxy-KBR 2738)	-	-	-	-
M08 (conjugate of 4-hydroxy-KBR 2738)+other hydroxy-KBR 2738 metabolites)	-	-	-	-
Total identified	**81.6**	**0.19**	**20.9**	**0.04**
diffuse radioactivity in dichloromethane phase 1	-	-	4.7	0.01
sum of hydrolysis products from aqueous phase 1 dissolved in ethyl acetate	n.d.	n.d.	0.9	<0.01
dioxane/HCl extract	3.0	≤0.01	-	-
unpolar radioactivity/hydrolysis products remaining in aqueous phase 2	-	-	8.2	0.02
diffuse radioactivity/hydrolysis products remaining in aqueous phase 2	-	-	13.7	0.03
TLC-origin (dichloromethane phase 1)	1.8	<0.01	2.8	<0.01
TLC-origin (dichloromethane phase 2)	-	-	2.8	<0.01
TLC-origin (ethyl acetate phase of hydrolysed aqueous phase 1)	0.7	<0.01	-	-
TLC-origin (aqueous phase 2)	-	-	6.0	0.01
radioactivity partitioned into aqueous phase after acidic hydrolysis of aqueous phase 1	9.5	0.02	13.1	0.03
radioactivity of the KOH extract	-	-	17.2	0.03
Total characterised [b]	**15.0**	**0.03**	**69.4**	**0.14**
Total extractable	**96.5**	0.22	**90.4**	**0.18**
Unextractable (post extraction solids, PES)	3.5	≤0.01	9.6	0.02
Accountability	100.0	0.23	100.0	0.20

III. Conclusions

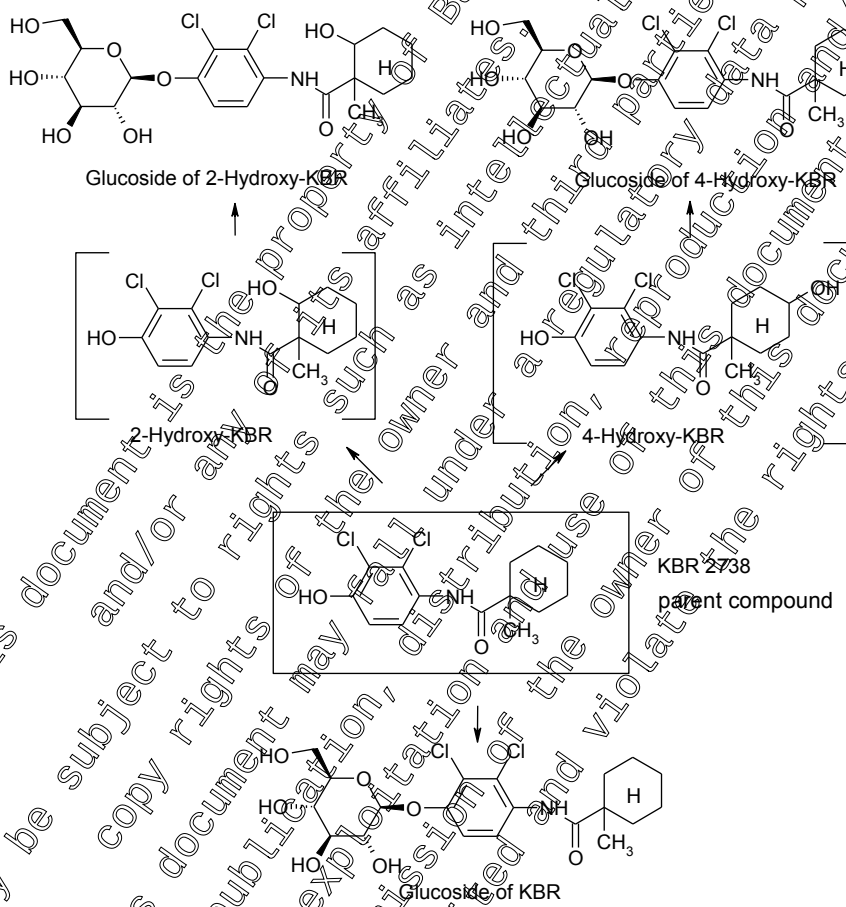
The metabolism of KBR 2738 in field pea proceeded via two basic pathways. The first was the conjugation of the parent compound with glucose at the aromatic hydroxyl group. The second was an oxidation of the cyclohexyl ring, leading to hydroxy-derivatives of the parent compound in the 2- and 4-positions followed by conjugation.

However, these metabolic changes occurred only to a small extent, the vast majority of radioactivity was mainly unchanged parent compound. From these results it can be concluded that only the parent compound is relevant for the residue definition.

This was in agreement with other metabolism studies conducted in grapes, tomatoes, apples and

lettuce. Also, the extracted radioactivity and the distribution into fractions was very similar and no cleavage of the amide structure was observed. Only dry seeds were more difficult to extract, but parent compound was also identified as the main component.
As a consequence, parent compound is considered to represent the relevant residue.
From the chromatograms and investigations it was concluded, that parent compound and metabolites were stable.

Figure 6.2.1-2: Proposed metabolic pathway of [phenyl-UL-^{14}C]KBR 2738 in field pea



Investigation on the possible metabolite DCHA (M34) in plants:

Report:	**KIIA 6.2.1 /03; [redacted], 1997**
Title:	Supplementary Report on the Investigation of 2,3-Dichloro-4-hydroxyaniline (DCHA) as a possible Metabolite of KBR 2738 in Plants.
Report No & Document No	MR-92/97 M-003792-01-1
Guidelines:	not applicable
GLP	no

Samples from the three plant metabolism studies in grapes, apples, and tomatoes were further investigated regarding 2,3-dichloro-4-hydroxyaniline (DCHA = M34) as a possible degradation product of fenhexamid in plants.

The majority of the extraction procedures and of the data in this study was already reported in the studies on the metabolism of fenhexamid in grapes, apples and tomatoes. Additionally, two hydrolysis experiments were conducted to confirm the hydrolytic stability of fenhexamid. Various soluble fractions were analysed for 2,3-dichloro-4-hydroxyaniline (DCHA) chromatographically (TLC and HPLC).

Surface wash solution, organic phase or aqueous phase of apples were analysed for DCHA by TLC with a very unpolar solvent system, well suited for chromatographic separation of DCHA from parent compound. Neither of the extracts contained DCHA. Additionally, the aqueous phase was treated enzymatically (β-glucosidase, cellulase) and with acidic hydrolysis. None of the treatments produced DCHA. The hydrolysis products detected were all derived from conjugates or cyclohexyl-hydroxylated derivatives of the parent compound. Examination of the detectable limits indicated that DCHA was not a metabolite in apples.

Similar investigations were conducted with extracts of grapes. The HPLC chromatogram of organic phase 1 showed that no DCHA was present. In the aqueous phase 1, the possible presence of trace amounts of DCHA was indicated by HPLC chromatography. However, the identity of this metabolite as DCHA was by no means definitively confirmed. But assuming this metabolite was DCHA then the total maximum amount of the TRR in grapes that could be possibly attributed to DCHA was only 0.12% (0.006 mg/kg).

Solutions of the tomato study were reanalysed by HPLC for the presence of DCHA. Metabolites in the aqueous phase were totally cleavable with enzymes to hydroxy compounds of the parent compound, thus showing that they were not DCHA. Therefore, this clearly showed that no DCHA was present in tomatoes.

For the hydrolysis experiments aliquots of [phenyl-UL-^{14}C]fenhexamid were evaporated to dryness and then heated under reflux with HCl and NaOH (both 1 mol/L), respectively. After cooling the solutions were neutralised, resolved in methanol and analysed by TLC and HPLC for DCHA. The HPLC investigation showed no DCHA in the solutions, but the TLC investigation indicated trace amounts (1.2% with NaOH, 2.2% with HCl). From the results of the hydrolysis experiments and the metabolism reports it was concluded that the amide group of fenhexamid was stable.

Extracted radioactivity and distribution into various fractions in apples, grapes and tomatoes was very similar. The vast majority of radioactivity was unchanged parent compound. No DCHA was detected in these plant metabolism studies, although from theoretical calculations trace amounts could have been present.

Note: The non-presence of DCHA was also confirmed by the lettuce metabolism study and the field pea metabolism study which were discussed above.

IIA 6.2.2 Poultry

A laying hen metabolism study was not conducted because the crops treated with fenhexamid like grapes or stone fruits are no feed item for laying hens.

IIA 6.2.3 Lactating ruminants (goat or cow)

A lactating goat metabolism study was conducted with [phenyl-UL-^{14}C]KBR 2738 and is presented in this dossier. The study was also included in the JMPR dossier prepared and submitted in 2005.

Report:	**KIIA 6.2.3 /01; [redacted], 1998**
Title:	[Phenyl-UL-^{14}C]KBR 2738 - Absorption, distribution, excretion and metabolism in the lactating goat
Report No & Document No	PF4387, date: 1998-09-01, amended 2000-09-13 M-004439-02-1
Guidelines:	EPA Pesticide Assessment Guidelines Subdivision O, Residue Chemistry, Series 171-4: Nature of Residue, Livestock (Ruminant) EPA 540/9-82-023, October 1982
GLP	yes

The kinetic behaviour and the metabolism of fenhexamid was investigated in the lactating goat. The test item [phenyl-UL-^{14}C]fenhexamid was administered in a tragacanth suspension to one lactating goat at the oral target dose level of 10 mg/kg body weight on three consecutive days in time intervals of 24 hours. Radioactivity was measured in the excreta, plasma and milk at different sampling intervals, and in the edible tissues kidney, liver, muscle and fat at sacrifice. The milk and edible tissues were analysed for parent compound and metabolites by extraction and chromatographic separation techniques (HPLC and TLC). The main radioactive compounds in extracts of tissues and milk were identified by chromatographic comparison with authentic reference compounds, by HPLC-MS/MS investigations or, in some cases, by NMR spectroscopic methods.

The goat was milked every morning prior to administration and every evening, 6 to 8 hours after the administration, and immediately before sacrifice. Sacrifice took place six hours after the goat had received the final dose, 54 hours after the first administration.

Until sacrifice (54 hours after the first administration), the excretion amounted to about 63.5% of the total radioactivity administered. The major excretory pathway of radioactive residues was via the faeces (38.6%), followed by excretion via the urine (24.9%). An extremely low amount (0.03% of the total dose) was secreted with the milk.

At sacrifice, 54 hours after the first administration (i.e. 6 hours after the last dosage), the total radioactive residues in the edible tissues and organs were measured or estimated to be about 0.58% of the total dose (see below).

The value for the total clearance amounted to Cl = 28 mL per min and kg body weight as calculated from plasma curve analysis with a three compartment disposition model assuming a complete absorption process.

The absorption process of the compound-related radioactivity administered in a 0.5% tragacanth suspension was characterised by a very fast onset (lag-time = 7 min.) followed by a short half-life of absorption of t_a = 13 min.

The radioactivity concentrations in the plasma showed a distinct maximum with a measured peak level of 1.14 µg/mL at 0.5 hours after the first administration, corresponding to only 11% of the equidistribution concentration of 10 µg/mL. The radioactivity was eliminated from the plasma with two half-lives. For the time period following the maximum up to about 2 hours, the elimination was

dominated by a half-life of about 0.5 hours. Thereafter, the elimination process declined and was governed by a half-life of about 7 hours.
At sacrifice (54 hours after the first administration), the highest equivalent concentration was measured in the liver (4.68 mg/kg wet tissue), followed by that obtained for the kidney (3.27 mg/kg). The concentrations corresponded to 0.47% (liver) and 0.038% (kidney) of the total dose. The results reflect the importance of these organs for metabolism and/or excretion of the compound. The concentrations in kidney and liver were followed in decreasing order by those obtained for the omental fat (0.126 mg/kg), perirenal fat (0.092 mg/kg), round muscle (0.039 mg/kg), subcutaneous fat (0.038 mg/kg), flank muscle (0.035 mg/kg) and loin muscle (0.032 mg/kg). Detailed results are given in Table 6.2.3-1.

Table 6.2.3-1 Residual radioactivity in edible tissues and organs of the lactating goat after repeated (3 x) oral administration of 10 mg/kg at sacrifice 54 hours after the first administration

Matrix	Fresh weight [g]	Equivalent concentration C (TRR) [mg/kg]	% of the radioactivity totally administered
Liver	1221.8	4.682	0.470
Kidney	142.9	3.267	0.038
Round muscle (sample)	2692.9	0.039	-
Flank muscle (sample)	366.4	0.035	-
Loin muscle (sample)	160.5	0.032	-
Total body muscle a)	12000.0	0.035 b)	0.035
Perirenal fat (sample)	392.7	0.092	-
Subcutaneous fat (sample)	76.2	0.038	-
Omental fat (sample)	669.3	0.126	-
Dissectable total body fat a)	4800.0	0.085 b)	0.034
Calculated/estimated residue in the edible tissues/organs			0.577

a) calculated from the body weight (40 kg at sacrifice);
assuming 30% and 12% of the body weight for total body muscle and dissectable total body fat, respectively
b) mean concentration of the three different types of muscle or fat

Equivalent concentrations of 0.212 µg/mL and 0.182 µg/mL were measured in the milk collected 8 hours after the first and second dosage, respectively. The first value represented the highest concentration measured during the whole test period. The values declined during the time period of 16 hours following the first and second administration to values of 0.048 µg/mL and 0.045 µg/mL, respectively. These findings indicate that there is no risk of a significant bioaccumulation of compound-related residues in milk after repeated dosage. The concentrations in milk were comparable to those determined in the plasma at the same times. In terms of amounts, an extremely low fraction of 0.03% of the dose administered in total was found in the milk during the whole test period.
The predominant metabolite in extracts from the milk sampled in the evening was KBR 2738 glucuronide (M17) accounting for about 71% of the TRR in the extracts or 0.134 mg/kg parent compound equivalents. In extracts from milk sampled in the morning, the predominant metabolite was KBR 2738 glucuronide (M17) accounting for 59% of the TRR, i.e. 0.026 mg/kg parent compound equivalents.

The two predominant radiolabelled compounds in extracts of liver were KBR 2738 and the equatorial (e) 4-hydroxy-KBR 2738 (M06), accounting for 54 and 28% of the TRR, respectively. The corresponding equivalent concentrations were 2.526 and 1.316 mg/kg.
The major radioactive component in kidney extracts was the KBR 2738 glucuronide (M17; 31% of the TRR) followed by 4-hydroxy-KBR 2738 (e) (M06; 24% of the TRR), by KBR 2738 (21% of the TRR) and by the axial (a) 4-hydroxy-KBR 2738 glucuronide (M18; 9% of the TRR). The corresponding equivalent concentrations were 1.016 mg/kg, 0.784 mg/kg, 0.687 mg/kg and 0.308 mg/kg. For the identification of the latter compound LC-MS/MS and NMR spectroscopic methods were used.
HPLC analysis of extracts from composite samples of round, flank and loin muscle revealed three main radiolabelled components: KBR 2738 glucuronide (M17), KBR 2738 and 4-hydroxy-KBR 2738 (e) (M06), which accounted for 24, 19 and 18% of the TRR in muscle, i.e. 0.009 mg/kg, 0.007 and 0.007 mg/kg.
HPLC investigations of the extract from composite samples of omental, subcutaneous and perirenal fat revealed three main radiolabelled compounds: KBR 2738 accounting for 36%, 4-hydroxy-KBR 2738 (e) (M06) accounting for 32% and KBR 2738 glucuronide (M17) accounting for 9% of the TRR. The corresponding equivalent concentrations were 0.031, 0.027 and 0.008 mg/kg.
The quantitative distribution of fenhexamid and its metabolites is summarised in Table 6.2.3-2.

Table 6.2.3-2 Quantitative distribution of fenhexamid and its metabolites in extracts from the edible tissues and milk of the lactating goat (mean values of two extractions)

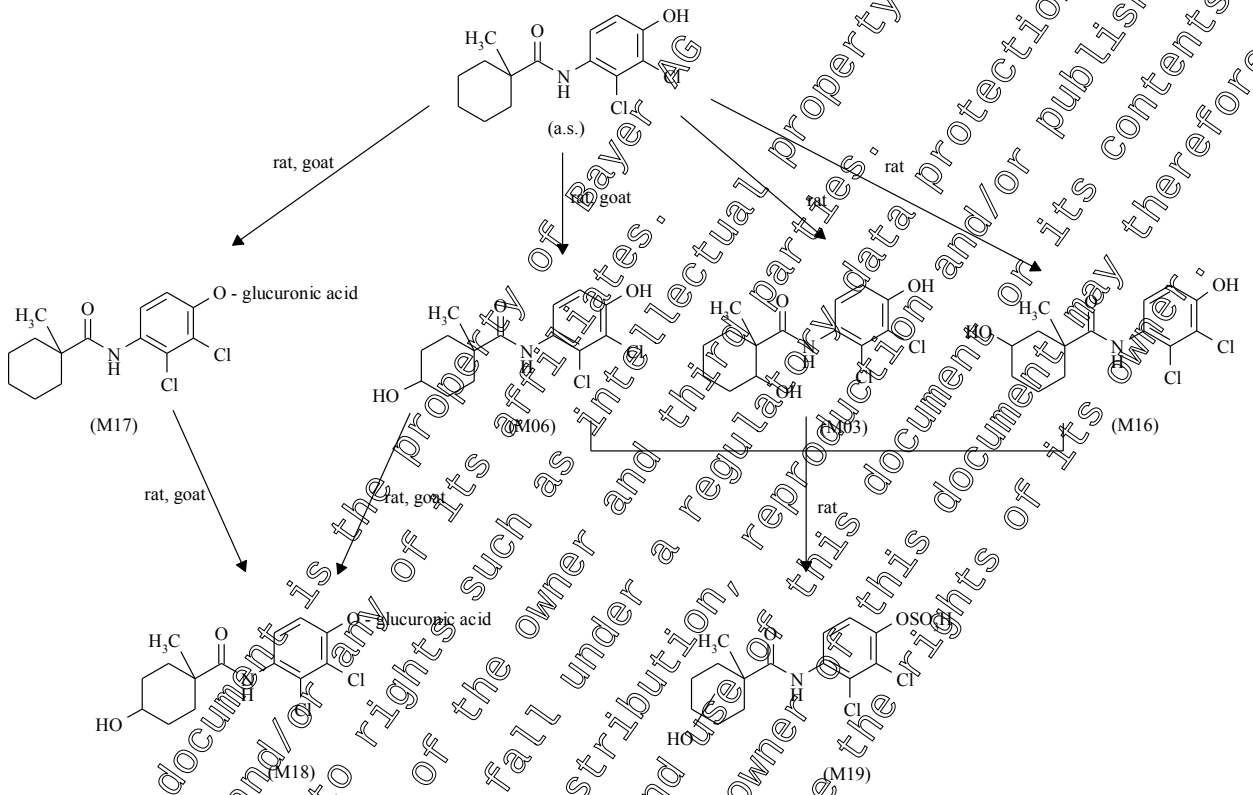
	Evening Milk		Morning Milk		Liver		Kidney		Muscle		Fat	
	% TRR	equiv. conc. [mg/kg]	% TRR	equiv. conc. [mg/kg]	% TRR	equiv. conc. [mg/kg]	% TRR	equiv. conc. [mg/kg]	% TRR	equiv. conc. [mg/kg]	% TRR	equiv. conc. [mg/kg]
TRR [a)]		0.189		0.044		4.682		3.267		0.035		0.085
a.s.	n.d.	n.d.	n.d.	n.d.	54.0	2.526	21.0	0.687	19.0	0.007	36.0	0.031
M06	n.d.	n.d.	n.d.	n.d.	28.1	1.316	24.0	0.784	18.1	0.007	31.5	0.027
M17	70.9	0.134	59.3	0.026	n.d.	n.d.	31.1	1.016	23.9	0.009	9.0	0.008
M18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.4	0.308	n.d.	n.d.	n.d.	n.d.
Sum identified	70.9	0.134	59.3	0.026	82.1	3.842	85.5	2.795	61.0	0.023	76.5	0.066

% TRR % of the TRR in the respective matrix, compare footnote a)
equiv. conc. Equivalent concentration of KBR 2738 and metabolites
n.d. not detected
a) TRR in organs/tissues after sacrifice
a.s. = fenhexamid (active substance)
M06 = equatorial 4-hydroxy-KBR 2738
M17 = glucuronide of KBR 2738
M18 = axial glucuronide of 4-hydroxy-KBR 2738

The unchanged parent compound was found in all tissue samples with the highest concentrations being detected in liver. The portion of parent compound in all tissues ranged from 19 to 54% of the TRR.
The metabolism of fenhexamid in the lactating goat proceeded via conjugation of the aromatic hydroxyl group and via hydroxylation of the cyclohexyl ring in the position 4. The resulting metabolites were the glucuronide of KBR 2738 (M17), the equatorial 4-hydroxy-KBR 2738 (M06) and the axial glucuronide of 4-hydroxy-KBR 2738 (M18). Both glucuronides were readily excreted with the urine. The parent compound and the metabolites were stable during the whole study period.

Thus the metabolism of fenhexamid in goat is comparable to metabolic routes already known from the rat. The proposed metabolic pathway is shown in Figure 6.2.3-1.

Figure 6.2.3-1: Proposed metabolic pathway of fenhexamid in rats and lactating goat



a.s. = fenhexamid
M03 = 2-hydroxy-KBR 2738
M06 = 4-hydroxy-KBR 2738
M16 = 3-hydroxy-KBR 2738
M17 = glucuronic of KBR 2738
M18 = glucuronide of 4-hydroxy-KBR 2738
M19 = sulfate of isomeric hydroxy-KBR 2738

IIA 6.2.4 Pigs

The metabolic pathway of fenhexamid was very similar in rat and goat. A laying hen metabolism study was not conducted. A pig metabolism study is not regarded as necessary.

IIA 6.2.5 Nature of residue in fish

As outlined in the EU-Directive 91/414/EEC, the EU Aquatic Guidance Document, as well as in EPA and PMRA guidelines, a log P_{ow} >3 should be used as a general trigger for a fish bioconcentration study. The study was summarised in the first dossier under KIIA 8.2.3 (bioconcentration).

Report:	**KIIA 6.2.5 /01; [redacted] 1997**
Title:	[Phenyl-UL-^{14}C]KBR 2738: Identification of Radioactive Residues in Bluegill Sunfish (Lepomis macrochirus)
Report No. & Document No.:	PF 4204 M-003791-01-1
Guidelines:	US-EPA § 165-4
GLP	Yes

Material and methods:
[^{14}C]-KBR 2738, radiochemical purity: >98 % (radio-HPLC, radio-thin-layer), KBR 2738, chemical purity: >99% (HPLC, UV-detector), Specification: (Lot No.: 1065/1), bluegill Lepomis macrochirus (lot F 3/95D), the study was performed with [phenyl-UL-^{14}C]-KBR 2738 on the bluegill sunfish with a tested water concentration of 210 ug/l (nominal) in a flow-through system. Duration of exposure was 7 and 14 days, respectively.

Findings and Observations:
The total radioactive residues at days 7 and 14 were determined as follows:

Table 6.2.5-1 Total radioactive residues of [phenyl-UL-^{14}C]KBR 2738 in bluegill sunfish

	Test A (7-day exposure)	Test B (14-day exposure)
Edible tissues	10.18 mg/kg	11.50 mg/kg
Viscera	77.17 mg/kg	113.36 mg/kg

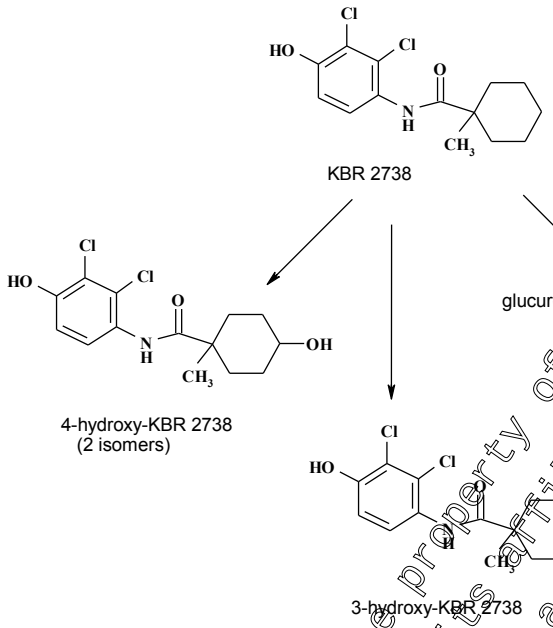
The parent compound KBR 2738 was the major component in all fish samples. Besides the parent compound three metabolites were identified and their amounts quantitated.

Table 6.2.5-2 Amount of parent compound and of metabolites in bluegill sunfish using [phenyl-UL-^{14}C]KBR 2738

	Test A (7-day exposure)				Test B (14-day exposure)			
	Edible tissues		Viscera		Edible tissues		Viscera	
	[%TTR]	[mg/kg]	[%TTR]	[mg/kg]	[%TTR]	[mg/kg]	[%TTR]	[mg/kg]
4-hydroxy-KBR 2738	3.12	0.32	2.82	2.18	3.87	0.45	3.71	4.21
3-hydroxy-KBR 2738	3.72	0.38	2.34	1.81	5.59	0.64	4.02	4.56
KBR 2738-glucuronide	11.97	1.22	3.37	2.60	10.65	1.22	2.90	3.29
KBR 2738	48.11	4.90	45.30	34.96	49.91	5.74	41.84	47.42
Total identified	66.92	6.82	53.83	41.55	70.02	8.05	52.47	59.48

The biotransformation of KBR 2738 in bluegill sunfish is characterized by 1.) conjugation of the aromatic hydroxyl group with glucuronic acid and 2.) hydroxylation of the cyclohexyl ring in the positions 3 and 4. The proposed metabolic pathway is shown in Figure 6.2.5-1.

Figure 6.2.5-1: Proposed metabolic pathway of [phenyl-UL-^{14}C] KBR 2738 in Bluegill Sunfish.



IIA 6.2.6 Chemical identity

This Annex point is not an EC data requirement according to Reg. 1107/2009/EC.

IIA 6.3 Residue trials (supervised field trials)

The representative uses chosen for the Annex I renewal are: grapes, strawberries and tomatoes and the GAPs supported for the inclusion renewal for these crops are the same as those evaluated for the first inclusion.

During the EU review further grape trials conducted in the EU were submitted and subsequently evaluated (ECCO Peer Review Meetings, 'Full Report on Fenhexamid' ECCO Team at BBA, Braunschweig of 28 February).

As a registration on grapes was granted in the USA during the Peer Review process, Bayer AG recommended reconsidering the grape MRL proposal given in the draft assessment report (2 mg/kg) by submitting the US data so that the MRL would also cover imported produce (ECCO Peer Review Meetings, 'Full Report on Fenhexamid' ECCO Team at BBA, Braunschweig of 28 February 2000, pages 155 – 186).

The data submitted were considered sufficient to derive processing factors, but one open point was the recalculation of material balances where the necessary data are available. Two processing studies on grapes are submitted with this AIR dossier providing information on mass balances (preparation of wine and raisins).

In the Report on the ECCO Peer Review Meeting a concern was addressed relative to the comparability of residue data for tomatoes and strawberries generated in greenhouses from the

northern and southern region. This issue has been addressed with a statement comparing greenhouse conditions in both European regions (Doc. no M-008470-01-1, report no. MR-140/99) and which was submitted during the evaluation process. In the meantime it has become common sense reflected in the "Guideline on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev.9) that for greenhouse uses only one zone in Europe may exist.
Also the fact that the strawberry trials were conducted under plastic tunnels in southern Europe was finally considered acceptable since the GAP involves a PHI of 1 day. Due to the short pre harvest interval the growing conditions either in the glasshouse or in a plastic tunnel are not considered to result in significantly different residue levels at harvest.

In the process of the MRL review program under Article 12/2 of the MRL Reg. 396/200, Tier I Summaries from all trials (original dossier, additional European grape trials and US data) were provided to the RMS (CRD) so that all necessary data are already available. Therefore, no residue data will be included in the amended Annex II dossier.

IIA 6.4 Livestock feeding studies

Livestock feeding studies were not conducted since the simulation of the feed-to-food transfer was regarded as not relevant due to the use pattern supported, neither with the first inclusion submission nor with the renewal application.

IIA 6.4.1 Poultry

No additional data necessary – please refer to statement under point IIA 6.4.

IIA 6.4.2 Lactating ruminants (goat or cow)

No additional data necessary – please refer to statement under point IIA 6.4.

IIA 6.4.3. Pigs

No additional data necessary – please refer to statement under point IIA 6.4.

IIA 6.4.4 Fish

This Annex point is not an EC data requirement according to Reg. 1107/2009/EC.

IIA 6.5 Effects of industrial processing and/or household preparation on

IIA 6.5.1 The nature of residue

A study from [redacted] (1996, PF4166) is available and was included in the Monograph 1998 in section B.6.8.1 Effects on the nature of the residue.
Fenhexamid was resistant against hydrolysis under conditions representative for pasteurization, baking, brewing, boiling and sterilization.

IIA 6.5.2 Distribution of the residue in peel/pulp

Please refer to the Annex point below (IIA 6.5.3).

IIA 6.5.3 Residue levels - balance studies on set of representative processes

In the Annex II dossier of fenhexamid several processing studies in grapes were submitted, but the description of the mass balances was not part of these studies.

The processing study presented below provides this information on mass balances for the preparation of wine and raisins.

Report:	**KIIA 6.5.3/01, [redacted], 2011**
Title:	Determination of the residues of fenhexamid in/on grape and the processed fractions (pomace, grape; must; wine at bottling; raisin waste; washings; raisin) after spraying of Fenhexamid WG 50 in the field
Report No. & Document No.:	10-3076, dated September 07, 2011 **M-413919-01-1**
Guidelines:	• EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed • EC guidance working document 7029/VI/95 rev. 5 (July 22, 1997) • OECD Guideline for the Testing of Chemicals, Magnitude of the Pesticide Residues in Processed Commodities, 508 (October 03, 2008)

I. Materials and Methods

In view of the existence of residues of fenhexamid on harvested grapes determined in samples from field residue trials performed according to the intended commercial use conditions (see point IIA 6.3.3), investigations on the effects of processing have been conducted. Two processing trials were conducted in Germany and France in order to determine the residues of fenhexamid in grapes (RAC) and in the processing products must, wine and raisins (10-3076; KIIA 6.5.3/01). The field trials were also conducted for RAC analysis and were reported in detail in report no. 10-2076, which can be provided at request.

Fenhexamid WG 50 was sprayed twice at application rates of approx. 750 g a.s./ha and water volumes of 200-800 L/ha, depending on the type of application (low or high-volume spraying). The last application was conducted at a pre-harvest interval of 14 days.

After processing (described below), residue analysis was performed according to the fenhexamid method 01180 (for more information, cf. point IIA 4.3). The limit of quantitation was 0.05 mg/kg for all matrices. Prior and parallel to the residue analysis, the method was validated by recovery experiments.

Preparation of must and wine:

Red and white grapes were processed to must and wine according to slightly different vinification techniques. The main steps during vinification are crushing, fermentation, racking, and bottling. Detailed information about the different vinification techniques is given in flow diagrams 6.5.3-1 and 6.5.3-2.

Preparation of raisins:

The preparation of raisins simulated the industrial practice in a laboratory scale. The destemmed fruit (grapes) were dried at a temperature of 60°C. The water content of the raisins ranged from 10 to 12%. After drying, the raisins were washed in standing water under slow movement. After washing, the water content of the raisins ranged from 13 to 19%; cf. diagram 6.5.3-3.

II. Findings

In concurrent recovery experiments, the sample materials were spiked with fenhexamid in concentrations of 0.05-100 mg/kg. The recovery data for the individual sample materials (all spiking levels) are summarised below in Table 6.5.3-1.

Table 6.5.3-1: Recovery data for fenhexamid in grapes

Matrix	FL [mg/kg]	n	Recoveries [%] (Single Values)						Min [%]	Max [%]	Mean [%]	RSD [%]
Bunch of grapes	0.05	5	85	95	98	99	101		85	101	96	6.6
	0.50	5	91	92	95	95	97		91	97	94	2.6
	4.0	1	80								80	
	overall	11							80	101	93	6.7
Raisin waste	0.05	5	77	78	78	83	86		77	86	80	4.9
	0.50	6	77	78	80	80	80	82	77	82	80	2.2
	5.0	1	92								92	
	25	1	82								82	
	50	1	84								84	
	100	1	86								86	
	overall	15							77	92	82	5.1
Raisins washings	0.05	5	95	102	104	105	106		95	106	102	4.3
	0.50	5	97	97	101	103	104		97	104	100	3.3
	3.9	1	89								89	
	overall	11							89	106	100	5.2
Raisin	0.05	5	97	98	98	104	104		97	104	100	3.5
	0.50	5	94	94	95	97	97		94	97	95	1.6
	39	1	93								93	
	10	1	83								83	
	20	1	86								86	
	overall	13							83	104	95	6.2
Pomace, grape	0.05	6	74	77	78	79	80	84	77	84	79	4.2
	0.50	6	81	86	87	88	88	101	81	101	89	7.5
	4.0	1	87								87	
	5.0	1	83								83	
	10	1	79								79	
	20	1	80								80	
	overall	16							74	101	83	7.7
Must	0.05	6	104	104	106	106	111	113	104	113	107	3.5
	0.50	6	101	104	106	109	109	111	101	111	107	3.5
	2.0	1	94						-	-	94	
	3.9	1	105						-	-	105	
	overall	14							94	113	106	4.5
Wine at bottling	0.05	6	89	92	97	99	100	105	89	105	97	5.9
	0.50	6	89	93	97	98	101	102	89	102	97	5.1
	1.9	1	80								80	
	3.9	1	96								96	
	overall	14							80	105	96	6.8

Must and wine:

Residues of fenhexamid in the harvested bunches of grapes at day 14 ranged from 0.85 to 2.0 mg/kg. The values in must ranged from 0.05-0.38 mg/kg and in wine at bottling from 0.30 to 1.2 mg/kg.

Mean transfer factors can be calculated from the residue levels as follows: 0.75 for must and 0.50 for young wine at bottling; cf. Table 6.5.3-2. As all of these transfer factors are <1, no concentration of fenhexamid during processing to wine is to be expected.

Raisins:

At day 14, fenhexamid residues from 0.85 to 2.0 mg/kg were measured in the bunch of grapes. In raisins, the residue levels were between 3.5 and 7.3 mg/kg.

A mean transfer factor of 3.9 was calculated for raisins. The transfer factor is >1, thus a concentration of the residues of fenhexamid will occur during processing to raisins.

Table 6.5.4-2: Summary of residue values and transfer factors in grape RACs and processed products following application of fenhexamid WG 50

Study Trial No. Trial SubID GLP	Country Year	PHI (days)	Portion analysed	Fenhexamid	
				Residues (mg/kg)	Transfer factor
10-3076 10-3076-01 GLP yes	Germany 2010	14	Bunch of grapes	2.0	--
			Pomace, grape	6.5	3.3
			Must	1.6	0.8
			Wine at bottling	1.2	0.6
			Raisin waste	25.0	13.0
			Raisin washings	0.17	0.09
			Raisin	7.3	3.7
10-3076 10-3076-02 GLP yes	France 2010	14	Bunch of grapes	0.85	--
			Pomace, grape	3.8	4.5
			Must	0.39	0.5
			Wine at bottling	0.30	0.4
			Raisin waste	11.0	13.0
			Raisin washings	0.09	0.1
			Raisin	3.5	4.1

Table 6.5.4-3: Mean transfer factors from processing in grape treated with fenhexamid on day 14

Portion analysed	Fenhexamid	
	Transfer factor (TF)	Mean TF
Bunch of grapes	--	--
Pomace, grape	3.3; 4.5	3.9
Must	0.8; 0.5	0.65
Wine at bottling	0.6; 0.4	0.50
Raisin waste	13; 13	13
Raisin washings	0.09; 0.1	0.095
Raisin	3.7; 4.1	3.9

Material balance

For the material balance of fenhexamid, the absolute residue **A** was calculated from the relative residue **R** according to the following equation:

Absolute Residue **A** = Relative Residue **R** in mg/kg * Weight **m** of Fraction in kg

Corrected weights were calculated for fractions which were not produced from the total amount of material available but only from a portion of the material. The absolute residue A for these fractions was calculated according to the following equation:

Absolute Residue **A** = Relative Residue **R** in mg/kg * Corrected Weight $\mathbf{m}_{corr.}$ of Fraction in kg

The material balances show, that 10 to 25% of the absolute residues of fenhexamid were recovered in wine at bottling, while 57 to 91% were recovered in raisins.

Overviews of the material balances and the percentage of residues recovered in the different processing fractions of the treated samples are given in Table 6.5.4-4 and Table 6.5.4-5.

Table 6.5.4-4: Material balances and percentage of residues of fenhexamid recovered in the processed fractions pomace, grape; must and wine at bottling

Sample Material	Relative Residue R [mg/kg]	Starting Material		Fraction			Residue	
		Total [kg]	Used [kg]	Weight m [kg]	[%]	Corrected Weight [kg]	Absolute Residue A [mg]	Percentage recovered [%]
Trial 10-3076-01								
Bunch of grapes (RAC)	2.0	-	-	38.36	100	-	77	100
Pomace, grape	6.5	38.36	38.36	10.04	26		65	85
Lees	-	38.36	38.36	4.26	11		-	-
Must	1.6	38.36	38.36	19.60	51		31	41
Wine at bottling	1.2	19.60	18.60	14.94	39	15.74 [1)]	19	25
Trial 10-3076-02								
Bunch of grapes (RAC)	0.85		-	67.80	100	-	58	100
Pomace, grape	3.8	67.80	67.80	8.36	12	-	32	55
Lees	-	67.80	67.80	3.12	5	-	-	-
Must	0.39	67.80	67.80	23.32	34	-	9	16
Wine at bottling	0.30	23.32	22.32	18.10	27	18.91 [1)]	6	10

Minor deviations may occur due to rounding. RAC: raw agricultural commodity.
[1)] Corrected weight = weight of fraction * (total amount of must obtained after clarification/amount used for fermentation)

Table 6.5.4-5: Material balances and percentage of residues of fenhexamid recovered in the processed fractions raisin waste, raisin and washings

Sample Material	Relative Residue R [mg/kg]	Starting Material		Fraction			Residue	
		Total [kg]	Used [kg]	Weight m [kg]	[%]	Corrected Weight [kg]	Absolute Residue A [mg]	Percentage recovered [%]
Trial 10-3076-01								
Bunch of grapes (RAC)	2.0	-	-	5.57	100	-	11	100
Berry	-	5.57	5.57	4.45	80	-	-	-
Raisin waste, undried		5.57	5.57	0.29	5	-	-	-
Raisin waste	25	0.29	0.29	0.071	1	-	1.8	16
Raisin, oven-dried	-	4.45	4.45	0.85	15	-	-	-
Raisin	7.3	0.85	0.85	0.87	16	-	6.4	57
Raisin washings	0.17	0.85	0.85	1.69	30	-	0.3	3
Trial 10-3076-02								
Bunch of grapes (RAC)	0.85	-	-	5.18	100	-	4.4	100
Berry	-	5.18	5.18	4.98	96	-	-	-
Raisin waste, undried		5.18	5.18	0.18	3	-	-	-
Raisin waste	25	0.18	0.18	0.052	1	-	0.6	13
Raisin, oven-dried	-	4.98	0.85	1.09	21	-	-	-
Raisin	7.3	1.09	1.09	1.15	22	-	4.0	91
Raisin washings	0.17	1.09	1.09	2.10	41	-	0.2	4

Minor deviations may occur due to rounding. RAC: raw agricultural commodity.

III. Conclusions

In order to determine transfer factors for residues of fenhexamid in must, wine and raisins, processing studies have been conducted.

The mean value of residue transfer factor for must was 0.65, for wine 0.50 and for raisins 3.9. Processing of grapes, except to raisins, yields a reduction of the levels of fenhexamid residues in the processed commodities as compared to the RAC's. Thus, for fenhexamid, processing to liquid products will not result in any concentration of the residues. Only in the case of raisins - in which the drying process would be expected to increase the relative residues via weight/water loss - is a concentration of residues evident.

Diagram 6.5.3-1: Flow chart for white wine processing

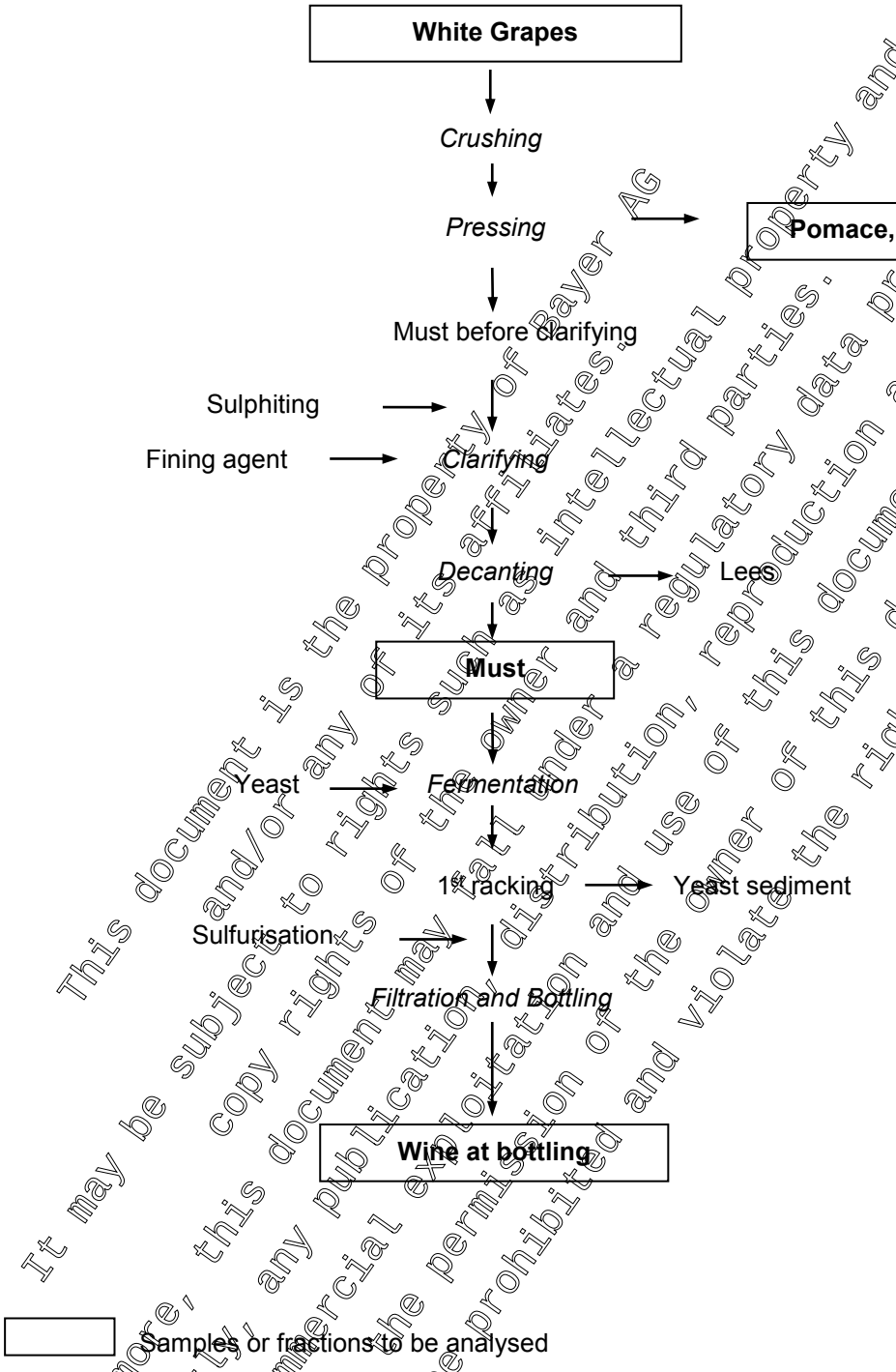


Diagram 6.5.3-2: Flow chart for red wine processing

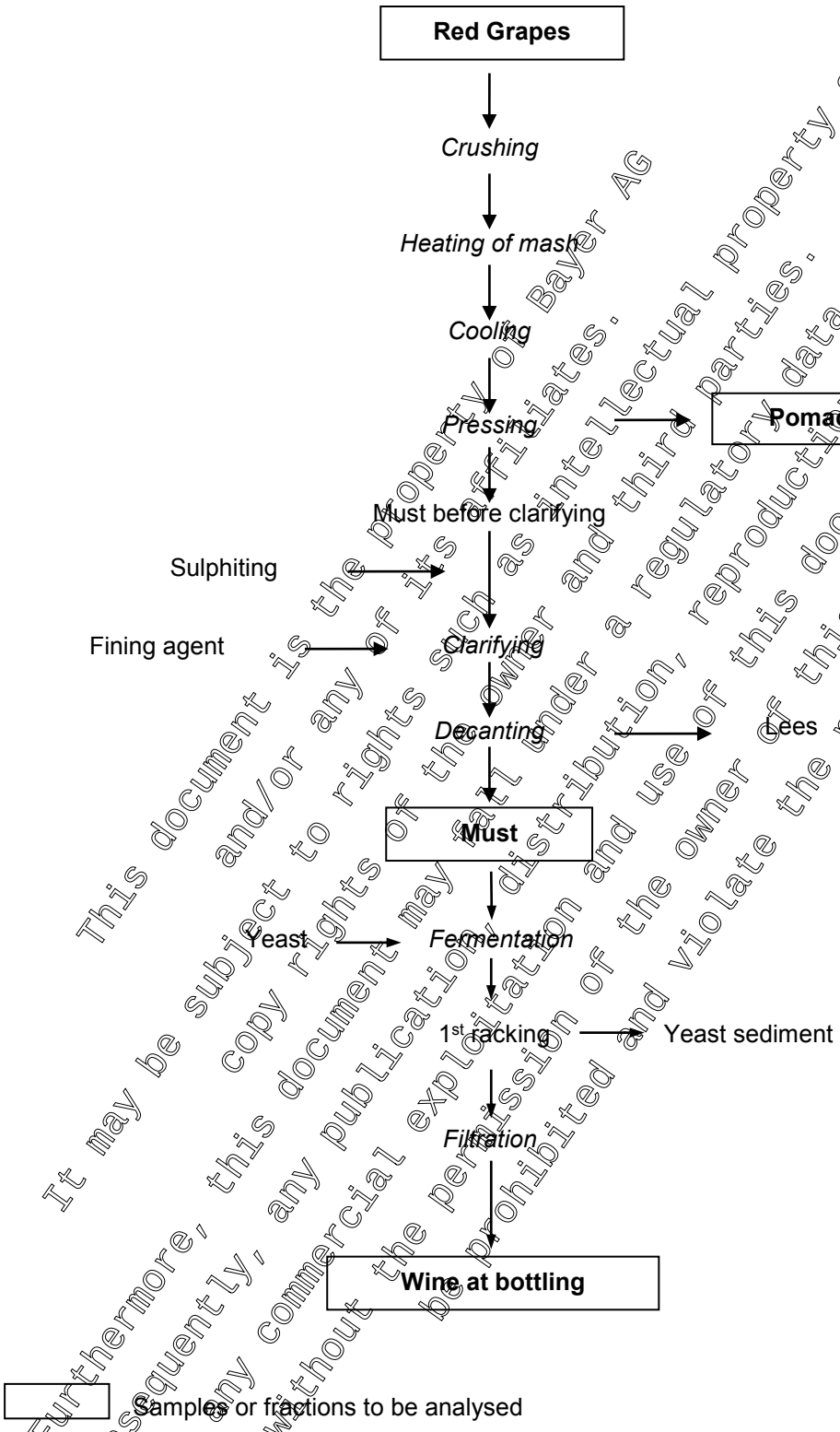
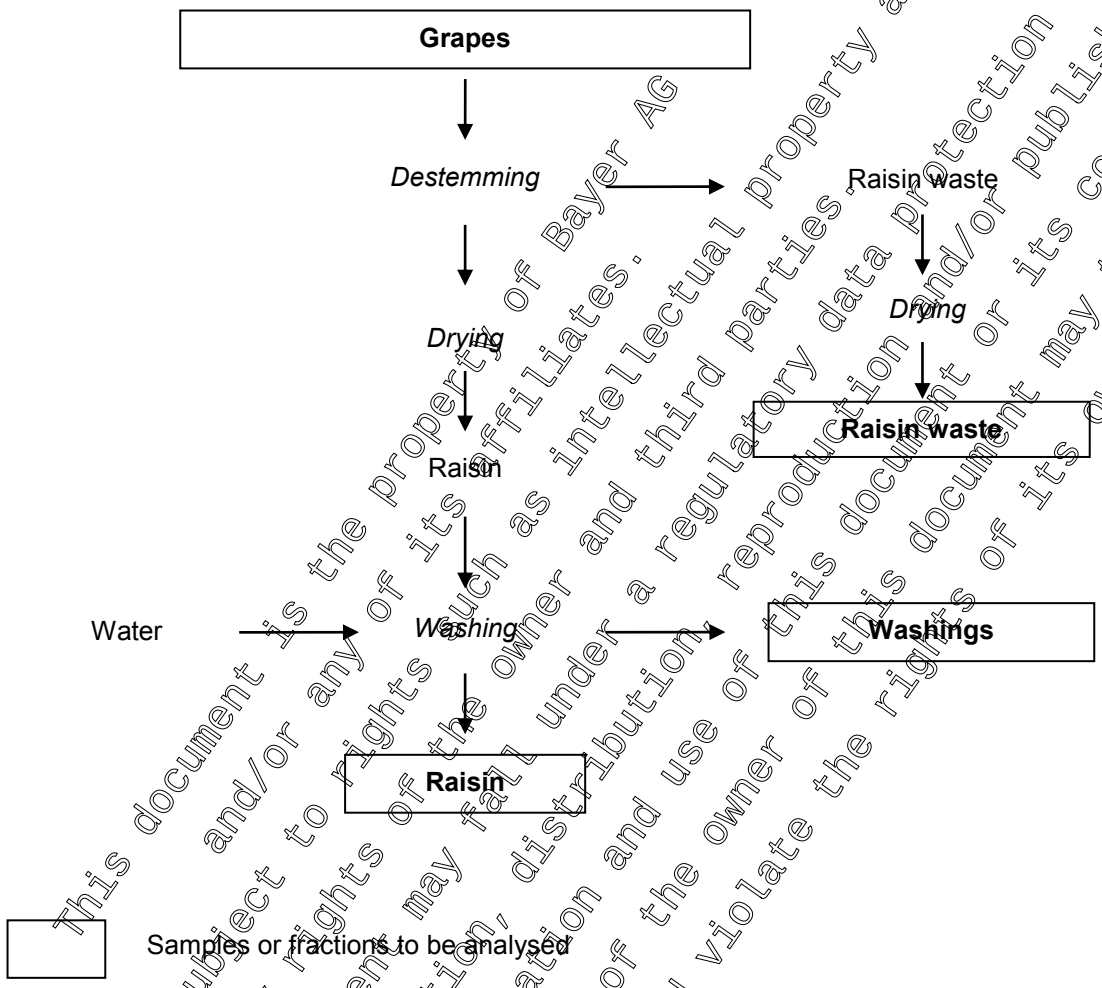


Diagram 6.5.3-3: Flow chart for raisins processing



IIA 6.5.4 Residue levels - follow-up studies; concentration or dilution factors

Please refer to the Annex point above (IIA 6.5.3).

IIA 6.6 Residues in succeeding crops

IIA 6.6.1 Theoretical consideration of the nature and level of the residue

The level and nature of residues in succeeding crops (confined rotational crops, field rotational crops) is influenced by the amount of active ingredient applied to the soil, by the degradation behaviour in soil, and by the uptake of parent compound and soil metabolites by the roots. Additionally parent compound and soil metabolites can be metabolised by the plants. Especially hydroxylation reactions and formation of conjugates are often observed.
The aerobic degradation of Fenhexamid (KBR 2738) in soil was investigated in laboratory studies and is described in the E-Fate section AII 7.1.
The metabolism of KBR 2738 was investigated in rotational crops (spring wheat, Swiss chard and turnips) following soil application of [phenyl-UL-^{14}C]KBR 2738. The application rates were slightly higher than in agricultural practice.

IIA 6.6.2 Metabolism and distribution studies on representative crops

Report:	**KIIA 6.6.2 /01; [redacted] 1997**
Title:	Confined Rotational Crop Study with KBR 2738
Report No & Document No	PF4240 M-003800-01-1
Guidelines:	US EPA Residue Chemistry Test Guideline OPPTS 860.1850
GLP	yes

Executive Summary

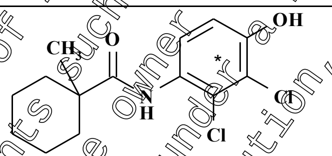
The metabolism of the fungicide KBR 2738 was investigated in the rotational crops wheat, Swiss chard and turnips from three consecutive rotations. [Phenyl-UL-^{14}C]KBR 2738 was formulated as a 50 WP and applied uniformly to the soil of a planting container (1 m²) by spray application (day 0). The application rate corresponded to 3460 g a.s./ha and was based on the projected annual field rate of 3360 g a.s./ha. Crops of the first, second and third rotation were sown at day 30, day 134 and day 314, respectively. Immature samples investigated were wheat forage and hay (soft dough stage). Wheat straw and grain, Swiss chard, turnip leaves and roots were harvested at maturity.
The total radioactive residues (TRRs) decreased significantly from the first to the second rotation and were even lower in the third rotation (details in Table 6.6.2-1). The maximum TRR (0.73 mg/kg) was observed for Swiss chard (day 75) sown 30 days after soil application. The TRRs from the second rotation were all ≤0.10 mg/kg. The TRRs of the third rotation ranged from ≤0.01 mg/kg (turnip roots) to 0.08 mg/kg (straw, day 477).
Generally, only a relatively small amount of the TRR was extracted using methanol/water, and the active ingredient, detected in the dichloromethane phase, was a minor compound of 2.0% of the TRR or even less. A major amount of the radioactivity (ca. 50% up to ca. 90%) was extracted by exhaustive extraction using dioxane/2N HCl 9:1 under reflux followed by 1N KOH at room temperature. As a result, the total amount of parent compound in the first rotation ranged from 0.4% (<0.01 mg/kg) in wheat forage to 3.7% (0.03 mg/kg) in Swiss chard (as a maximum of all plant samples of all rotations). The distribution of radioactivity into the four special fractions, which are characterised by the extraction procedure, indicated the presence of a number of components of different polarity and structure. This was conclusively shown by TLC of phases and extracts, where possible, proving that numerous minor compounds contributed to the metabolite pattern. Based on the extraction results, it

was concluded that major amounts of the TRR were bound to the lignin and hemicellulose fractions of the plant matrix.
As an example, the individual amounts of more than 30 components of the TRR in Swiss chard were either very low (e.g. 0.04 mg/kg as a maximum assigned to metabolite group 1) or the radioactivity remained at the TLC-origin (e.g. 0.25 mg/kg released from the lignin fraction using dioxane/HCl). Three metabolites were characterised as soil metabolites (dimer and trimers of the parent compound) each amounting to ≤1.5% (≤0.01 mg/kg).
A total hydrolysis experiment was conducted to analyse for the maximum amount of 4-hydroxy-KBR (cyclohexyl-hydroxylated derivative of the parent compound) in Swiss chard, which resulted in 1.2% (≤0.01 mg/kg). Intensive efforts were made to analyse for 2,3-dichloro-4-hydroxyaniline (DCHA). It was clearly shown using Swiss chard and straw, representing the maximum TRRs, that DCHA was not detectable.
The results of the metabolism of KBR 2738 in rotational crops are summarised and illustrated by the proposed metabolic pathway.

I. Material and Methods

A. Materials

1. Test Material

Chemical structure		* position of the radiolabel
Radiolabelled test material	[phenyl-UL-^{14}C]KBR 2738	
Specific radioactivity	1.90 MBq/mg (51.4 µCi/mg)	
Radiochemical purity	> 98% (HPLC and TLC)	
Application rate	one spray application to soil at 3.460 kg a.s./ha	
Preparation of application solution	The WP 50 formulation was prepared by homogenising the active ingredient with the blank formulation of the WP 50. The application solution was prepared by dissolving the formulation in 44 ml of water.	

2. Soil:

Monheim 3 (Germany), sandy loam soil, 1.98% organic carbon, pH 6.3 ($CaCl_2$), cation exchange capacity (CEC) 10 [meq/100 g]

3. Plants:

Rotational crop	Variety	Representative for crop group
spring wheat	Kadett	small grain
Swiss chard	Lucullus	leafy vegetable
turnips	Vollenda	root vegetable

B. Study Design

Experimental conditions:

Application:

The application conditions simulated the proposed annual maximum rate of 3360 g a.s./ha. In the rotational crop study one soil application (day 0) was performed. A computer controlled track sprayer with a flat fan nozzle was used for application. As a result, 346.0 mg a.s. was applied to the soil corresponding to 3460 g a.s./ha. The uniformity of application was confirmed using 10 filter discs (ca. 1.5 cm diameter) placed evenly over the soil surface before spraying and the radioactivity determined by combustion.

Growth:

Plants were cultivated in a planting container which was filled with a sandy loam soil. Approximately 0.5 m² of the area was used for the sowing of spring wheat, 0.25 m² for Swiss chard, and 0.25 m² for turnips. During the first rotation plants were grown similar to natural temperature and light conditions in a vegetation area and for the remaining period in a greenhouse (see details in tables). The glass roof of the vegetation area was open during the sunshine periods and was automatically closed during rainfall. Plants were irrigated as needed.

Growth of confined rotational	Month	Average temp. (° C)	Sunshine hours (h)
crops in the vegetation area (first rotation)	May	10.4	132
	June	16.2	224
	July	16.0	222
	August	15.8	207
	September	9.9	140
	October	7.9	123

Growth of confined rotational	Month	Temp. (° C)	Day	Temp. (° C)	Night
crops in the greenhouse (second and third rotation)	October to July	20	6.00 am - 8.00 pm	14	8.00 pm - 6.00 am

Sampling:

The sampling dates are given in Table 6.6.2-1. Wheat forage and hay (soft dough stage) represented immature samples. Wheat straw and grain, Swiss chard, and turnips were harvested at maturity.
Wheat grains were collected manually. The remaining ears and chaff were combined with straw which was cut into pieces and homogenised with liquid nitrogen.
Turnips were separated into leaves and roots and cut into pieces.
Aliquots of all samples were either extracted immediately or after a few days of storage at -20°C or below.

C. Analytical Procedures

Extraction:

An aliquot of forage was successively macerated with methanol (2 x) and methanol/water 1:1 (v/v) using a Polytron homogeniser. The suspension was filtered by suction yielding the methanol/water extract (combined filtrates) and solids 1. The methanol/water extract was evaporated to the aqueous remainder at ca. 40°C using a rotary evaporator. The aqueous remainder was partitioned with dichloromethane (3 x) leaving the aqueous phase. The dichloromethane solution was concentrated yielding the dichloromethane phase. The remaining solids 1 were further exhaustively extracted.

The TRR value of each RAC was determined by summation of the radioactivity measured in the combined methanol/water extracts and in the corresponding solids (solids 1) remaining after this conventional extraction.
The radioactivity in extracts was determined by liquid scintillation counting (LSC). The radioactivity in solids was determined by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and quantified by LSC.

Exhaustive Extraction 1 (Acidic Hydrolysis of Solids 1)
Wheat forage: An aliquot of solids 1 was further extracted using dioxane/2N HCl 9:1 (v/v) under reflux for 2 hours. The suspension was filtered by suction and the filter cake washed using dioxane. This gave the dioxane/HCl extract and the solids 2.
Exhaustive Extraction 2 (Alkaline Hydrolysis of Solids 2)
Wheat forage: Solids 2 were further extracted using 1N KOH at room temperature for 2 hours. The suspension was filtered by suction and the filter cake washed using water. This gave the KOH extract and the solids (non-extractable residue). Aliquots from the dioxane/HCl and KOH extracts and solids were taken for radioactivity measurement.
Extraction of other crops
Other crops were extracted analogously as described for forage.

Enzymatic treatment of the aqueous phase of Swiss chard with Cellulase
The enzyme solution was prepared using cellulase in sodium acetate buffer (pH 5.0) containing 0.02 % NaN_3. An aliquot of the aqueous phase of Swiss chard of the first rotation was evaporated to dryness under a stream of nitrogen in a reacti-vial. To the dried residue an aliquot of the cellulase enzyme solution was added and stirred for 16 hours at 37°C. The aqueous phase (before and following cellulase treatment) was analysed by TLC.

Partitioning of radioactivity in the KOH extract of grain
An aliquot of the KOH extract of grain was partitioned using dichloromethane (2 x), the mixture was centrifuged, the organic phase was separated and concentrated. The radioactivity of the organic phase was determined. The remaining aqueous phase was neutralised with 2N HCl and partitioned using dichloromethane (2 x). The mixture was centrifuged, the organic phase separated, concentrated and the radioactivity determined.

Total hydrolysis of Swiss chard
An aliquot of homogenised Swiss chard was stirred and heated under reflux for 2 hours with dioxane/2N HCl (9:1). After cooling, the solution was filtered by suction and the solids were dried at room temperature. The radioactivity of the dioxane/HCl extract and of the solids was measured.
The dioxane/HCl extract was mixed with water and concentrated using a rotary evaporator at ca. 40°C. The aqueous remainder was extracted with dichloromethane (3 x). The combined dichloromethane solutions were concentrated and the phases analysed by TLC.

Total hydrolysis of straw
The hydrolysis conditions for straw were very similar as for Swiss chard, however greater volumes of solvent were used.

Quantitation:
Parent compound and metabolites in the extracts were quantified by TLC.

Identification and characterisation:

Parent compound and metabolites were identified by TLC. HPLC was used for the chromatographic comparison of metabolites and fractionation. The HPLC was equipped with a DAD-Detector (wave length 254 nm) and a radioactivity flow through monitor with a solid scintillator glass cell.

Storage stability:

The rotational crop samples were all extracted either immediately after sampling or after short storage (nine days as a maximum) at -20°C or below. The investigation of extracts or phases and the hydrolysis experiments gave no indication for a decomposition of metabolites. From all experimental data it was concluded that the extraction data and the metabolite pattern were not influenced from storage of samples.

II. Results and Discussion

Swiss chard of the first rotation harvested at maturity (day 75) revealed the maximum TRR (0.73 mg/kg) of all crops of all rotations. The lowest TRR from the first rotation was determined in turnip roots and leaves, both 0.06 mg/kg at maturity (Table 6.6.2-1). The TRRs from the second rotation were significantly lower. This was especially evident for Swiss chard showing a decline to 0.02 mg/kg. The ^{14}C-levels of the second rotation were close to each other ranging from 0.02 mg/kg (wheat forage, Swiss chard, turnip leaves and roots) to 0.10 mg/kg (straw). The TRRs from the third rotation were even lower than the second and only one crop (wheat hay) remained unchanged at 0.03 mg/kg. The TRRs from the third rotation ranged from <0.01 mg/kg (turnip roots) to 0.08 mg/kg (straw).

Table 6.6.2-1: Total radioactive residues (TRRs) in confined rotational crops following soil application of [phenyl-UL-^{14}C]KBR 2738 at 3.460 kg a.s./ha

	Total Radioactive Residue (TRR)					
rotational crop	**first rotation**		**second rotation**		**third rotation**	
	mg/kg	**sampling day**	**mg/kg**	**sampling day**	**mg/kg**	**sampling day**
wheat forage	0.14	63	0.02	177	0.01	352
wheat hay	0.17	89	0.03	239	0.03	406
wheat straw	0.52	131	0.10	299	0.08	447
wheat grain	0.17	131	0.04	299	0.03	447
Swiss chard	0.73	75	0.02	191	0.01	363
turnip leaves	0.06	110	0.02	237	0.01	390
turnip roots	0.06	110	0.02	237	≤0.01	390

As Swiss chard from the first rotation showed the maximum TRR (0.73 mg/kg) of all crops, the metabolite pattern of this sample was investigated more intensively. The complete analysis is summarised in Table 6.6.2-2. A comparison of the amount of parent compound in all crops of the first rotation is given in Table 6.6.2-3. The stepwise extraction procedure for the investigation and characterisation of the nature of the residues, and the resulting data can be described as follows:

The conventionally extracted radioactivity of the combined methanol/water extracts (generally ca. 50 % of the TRR) was partitioned into the dichloromethane phase and aqueous phase to facilitate the characterisation of the metabolites according to their polarity, and to quantify them in appropriate TLC solvent systems. The radioactivity in the dichloromethane phase of crops from the first rotation ranged from 1.1% (grain) to 22.1% (Swiss chard) and in the aqueous phase from 9.0% (grain) to 37.1% (wheat forage). Individual data and the unextracted amount of radioactivity (solids 1) following

methanol/water extraction of each crop is given in Table 6.6.2-4 to Table 6.6.2-9 (in % and mg/kg).

Table 6.6.2-2: Identification and characterisation of radioactive residues in Swiss chard of the first rotation following soil application of [phenyl-UL-^{14}C]KBR 2738 at 3.460 kg a.s./ha

	% of TRR	mg/kg
identified *		
KBR 2738, parent compound (subtotal)	(3.7)	(0.03)
KBR 2738, dichloromethane phase	2.0	0.01
KBR 2738, dioxane/HCl extract	1.7	0.01
characterised (subtotal)	(87.8)	(0.64)
characterised by comparison with soil metabolites (subtotal)	(3.3)	(0.02)
mono-deschloro trimer, BBJ 98-12, (M23), dichloromethane phase	1.5	0.01
trimer of KBR 2738, BBJ 98-9, (M22), dichloromethane phase	1.1	<0.01
[C-O-C] dimer of KBR 2738, BBJ 98-11, (M29), dichloromethane phase	0.7	<0.01
characterised by extraction procedure and TLC analysis (subtotal)	(74.0)	(0.54)
metabolite group 1, dichloromethane phase	5.3	0.04
diffuse radioactivity 3, dichloromethane phase	2.1	0.02
at least 10 unknown components of the dichloromethane phase, each ≤ 1.9 %, ≤ 0.01 mg/kg	9.4	0.07
metabolite 16, aqueous phase	2.6	0.02
metabolite group 17, aqueous phase	2.4	0.02
diffuse radioactivity 6, aqueous phase	2.8	0.02
at least 6 components of the aqueous phase, each ≤ 1.8 %, ≤ 0.01mg/kg	8.3	0.06
TLC-origin, aqueous phase	4.9	0.04
unpolar compounds, dioxane/HCl extract (lignin-fraction)	2.4	0.02
polar compounds, dioxane/HCl extract (lignin-fraction), mainly TLC-origin	33.8	0.25
characterised by extraction procedure		
KOH extract (hemicellulose fraction), high matrix content, not chromatographed	10.5	0.08
solids (non-extractable residue after two exhaustive extraction steps)	8.5	0.06
total residue (TRR)	**100.0**	**0.73**

* further 1.2 % (≤0.01 mg/kg) of the TRR were identified as 4-OH-KBR following total hydrolysis of Swiss chard (conducted and analysed as a separate experiment)

Table 6.6.2-3: Comparison of TRR and amount of parent compound in rotational crops (first rotation) grown in soil treated with [phenyl-UL-^{14}C]KBR 2738

	TRR	KBR 2738	
	mg/kg	%	mg/kg
wheat, forage	0.14	0.4	<0.01
wheat, hay	0.17	0.7	<0.01
wheat, straw	0.52	0.7	<0.01
wheat, grain	0.17	2.5	<0.01
Swiss chard	0.73	3.7	0.03
turnip leaves	0.06	0.5	<0.01
turnip roots	0.06	0.5	<0.01

Preliminary hydrolysis experiments (acid and base including mixtures with different solvents, variation of temperature) were conducted using solids 1 of Swiss chard from the first rotation to

develop the most effective extraction and possibly achieve a one step procedure. However, a sequence of two exhaustive hydrolysis steps was necessary to minimise the non-extractable residues.
As a result, the exhaustive extraction was conducted using dioxane/2N HCl 9:1 (v:v) under reflux for 2 hours followed by 1N KOH at room temperature for 2 hours. The non-extractable residues (solids 3) following both treatments were mostly ≤0.01 mg/kg except for forage (0.02 mg/kg), straw (0.06 mg/kg) and Swiss chard (0.06 mg/kg) from the first rotation, and straw (0.02 mg/kg) from the second rotation. Some crops of the second and third rotation were not further extracted since solids 1 were ≤0.01 mg/kg following methanol/water extraction (e.g. turnips).

Table 6.6.2-4: Characterisation of the extraction behaviour of ^{14}C-residues in wheat samples of the first rotation

	Wheat							
	Forage (day 63)		Hay (day 89)		Straw (day 131)		Grain (day 131)	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
dichloromethane phase	7.6	0.01	12.7	0.02	9.2	0.05	1.1	<0.01
aqueous phase	37.1	0.05	23.7	0.04	32.5	0.17	9.0	0.02
(solids 1) subtotal, further extracted	(55.3)	(0.08)	(63.5)	(0.11)	(58.3)	(0.30)	(89.8)	(0.15)
dioxane/HCl extract	25.6	0.04	33.3	0.06	38.1	0.20	7.9	0.01
KOH extract	18.7	0.03	22.1	0.04	8.2	0.04	77.8	0.13
solids (non-extractable residue)	10.9	0.02	8.1	0.01	12.0	0.06	4.2	≤0.01
Total radioactive residue	100.0	0.14	100.0	0.17	100.0	0.52	100.0	0.17

Table 6.6.2-5: Characterisation of the extraction behaviour of ^{14}C-residues in Swiss chard and turnip samples of the first rotation

	Swiss chard (day 75)		Turnip leaves (day 110)		Turnip roots (day 110)	
	%	mg/kg	%	mg/kg	%	mg/kg
dichloromethane phase	22.1	0.16	14.7	≤0.01	11.2	≤0.01
aqueous phase	21.0	0.15	33.4	0.02	28.5	0.02
(solids 1) subtotal, further extracted	(56.9)	(0.42)	(51.9)	(0.03)	(60.3)	(0.04)
dioxane/HCl extract	37.9	0.28	19.3	0.01	24.6	0.02
KOH extract	10.5	0.08	23.1	0.01	23.2	0.01
solids (non-extractable residue)	8.5	0.06	9.5	≤0.01	12.5	≤0.01
Total radioactive residue	100.0	0.73	100.0	0.06	100.0	0.06

As dioxane/HCl mixtures and increased temperature cleave e.g. ethers of aromatic alcohols, representing essential substructures of lignin, this extraction is suitable for the characterisation of radioactivity covalently bound into the lignin matrix or incorporated into lignin. A significant amount of solids 1 of the rotational crops was solubilised by treatment with dioxane/HCl. As an example, the portion of dioxane/HCl-extractable radioactivity in wheat was 25.6% in forage, 33.3% in hay and 38.1% in straw. However, only 7.9% was released from grain which is a typical storage organ of starch.
The undissolved radioactivity following dioxane/HCl extraction (solids 2) was treated with aqueous KOH reflecting typical extraction conditions for the characterisation of residues in hemicellulose fractions. As shown in Table 6.6.2-4, a significant portion of the TRR of wheat samples was solubilised in the KOH extract, especially from grain (77.8%, 0.13 mg/kg). This was obviously due to the higher water solubility of the radioactivity, possibly from partially hydrolysed carbohydrate

oligomers or polymers and probably to some extent to mineralised metabolites (e.g. $^{14}CO_2$) incorporated into starch and similar natural compounds.

Swiss chard revealed a significant portion (37.9%, 0.28 mg/kg) of the TRR in the dioxane/HCl extract. The amount of radioactivity in the dioxane/HCl extracts and KOH extracts of turnip leaves and turnip roots was very similar (ca. 23%, Table 6.6.2-5).

Table 6.6.2-6: Characterisation of the extraction behaviour of ^{14}C-residues in wheat samples of the second rotation

	Wheat							
	Forage (day 177)		Hay (day 239)		Straw (day 299)		Grain (day 299)	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
dichloromethane phase	20.8	≤0.01	8.2	<0.01	5.8	≤0.01	1.2	<0.01
aqueous phase	22.8	≤0.01	27.6	≤0.01	28.4	0.03	8.2	<0.01
(solids 1) subtotal, further extracted	(56.4)	(0.01)	(64.2)	(0.02)	(65.8)	(0.07)	(90.7)	(0.04)
dioxane/HCl extract	17.9	<0.01	30.3	≤0.01	34.1	0.03	9.7	<0.01
KOH extract	26.9	≤0.01	13.9	0.01	9.7	0.01	77.5	0.03
Solids (non-extractable residue)	11.6	<0.01	20.0	≤0.01	22.0	0.02	3.5	<0.01
Total radioactive residue	100.0	0.02	100.0	0.03	100.0	0.10	100.0	0.04

Table 6.6.2-7: Characterisation of the extraction behaviour of ^{14}C-residues in Swiss chard and turnip samples of the second rotation

	Swiss chard (day 191)		Turnip leaves (day 237)		Turnip roots (day 237)	
	%	mg/kg	%	mg/kg	%	mg/kg
dichloromethane phase	14.4	<0.01	12.8	≤0.01	3.0	<0.01
aqueous phase	33.6	≤0.01	40.3	≤0.01	57.3	0.01
solids 1 (subtotal)	(52.0)	(0.01)	46.9	0.01	39.7	≤0.01
dioxane/HCl extract	9.8	<0.01	*	*	*	*
KOH extract	32.5	≤0.01	*	*	*	*
Solids (non-extractable residue)	9.7	<0.01	*	*	*	*
Total radioactive residue	100.0	0.02	100.0	0.02	100.0	0.02

* no further extraction

Table 6.6.2-8: Characterisation of the extraction behaviour of ^{14}C-residues in wheat samples of the third rotation

	Wheat							
	Forage (day 352)		Hay (day 406)		Straw (day 447)		Grain (day 447)	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
dichloromethane phase	12.3	<0.01	20.9	≤0.01	10.2	≤0.01	1.7	<0.01
aqueous phase	24.7	<0.01	49.9	0.02	40.1	0.03	9.9	<0.01
(solids 1) subtotal, further extracted	63.0	≤0.01	29.2	0.01	(49.7)	(0.04)	(88.3)	(0.02)
dioxane/HCl extract	*	*	*	*	30.2	0.02	10.7	<0.01
KOH extract	*	*	*	*	7.5	≤0.01	73.0	0.02
solids (non-extractable residue)	*	*	*	*	12.1	0.01	4.6	<0.01
Total radioactive residue	100.0	0.01	100.0	0.03	100.0	0.08	100.0	0.03

* no further extraction

Metabolite pattern in Swiss chard:
Radio-TLC analysis of the dichloromethane phase (22.1%, 0.16 mg/kg) indicated numerous components. Unchanged parent compound accounted for only 2.0% (0.01 mg/kg) of the TRR. The dichloromethane phase was further analysed using characterised metabolites from a soil metabolism study as reference compounds. The reference soil metabolites were applied as partly overlapping zones. Three metabolites were assigned as BBJ 98-9 (mono-deschloro trimer of KBR 2738, M23), BBJ 98-12 (trimer of KBR 2738, M22) and BBJ 98-11 ([C-O-C] dimer of KBR 2738, M20), respectively. Each of the soil metabolites contributed ≤1.5% (≤0.01 mg/kg) of the TRR as given in Table 6.6.2-2.

Table 6.6.2-9: Characterisation of the extraction behaviour of ^{14}C-residues in Swiss chard and turnip samples of the third rotation

	Swiss chard (day 363)		Turnip leaves (day 390)		Turnip roots (day 390)	
	%	mg/kg	%	mg/kg	%	mg/kg
dichloromethane phase	7.8	<0.01	11.2	<0.01	6.2	<0.01
aqueous phase	46.7	≤0.01	32.3	<0.01	45.6	≤0.01
solids 1	45.5	≤0.01	56.4	≤0.01	48.2	≤0.01
dioxane/HCl extract	*	*	*	*	*	*
KOH extract	*	*	*	*	*	*
Total radioactive residue	100.0	0.01	100.0	0.01	100.0	≤0.01

* no further extraction

The radio-TLC analysis of the aqueous phase (21.0%, 0.15 mg/kg) of Swiss chard revealed numerous metabolites. The amount of each single compound was ≤0.02 mg/kg. For further characterisation, the aqueous phase was tentatively treated with cellulase.
The aqueous phase of Swiss chard remained practically unchanged with cellulase. To obtain further details of possible significant basic structures by chemical methods, a total hydrolysis of Swiss chard was additionally conducted with an aliquot of the original sample using dioxane/HCl (described below).

Metabolite pattern in other crops:
Following conventional extraction the amount of unchanged KBR 2738 in the <u>dichloromethane phase</u> was low for wheat forage, hay, straw, turnip leaves and roots (each ≤0.01 mg/kg a.s., Table 6.6.2-3). The radio-TLC comparison of the <u>aqueous phases</u> from the first rotation, including grain, showed that most of the metabolites accounted for <0.01 mg/kg each and the maximum metabolite amounted to 0.03 mg/kg in straw.
The analysis of the <u>dioxane/HCl extracts</u> showed that parent compound was only detectable in Swiss chard (1.7 %, 0.01 mg/kg) and grain (2.5%, <0.01 mg/kg). Therefore, KBR 2738 constituted only a small part of the lignin fraction.

DCHA discussion:
DCHA (2,3-dichloro-4-hydroxyaniline) was not detected by TLC in the dioxane/HCl extract of any sample. This was confirmed for forage, hay, straw, grain and turnips using a further solvent system. As traces of DCHA could have been present in Swiss chard, the dichloromethane phase of the total hydrolysis experiment was analysed by 2-dimensional TLC and provided further evidence that no DCHA was present.
The aqueous KOH extracts were highly viscous, dark coloured and heavily loaded with matrix. Based

on the measurement of radioactivity and from the chromatographic analyses of the organic phases (dichloromethane phases and dioxane/HCl-extracts) it proved impossible to quantify single components.

Further characterisation of the KOH extract from grain:
Due to the importance of grain for human consumption, and because of the relatively high percentage of the TRR present in the KOH extract, partitioning with dichloromethane was investigated. However, no radioactivity was measurable in the organic phase proving that the radioactivity remained in the aqueous KOH extract. The same was observed following neutralisation of the KOH extract.
From these results it was concluded that the radioactivity in grain was not due to parent compound or similar organic compounds but probably consisted of polar plant metabolites or polar compounds taken up by the roots, probably after degradation or mineralisation of the active ingredient in the soil.

Total hydrolysis of Swiss chard and discussion of 2,3-dichloro-4-hydroxyaniline (DCHA):
The total hydrolysis of an aliquot of Swiss chard confirmed the absence of DCHA even under drastic conditions using dioxane/HCl 9:1 for 2 hours under reflux, and confirmed the low percentages of parent compound and 4-OH-KBR which might be released from possible conjugates or bound residues. The HPLC investigation of the dichloromethane phase following total hydrolysis of Swiss chard was conducted analogously to the apple metabolism study. The 4-OH-KBR fraction was sampled and analysed by TLC. As a result, the metabolite 4-OH-KBR was detected in relatively small amounts (1.2 %, ≤0.01 mg/kg). From the total hydrolysis experiment it was therefore concluded that 4-OH-KBR or possible conjugate precursors were present in Swiss chard, but were of minor importance. DCHA (reference compound BNF 5537C) was not detectable.
The dichloromethane phase of the total hydrolysis experiment was also analysed by two-dimensional TLC. The chromatogram clearly proved that DCHA (BNF 5537C) was not present as a metabolite and furthermore, was not formed from any conjugate or plant matrix under the drastic hydrolysis conditions. The detected radioactivity of the integrated test area of DCHA was so low, that the value could not be distinguished from the background radioactivity. It was therefore concluded that DCHA was not present as a metabolite even following drastic hydrolysis conditions.

Total hydrolysis of straw and discussion of DCHA:
The total hydrolysis and 2-dimensional TLC-analysis for DCHA was also conducted for straw and the non-occurrence of DCHA was as clearly demonstrated as for Swiss chard.

DCHA discussion for other crops:
Grain:
The corresponding total hydrolysis of grain and the analysis for DCHA was not conducted due to the following experimental facts available from the extraction procedure.
The special investigations in Swiss chard and straw (highest TRRs) clearly proved the absence of DCHA. The extraction of grain (methanol/water and dioxane/HCl) showed that only an extremely low portion of radioactivity was dissolved and the main portion of the TRR was detected in the aqueous KOH extract. The radioactivity of the KOH extract could not be distributed into dichloromethane with or without neutralisation. Therefore, it was concluded from the chemical behaviour of the extracted radioactivity, that DCHA was not present in grain.

Forage, hay and turnips:
As DCHA was not found in the most important crops with the highest TRRs, and the metabolite

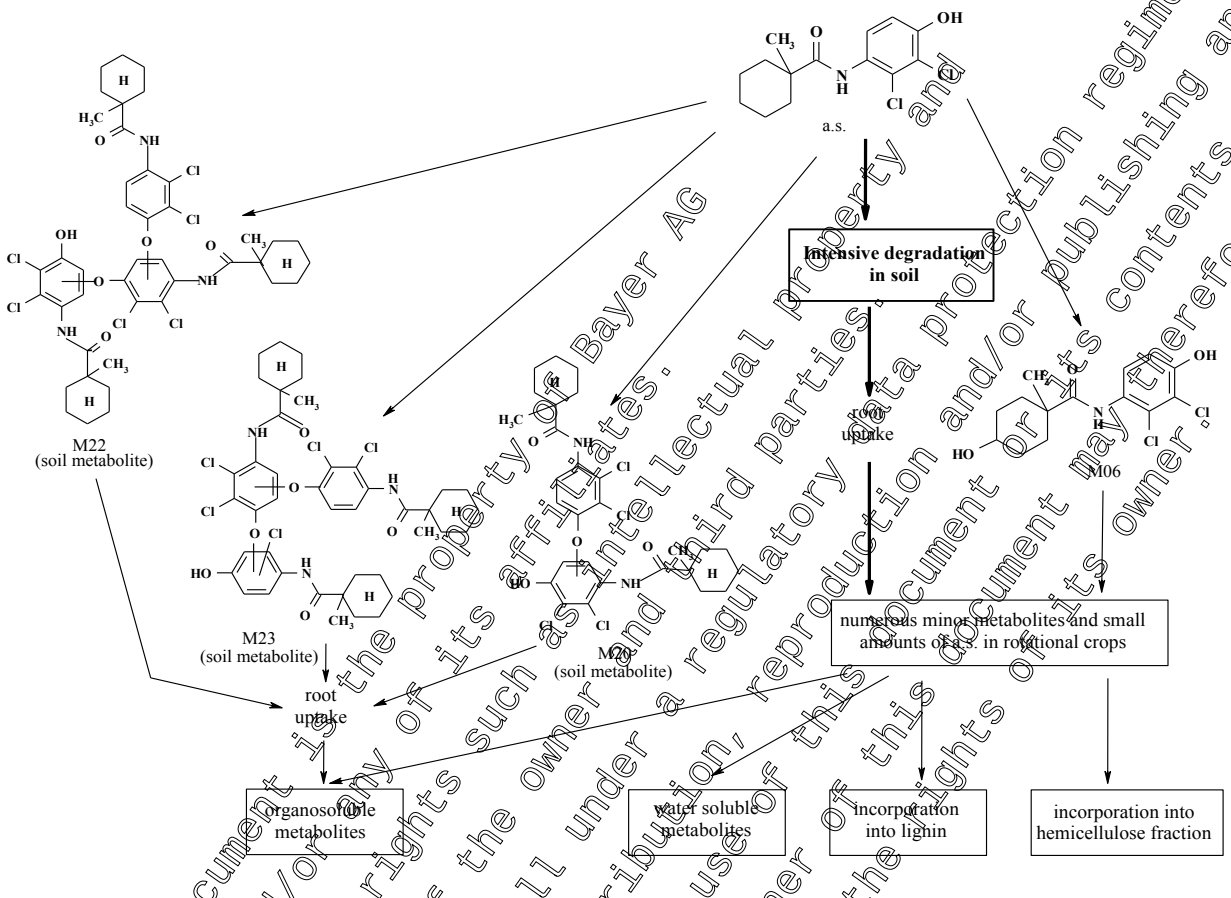
pattern indicated no significant differences, the remaining crops (forage, hay and turnips) with much lower residues, were not further investigated.

Metabolic pathway in confined rotational crops:
KBR 2738 was a relatively minor but significant compound in rotational crops and 4-OH-KBR was present in traces. The TRR consisted of numerous minor metabolites distributed in the dichloromethane phase, aqueous phase, dioxane/HCl-extract and KOH extract. The composition of the TRRs was strongly influenced by the degradation of KBR 2738 in soil. The proposed metabolic pathway is given in Figure 6.6.2-1.

III. Conclusions

The metabolism of KBR 2738 was investigated in rotational crops (wheat, Swiss chard and turnips) following soil application of phenyl-UL-^{14}C radiolabelled active ingredient. The TRRs were very low compared with the applied amount (3.46 kg a.s./ha). Unchanged active ingredient represented only 3.7 % or less of any TRR following conventional and exhaustive extraction indicating intensive degradation in soil before root uptake. A major amount of the radioactivity (ca. 50% up to ca. 90%) was not extractable using methanol/water. However significant amounts of radioactivity were solubilised by exhaustive extraction using dioxane/HCl 9:1 under reflux followed by 1N KOH. Based on extraction experiments and the final extraction procedure, it was concluded that major amounts of the TRR were characterised as compounds incorporated into the lignin fraction or hemicellulose fraction of the plant matrix. The extracts and phases were analysed by TLC, whenever possible, and the chromatographic investigation showed that the radioactivity was distributed between many minor compounds.
The highest TRRs were observed in crops from the first rotation sown 30 days after application. The residues declined significantly from the first to the second rotation and were even lower in the third rotation.
The composition of the TRR in rotational crops was obviously substantially influenced by the metabolism of KBR 2738 in soil and led to differences in the results obtained from the plant metabolism studies, where the radioactivity was easily extracted and consisted of mainly parent compound. The hydroxylated derivative of the parent compound (4-OH-KBR) was also of very minor importance in rotational crops. Although the parent compound was intensively degraded in soil, no DCHA was detectable, even in special investigations conducted in Swiss chard and straw. This was consistent with the results of the plant and soil metabolism studies.
From the results of the confined rotational crop study it was further concluded that for the majority of samples (wheat forage, hay, straw, grain, turnip leaves, roots) the amount of parent compound was <0.01 mg/kg when sown 30 days after soil application at the applied rate (3460 g). For Swiss chard, the amount of parent compound was 0.03 mg/kg when sown at day 30 from a TRR of 0.73 mg/kg. The TRR declined rapidly to 0.02 mg/kg after sowing Swiss chard at day 134. Therefore, considering this rapid decline, the proposed annual field rate of 3360 g a.i./ha distributed over several treatments and a generally faster degradation under field conditions, it is estimated that the time interval required to reach 0.01 mg/kg parent compound in Swiss chard would be nearer to 30 days than 134 days after application.

Figure 6.6.2-1 Proposed metabolic pathway of [phenyl-UL-^{14}C]KBR 2738 in confined rotational crops



a.s. = fenhexamid
M06 = 4-hydroxy-KBR 2738
M20 = [C-C]-C] dimer of KBR 2738
M22 = trimer of KBR 2738
M23 = mono-deschloro trimer of KBR 2738

IIA 6.6.3 Field trials on representative crops

Please refer to the statements given under Annex points above (IIA 6.6.1 and IIA 6.6.2).

IIA 6.7 Proposed residue definition and maximum residue levels

IIA 6.7.1 Proposed residue definition

The proposed residue definition - for risk assessment and monitoring purpose - is the parent only and applies for both - plant and animal matrices.

IIA 6.7.2 Proposed maximum residue levels (MRLs) and justification

Maximum Residue Limits (MRLs) for fenhexamid were set at European Level under several Commission Regulations the latest one being Commission Regulation 508/2011 of 24 July 2011 amending Annexes II and III of regulation EC 396/2005. In the process of the MRL review program under Article 12/2 of the MRL Regulation 396/200, Tier I Summaries from all trials (original dossier, additional European grape trials and US data) which were the basis for the MRL setting were provided to CRD. Thus, all necessary data are already available to the RMS.

Relative to the supported representative uses in/on grapes, strawberries and tomatoes the data sets forming the basis for the EU MRLs were reported in the original AII dossier and/or submitted and evaluated during the EU review process for Annex I inclusion and therefore, no residue data are reported in the amended Annex II dossier.

For easy reference the current harmonized temporary EU-MRL values for fenhexamid are shown in Table 6.7.2-1.

Table 6.7.2-1: Harmonized temporary EU-MRL values for fenhexamid

Code number	Groups and examples of individual products to which the MRLs apply (a)	Fenhexamid
100000	1. FRUIT FRESH OR FROZEN; NUTS	
110000	(i) Citrus fruit	0.05*
120000	(ii) Tree nuts (shelled or unshelled)	0.05*
130000	(iii) Pome fruit	0.05*
140000	(iv) Stone fruit	
140010	Apricots	5
140020	Cherries (sweet cherries, sour cherries)	5
140030	Peaches (Nectarines and similar hybrids)	5
140040	Plums (Damson, greengage, mirabelle)	1
140990	Others	0.05*
150000	(v) Berries & small fruit	
151000	(a) Table and wine grapes	5
152000	(b) Strawberries	5
153000	(c) Cane fruit	10
153010	Blackberries (cloudberries)	10
153020	Dewberries (Loganberries, Boysenberries, and Cloudberries)	10
153030	Raspberries (Wineberries, artic bramble/raspberry, (rubus articus), nectar rapberries	10
153090	Others	10
154000	(d) Other small fruit & berries	
154010	Blueberries (Bilberries)	5
154020	Cranberries (Cowberries (red bilberries))	5
154030	Currants (red, black and white)	5
154040	Gooseberries (Including hybrids with other ribes species)	5

Code number	Groups and examples of individual products to which the MRLs apply (a)	Fenhexamid
154050	Rose hips	5
154060	Mulberries (arbutus berry)	10
154070	Azarole (Mediterranean medlar) (Kiwiberry (Actinidia arguta)	5
154080	Elderberries (Black chokeberry (appleberry), mountain ash, buckthorn (sea sallowthorn), hawthorn, service berries, and other treeberries)	5
154990	Others	5
160000	(vi) Miscellaneous fruit	
161000	(a) Edible peel	0.05*
162000	(b) Inedible peel, small	
162010	Kiwi	10
162020	Lychee (Litchi) (Pulasan, rambutan (hairy litchi), mangosteen)	0.05*
162030	Passion fruit	0.05*
162040	Prickly pear (cactus fruit)	0.05*
162050	Star apple	0.05*
162060	American persimmon (Virginia kaki) (Black sapote, white sapote, green sapote, canistel (yellow sapote), and mammey sapote)	0.05*
162090	Others	0.05*
163000	(c) Inedible peel, large	0.05*
200000	2. VEGETABLES FRESH OR FROZEN	
210000	(i) Root and tuber vegetables	0.05*
220000	(ii) Bulb vegetables	
220010	Garlic	0.05*
220020	Onions (Silverskin onions)	0.6
220030	Shallots	0.05*
220040	Spring onions (Welsh onion and similar varieties)	0.05*
220990	Others	0.05*
230000	(iii) Fruiting vegetables	
231000	(a) Solanacea	
231010	Tomatoes (Cherry tomatoes, Tree tomatoes, Physalis, Gojiberry, Wolfberry (Lycium barbarum and L. chinense))	1
231020	Peppers (Chilli peppers)	2
231030	Aubergines (egg plants) (Pepino)	1
231040	Okra, lady's fingers	0.05*
231990	Others	0.05*
232000	(b) Cucurbits - edible peel	1
232010	Cucumbers	1
232020	Gherkins	1
232030	Courgettes (Summer squash, marrow (patisson))	1
232990	Others	1
233000	(c) Cucurbits - inedible peel	0.05*
234000	(d) Sweet corn	0.05*
239000	(e) Other fruiting vegetables	0.05*
240000	(iv) Brassica vegetables	
241000	(a) Flowering brassica	0.05*
242000	(b) Head brassica	0.05*
243000	(c) Leafy brassica	0.05*
244000	(d) Kohlrabi	0.05*
250000	(v) Leaf vegetables & fresh herbs	
251000	(a) Lettuce and other salad plants including Brassicacea	
251010	Lamb's lettuce (Italian cornsalad)	30
251020	Lettuce (Head lettuce, lollo rosso (cutting lettuce), iceberg lettuce, romaine	40

Code number	Groups and examples of individual products to which the MRLs apply (a)	Fenhexamid
	(cos) lettuce)	
251030	Scarole (broad-leaf endive) (Wild chicory, red-leaved chicory, radicchio, curled leave endive, sugar loaf)	30
251040	Cress	30
251050	Land cress	30
251060	Rocket, Rucola (Wild rocket)	30
251070	Red mustard	30
251080	Leaves and sprouts of Brassica spp. (Mizuna, leaves of peas and radish and other babyleaf brassica crops (crops harvested up to 8 true leaf stage)	30
251990	Others	30
252000	(b) Spinach & similar (leaves)	0.05*
253000	(c) Vine leaves (grape leaves)	0.05*
254000	(d) Water cress	0.05*
255000	(e) Witloof	0.05*
256000	(f) Herbs	30
256010	Chevil	30
256020	Chives	30
256030	Celery leaves (Fennel leaves, Coriander leaves, dill leaves, Caraway leaves, lovage, angelica, sweet cicely and other Apiacea leaves)	30
256040	Parsley	30
256050	Sage (Winter savory, summer savory,)	30
256060	Rosemary	30
256070	Thyme (Marjoram, oregano)	30
256080	Basil (Balm leaves, mint, peppermint)	30
256090	Bay leaves (laurel)	30
256100	Tarragon (Hyssop)	30
256990	Others (Edible flowers)	30
260000	(vi) Legume vegetables (fresh)	
260010	Beans (with pods) (Green bean (french beans, snap beans), scarlet runner bean, slicing bean, yardlong beans)	2
260020	Beans (without pods) (Broad beans, Flageolets, jack bean, lima bean, cowpea)	0.05*
260030	Peas (with pods) (Mangetout (sugar peas))	0.05*
260040	Peas (without pods) (Garden pea, green pea, chickpea)	0.05*
260050	Lentils	0.05*
260990	Others	0.05*
270000	(vii) Stem vegetables (fresh)	0.05*
280000	(viii) Fungi	0.05*
290000	(ix) Sea weeds	0.05*
300000	3. PULSES, DRY	0.05*
400000	4. OILSEEDS AND OILFRUITS	
401000	(i) Oilseeds	0.1*
402000	(ii) Oilfruits	
402010	Olives for oil production	0.05*
402020	Palm nuts (palmoil kernels)	0.1*
402030	Palmfruit	0.1*
402040	Kapok	0.1*
402990	Others	0.1*
500000	5. CEREALS	0.05
600000	6. TEA, COFFEE, HERBAL INFUSIONS AND COCOA	0.1*
700000	7. HOPS (dried) , including hop pellets and unconcentrated powder	0.1*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Fenhexamid
800000	8. SPICES	0.1*
900000	9. SUGAR PLANTS	0.05*
1000000	10. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS	
1010000	(i) Meat, preparations of meat, offals, blood, animal fats fresh chilled or frozen, salted, in brine, dried or smoked or processed as flours or meals other processed products such as sausages and food preparations based on these	0.05*
1020000	(ii) Milk and cream, not concentrated, nor containing added sugar or sweetening matter, butter and other fats derived from milk, cheese and curd	0.05*
1030000	(iii) Birds' eggs, fresh preserved or cooked Shelled eggs and egg yolks fresh, dried, cooked by steaming or boiling in water, moulded, frozen or otherwise preserved whether or not containing added sugar or sweetening matter	0.05*
1040000	(iv) Honey (Royal jelly, pollen)	0.05*
1050000	(v) Amphibians and reptiles (Frog legs, crocodiles)	0.05*
1060000	(vi) Snails	0.05*
1070000	(vii) Other terrestrial animal products	0.05*

* indicates lower limit of analytical determination

Substance	**Fenhexamid**
Legislation	Reg. (EC) No 508/2011
Entry in to force	24.07.2011

IIA 6.8 Proposed pre-harvest intervals. re-entry or withholding periods

IIA 6.8.1 Pre-harvest interval (in days) for each relevant crop

Crop	Zone/Country or specific situation	PHI (days)
Grapes	field (Germany)	14 (table grapes) 21 (wine grapes)
Strawberries	field (Germany)	3
	field (Belgium; Spain)	1
	greenhouse (Spain)	1
Tomatoes	field and greenhouse (Spain)	1

IIA 6.8.2 Re-entry period (in days) for livestock to areas to be grazed

Not applicable – no use on crops which are fed to livestock.

IIA 6.8.3 Re-entry period for man to crops, buildings or spaces treated

Not applicable.

IIA 6.8.4 Withholding period (in days) for animals feedingstuffs

Not applicable.

IIA 6.8.5 Waiting period between last application and sowing or planting

Not applicable.

IIA 6.8.6 Waiting period between application and handling treated products

Not applicable.

IIA 6.8.7 Waiting period between last application and sowing/planting succeeding crops

Not applicable - Fenhexamid is absolutely safe for any succeeding crop.

IIA 6.9 Estimation of exposure through diet and other means

The active substance fenhexamid was included in Annex I of Directive 91/414/EEC (Directive 2001/28/EC) with Entry-into-Force of June 1, 2001.

The EU toxicolgical endpoints relevant for the dietary risk assessment of fenhexamid as concluded during the EU review process are summarized in Table 6.9-1 below.

Table 6.9-1: Summary of fenhexamid EU toxicological end-points relevant for dietary risk assessment

EU End-Point	European Commission Fenhexamid 6497/VI/99-rev.2, 19 October 2000, Appendix II
Acceptable Daily Intake (ADI)	0.2 mg/kg bw/day 52 week dog study, safety factor: 100
Acute Reference Dose (ARfD)	Not allocated. Not considered necessary.

The dietary exposure of consumers to fenhexamid derived residues was evaluated using the EFSA PRIMo model (revision 2). This model was initially developed for the evaluation of the harmonized EU MRLs and includes chronic and acute consumption data for adults and children. For the evaluation of the chronic exposure the model uses 5 WHO diets relevant to the EU and 22 national diets from 13 different EU Member States. For the evaluation of the acute exposure 19 national diets from 11 different EU Member States are used.

The Acceptable Daily Intake (ADI) of 0.2 mg/kg bw/ was adopted during the initial inclusion of fenhexamid in Annex I (Standing Committee on Plant Health on 19 October 2000). Furthermore no Acute Reference Dose (ARfD) was set due to the low toxicity of fenhexamid.

IIA 6.9.1 TMDI calculations

For TMDI calculation all food items of plant and animal origin were considered to contain residues of fenhexamid at the proposed EU MRLs. All MRLs were considered even those set at the LOQ (see Table 6.7.2-1).

As shown in Table 6.9.1-1 the TMDIs of fenhexamid calculated according to the EFSA PRIMo model were found to range between 2.5% and 20.4% of the ADI of 0.2 mg/kg bw/day, which demonstrates a sufficient margin of safety. Therefore, temporary proposed MRLs of fenhexamid do not cause unacceptable risks to consumers due to chronic dietary exposure to fenhexamid residues.

Table 6.9.1-1: TMDIs of fenhexamid calculated according to EFSA/PRAPeR model

TMDI (% of ADI)	MS diet	Highest contributor to MS diet (in % of ADI)	Highest contributor to MS diet commodity / group of commodity
20.4	WHO Cluster diet B	7.2	Lettuce
17.5	FR all population	10.3	Table and wine grapes
14.7	ES adult	10.7	Lettuce
13.9	DE child	3.2	Table and wine grapes
13.9	IT adult	7.5	Lettuce
12.7	NL child	3.1	Scarole
12.6	WHO regional diet	7.5	Lettuce
12.4	IE adult	2.9	Table and wine grapes
11.5	IT kids/toddler	5.8	Lettuce
11.4	ES child	8.3	Lettuce
11.2	WHO Cluster diet E	4.5	Table and wine grapes
9.8	WHO Cluster diet F	6.0	Lettuce
9.2	PT general population	6.9	Table and wine grapes
8.5	NL general	2.4	Lettuce
6.9	DK child	2.8	Lettuce
6.8	FR toddler	1.6	Strawberries
6.7	UK vegetarian	2.8	Lettuce
6.5	UK adult	2.8	Table and wine grapes
5.3	WHO Cluster diet D	1.4	Table and wine grapes
5.2	UK toddler	0.7	Table and wine grapes
5.1	DK adult	3.7	Table and wine grapes
4.8	SE general population 90th percentile	0.7	Kiwi
4.1	FR infant	1.2	Strawberries
3.8	FI adult	1.6	Lettuce
3.6	UK infant	1.0	Products of animal origin
2.8	PL general population	0.8	Table and wine grapes
2.5	LT adult	1.3	Lettuce

IIA 6.9.2 NEDI calculations

Since the TMDI calculations for fenhexamid demonstrate a considerable margin of safety, it was not deemed necessary to perform NEDI calculations in order to refine the dietary risk assessment.

IIA 6.9.3 NESTI calculations

As no ARfD was derived – no acute exposure is necessary to be calculated.

IIA 6.10 Other/special studies

No additional studies are available.

IIA 6.11 Summary and evaluation of residue behaviour and reasonable grounds

IIA 6.11.1 Summary and evaluation of residue behaviour

Summary of plant metabolism

The metabolism of Fenhexamid (KBR 2738) was investigated in five target crops (grapes, tomatoes, apples, lettuce and field pea) following application of [phenyl-UL-^{14}C]-KBR 2738. The metabolism proceeded via two basic pathways. The first was the conjugation of the parent compound with glucose (and glucose-malonic acid) at the aromatic hydroxyl group. The second was an oxidation of the cylohexyl ring, leading to hydroxy-derivatives of the parent compound in the 2- and 4-positions followed by conjugation. Additionally, unknown conjugates were formed at different hydroxy groups. However, these metabolic changes occurred only to a small extent. The vast majority of radioactivity in target crops was mainly unchanged parent compound. From these results it can be concluded that only the parent compound is relevant for the residue definition. The extracted radioactivity and the distribution into solvent fractions was very similar and no cleavage of the amide structure was observed. In target crops only dry seeds of field peas were more difficult to extract.

The metabolism in confined rotational crops was intensively influenced by the degradation of Fenhexamid in soil. Only a low portion of the applied active substance and of radioactive soil residues was taken up with the roots. The resulting total radioactive residues (TRRs) in plants were therefore very low. Fenhexamid was the most important individual component in the TRRs of confined rotational crops. The quantities of metabolites were negligible.

From the metabolism investigations it was concluded that the reported metabolite pattern represented the residues at sampling or harvest and that parent compound and metabolites were stable. In total a high extraction rate and identification rate was achieved for all samples (target crops and CRCs). The following Figure (6.11.1-1) shows schematically the positions of the molecule which were involved in the metabolic reactions. The metabolic degradation pathways are shown in in the corresponding chapters of the first EU dossier and in this dossier. A common pathway is shown in Figure 6.11.1-2.

Based on the obtained results unchanged parent compound is the proposed residue definition for all target crops and rotational crops (risk assessment and monitoring).

Figure 6.11.1-1: Schematic picture of the positions indicating metabolic reactions of fenhexamid (KBR 2738) in target crops and confined rotational crops

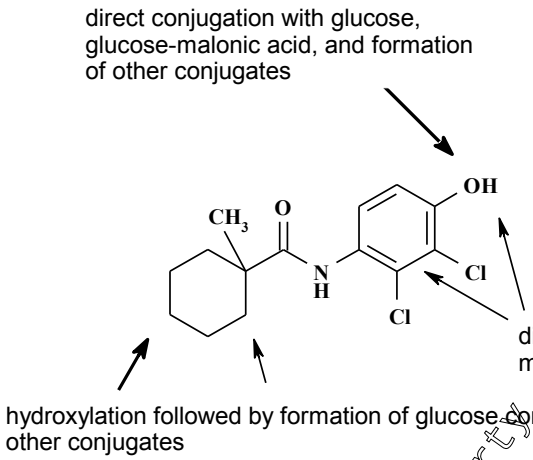
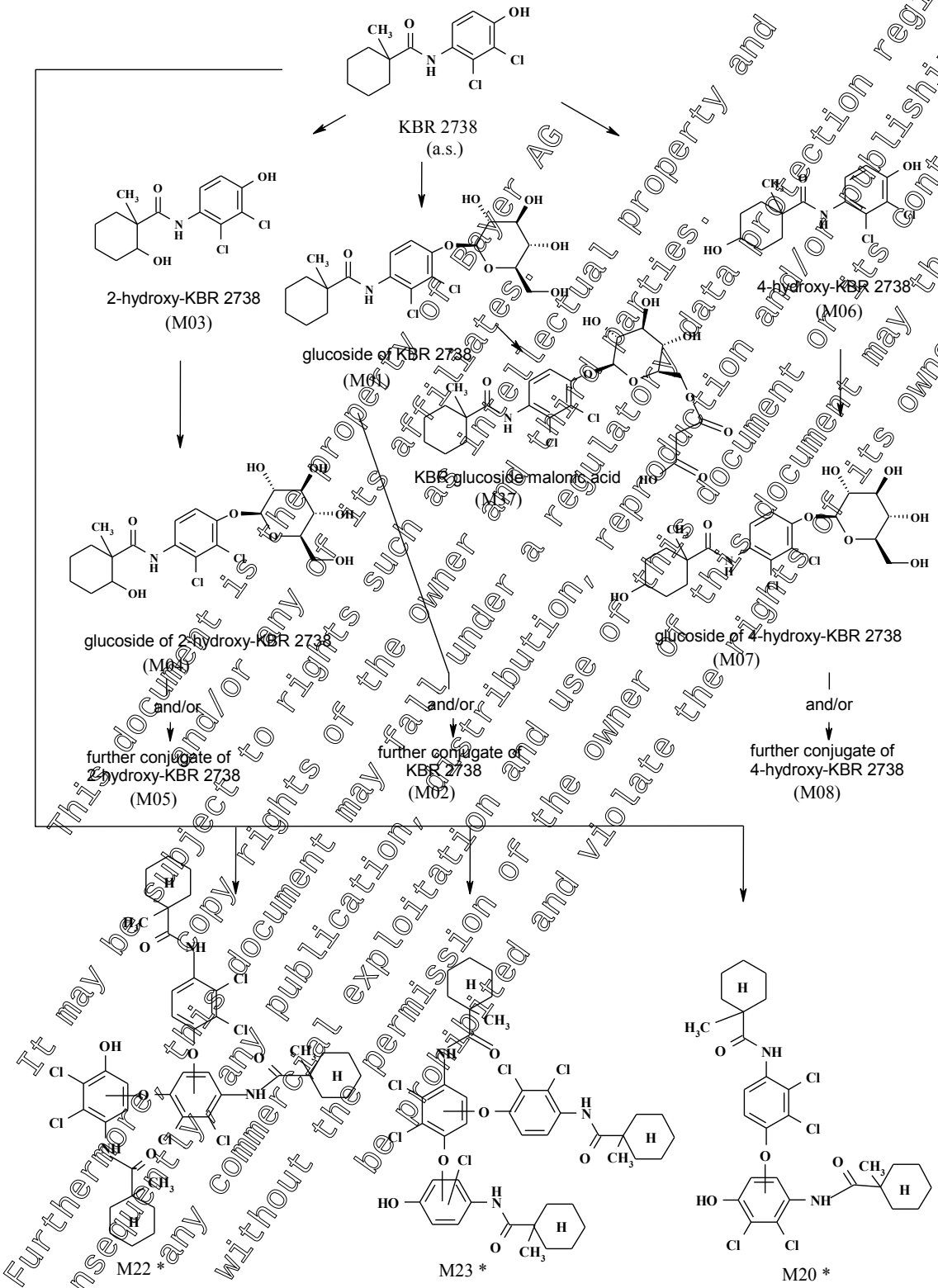


Figure 6.11.1-2: Proposed metabolic pathway of fenhexamid (KBR 2738) in plants (target crops and confined rotational crops)



* minor metabolites following root uptake from soil
M20 = [C-O-C] dimer of KBR 2738
M22 = trimer of KBR 2738
M23 = mono-deschloro trimer of KBR 2738

Summary of livestock metabolism

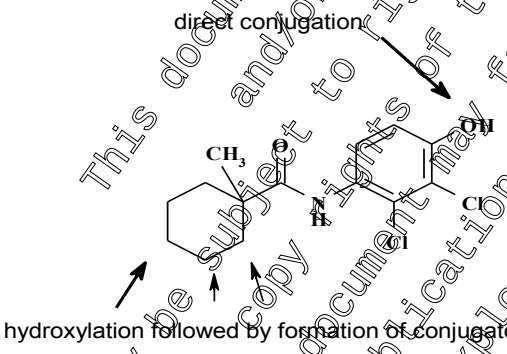
A livestock metabolism study was performed in the lactating goat. The main pathway of transformation proceeded via conjugation of the aromatic hydroxyl group with glucuronic acid. This compound is well suited for excretion.

Another site for enzyme action in the goat was the cyclohexyl ring. Hydroxylation took place at the position 4.

Comparison of the metabolism in plants and animals

The metabolism in plants and in animals was well comparable. Parent compound was the main residue in target crops, goat liver and goat fat. The parent compound was directly conjugated with glucose and glucose-malonic acid in plants (M01, M37). The corresponding glucuronic acid (M17) was formed in goat and rat. The parent compound was also oxidised (hydroxylated) at the 4-position of the cyclohexyl ring (M06) in animals and in plants. Examples of comparable conjugates are the glucuronic acid (M18) in the goat and rat and the glucoside (M07) in plants. Another comparable metabolisation was the hydroxylation at the 2-position of the cyclohexyl ring (M03) in the rat and in plants. An important common behaviour was that no cleavage products were formed in animals and plants. Further significant identified metabolites showing the detoxification were M16 and M19 in the rat. Therefore, no additional metabolism studies were deemed necessary with another radiolabel. In total it can be concluded that the metabolism in animals and plants was very similar.

Figure 6.11.1-3: Schematic picture of the positions indicating metabolic reactions of fenhexamid (KBR 2738) in animals and plants



Residues in raw agricultural commodities and processed fractions of grapes

The representative uses chosen for the Annex I renewal are: grapes, strawberries and tomatoes and the GAPs supported for the inclusion renewal are the same as those evaluated in the first inclusion.

During the EU review process, further grape trials conducted in the EU and US were submitted and subsequently evaluated (ECCO Peer Review Meetings, 'Full Report on Fenhexamid' ECCO Team at BBA, Braunschweig of 28 February 2000). Based on these data an appropriate MRL was set to cover also imported products into the EU.

The data submitted were considered sufficient to derive processing factors, but one open point was the recalculation of material balances where the necessary data are available. Two processing studies on grapes are submitted with this AIR dossier providing information on mass balances (preparation of wine and raisins).

Relative to the metabolism and residue section all further data requirements addressed in the 'Full Report on Fenhexamid' (ECCO Peer Review Meetings, ECCO Team at BBA, Braunschweig of 28 February) were fulfilled.

Residue definition and MRL

The proposed residue definition - for risk assessment and monitoring purpose - is the parent active substance only – as the only quantitatively significant substance detected in any plant commodity in plant metabolism studies.

Estimation of exposure through diet

The TMDI calculations using the EFSA PRIMo model (rev. 2) yielded a maximum usage of the ADI of 20 %. The estimate of the short term exposure was not considered necessary and thus not performed since an ARfD is not allocated. It can be concluded that a risk for the consumer does not arise from the long term or short term exposure to fenhexamid.

IIA 6.11.2 Reasonable grounds in support of the petition

Not considered since this is not an EC data requirement under Reg. 1107/2009/EC.