Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

pyridalyl (ISO); 2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether

EC Number: -

CAS Number: 179101-81-6

Index Number: not allocated

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1 PHYSICAL HAZARDS

1.1 Explosives

1.1.1 Study 1

Reference: Sweetapple 2002b

Test type: Guideline OPPTS 830.6316

Material and methods

Test material: S-1812 (pyridalyl) technical, batch PS-98041G, purity 93.7%.

The study evaluated the thermal explodability and impact explodability (Bureau of Explosives impact apparatus). For the impact explodability a small quantity of test substance was places in the liquid sample holder and impacted with an 3.63 kg weight from a drop heigh of 82.55 cm.

Results

- S-1812 did not exhibit impact explodability behaviour as tested at 25°C.
- S-1812 did not exhibit thermal explodability behaviour as tested up to 200°C.

1.2 Flammable gases (including chemically unstable gases)

Not applicable.

1.3 Oxidising gases

Not applicable.

1.4 Gases under pressure

Not applicable.

1.5 Flammable liquid

1.5.1 Study 1 – Flammability

Reference: Reitz 2002

Test type: Guidance EEC A.9, OPPTS 830.6315

Material and methods

Test material: pyridalyl technical, batch PS-98041G, purity 93.7%.

Measurements were made using an Erdco RapidTester Model. Two-ml samples were used for flash point measurements at 100°C or lower, and four-ml samples were used for determinations above 100°C.

Results

The measured flash point was 111°C. The barometric pressure was 759 mm Hg, so no correction for barometric pressure was required.

1.5.2 Study 2 – boiling point

Reference: Sweetapple, 2002

Test type: Guideline: EEC A.2, OPPTS 830.7220

Material and methods:

Test material: pyridalyl technical, batch AS 1817e, purity 99.1%.

Results:

S1812 degraded (as indicated by discoloration) at about 227°C before it boiled. Therefore, the degradation temperature is reported instead of a boiling temperature.

1.6 Flammable solids

Not applicable.

1.7 Self-reactive substances

Not applicable.

1.8 Pyrophoric liquids

No data.

1.9 Pyrophoric solid

Not applicable.

1.10 Self-heating substances

Not applicable.

1.11 Substances which in contact with water emit flammable gases

No data.

1.12 Oxidising liquids

1.12.1 Study 1

Reference: Reitz, 2002

Test type: OPPTS 830.6314, study design and performance does not enable any conclusions with regard to the oxidising properties as defined in EC A.21

Material and methods

Test material: pyridalyl technical, batch PS-98041G, purity 93.7%.

The incompatibility of the test substance with common oxidizing, reducing and fire extinguishing agents was evaluated. The test would indicate any hazardous reactions which can occur resulting from contact of the test substance with these reagents. The incompatibility of the test substance with water, monoammonium phosphate, granular zinc and a 1% (w/v) solution of potassium permanganate was assessed.

Results

The result showed that pyridalyl is compatible with the reagents.

1.13 Oxidising solids

Not applicable.

1.14 Organic peroxides

Not applicable.

1.15 Corrosive to metals

No data.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 Study 1

Characteristics

Reference	:	IIA 5.1.1/01, project No. 2144	exposure	:	single by gavage
type of study	:	Absorption, distribution and excretion	doses	:	5 mg/kg bw [phenyl-^{14}C]S-1812 500 mg/kg bw [phenyl-^{14}C]S-1812 5 mg/kg bw [propenyl-^{14}C]S-1812 500 mg/kg bw [propenyl-^{14}C]S-1812
year of execution	:	2001/2002	vehicle	:	Corn oil
test substances	:	[phenyl-^{14}C]S-1812 (pyridalyl), lot no. RIS98015, chemical purity 98.1%, radiochemical purity >97% (after repurification). [propenyl-^{14}C]S-1812, lot no. RIS98018, chemical purity 98.5%, radiochemical purity.	GLP statement	:	Yes
Route	:	Oral	guideline	:	US EPA (1989), MAFF
Species	:	Sprague-Dawley CD rat (144-202 g)	acceptability	:	Acceptable
group size	:	15 male and 15 female rats per dose group, in total 60 male and 60 female rats see table 2.1.1-01.			

Study design

Pharmacokinetic parameters of ^{14}C-S1812 (pyridalyl) were investigated after a single low (5 mg/kg bw) and high (500 mg/kg bw) oral dose of [phenyl-^{14}C]S-1812 or [propenyl-^{14}C]S-1812. All animals received food *ad libitum* throughout the study. Exposure and sampling of blood has been described in table 2.1.1-01.

Table 2.1.1-01: Experimental groups for each dose level

Group no.	No. of animals*	Treatment	Sampling times (h after dosing)	Sacrifice time (h after last dose)
1A	5 M & 5 F	5 mg/kg bw [phenyl-^{14}C]S-1812	Blood at 0, 1, 4, 12 and 72 hours	72
1B	5 M & 5 F	5 mg/kg bw [phenyl-^{14}C]S-1812	Blood at 0.25, 2, 6, 24 and 96 hours	96
1C	5 M & 5 F	5 mg/kg bw [phenyl-^{14}C]S-1812	Blood at 0.5, 3, 8, 48 and 120 hours	120
2A	5 M & 5 F	500 mg/kg bw [phenyl-^{14}C]S-1812	Blood at 0, 1, 4, 12 and 72 hours	72
2B	5 M & 5 F	500 mg/kg bw [phenyl-^{14}C]S-1812	Blood at 0.25, 2, 6, 24 and 96 hours	96
2C	5 M & 5 F	500 mg/kg bw [phenyl-^{14}C]S-1812	Blood at 0.5, 3, 8, 48 and 120 hours	120
3A	5 M & 5 F	5 mg/kg bw [propenyl-^{14}C]S-1812	Blood at 0, 1, 4, 12 and 72 hours	72
3B	5 M & 5 F	5 mg/kg bw [propenyl-^{14}C]S-1812	Blood at 0.25, 2, 6, 24 and 96 hours	96
3C	5 M & 5 F	5 mg/kg bw [propenyl-^{14}C]S-1812	Blood at 0.5, 3, 8, 48 and 120 hours	120
4A	5 M & 5 F	500 mg/kg bw [propenyl-^{14}C]S-1812	Blood at 0, 1, 4, 12 and 72 hours	72
4B	5 M & 5 F	500 mg/kg bw [propenyl-^{14}C]S-1812	Blood at 0.25, 2, 6, 24 and 96 hours	96
4C	5 M & 5 F	500 mg/kg bw [propenyl-^{14}C]S-1812	Blood at 0.5, 3, 8, 48 and 120 hours	120

* extra animals were dosed per dose group: Phenyl low and high dose group 2 extra male and 1 extra female rat; Propenyl low dose group 2 extra male and 1 extra female rat; Propenyl high dose group 1 extra male and 1 extra female.

Radioactivity in whole blood, plasma and red blood cells was quantified by liquid scintillation counting (LSC).

Results

Radiochemical purity analysis pre and post dosing showed that radiochemical purity of [phenyl-^{14}C]S-1812 was between 98.6 and 100.0%. Achieved doses were 108% of nominal for the low dose group and 91-105% of nominal for the high dose group.

Radiochemical purity analysis pre and post dosing showed that radiochemical purity of [propenyl-^{14}C]S-1812 was between 99.3 and 100.0%. Achieved doses were 102-115 % of nominal for the low dose group and 98-104% for the high dose group.

Blood residue concentrations

Combustion analysis of whole blood, plasma and red blood cells was performed. The results demonstrate that absorption into blood occurred at approximately 2 hours after dosing for both treatments and both sexes. Individual concentrations measured at the different time points differed substantially. Data outlier corrections were performed for all data as the rate of uptake of the compound in each animal was idiosyncratic and disparate within the sampling group. The three animals with the lowest deviation from the mean concentration were selected to generate the Representative Group mean per time point.

Radioactivity was found between 0.5 hours after dosing and 72 hours after dosing with 5 mg/kg bw [phenyl-^{14}C]S-1812; between 2 hours and 120 hours after dosing with 500 mg/kg bw [phenyl-^{14}C]S-1812; between 2 hours after dosing and 120 hours after dosing with 5 mg/kg bw [propenyl-^{14}C]S-1812; between 0.5 hours and 120 hours after dosing with 500 mg/kg bw [propenyl-^{14}C]S-1812.

Blood-plasma ratios ranged from 0.03 to 1.40 after dosing with 5 mg/kg bw [phenyl-^{14}C]S-1812; from 0.10 to 3.15 after dosing with 500 mg/kg bw [phenyl-^{14}C]S-1812; from 0.32 to 1.80 after dosing with 5 mg/kg bw [propenyl-^{14}C]S-1812; from 0.48 to 2.09 after dosing with 500 mg/kg bw [propenyl-^{14}C]S-1812.

Plasma pharmacokinetic parameters

The pharmacokinetic parameters are presented in Table 2.1.1-02. Peak [phenyl-^{14}C]S-1812 concentrations in blood were observed after 6-8 hours in both sexes. The half-life of [phenyl-^{14}C]S-1812 was approximately 18 hours after a dose of 5 mg/kg bw and 35 hours after a dose of 500 mg/kg bw. C_{max} was 0.255 ppm (males) and 0.212 ppm (females) after the low dose and 21.04 ppm (males) and 21.15 ppm (females) after the high dose. The area under the curve (AUC) was 5.54 µg*h/g (males) and 3.15 µg*h/g (females) after the low

dose and 568.09 µg*h/g (males) and 481.10 µg*h/g (females) and after the high dose. Both C_{max} and AUC increased dose proportional. No difference between sexes was observed.

Peak [propenyl-^{14}C]S-1812 concentrations in blood were observed after 6-12 hours in both sexes after the low dose and 12-24 hours in both sexes after the high dose. The half-life of [propenyl-^{14}C]S-1812 was 60 hours (males) and 52 hours (females) after a dose of 5 mg/kg bw and 80 hours (males) and 91 hours (females) after a dose of 500 mg/kg bw. C_{max} was 0.412 ppm (males) and 0.324 ppm (females) after the low dose and 34.75 ppm (males) and 32.35 ppm (females) after the high dose. The area under the curve was 23.33 µg*h/g (males) and 18.38 µg*h/g (females) after the low dose and 3597 µg*h/g (males) and 4116 µg*h/g (females) after the high dose. C_{max} increased dose proportional, whereas AUC increased dose proportional in male rats and more than dose proportional in female rats. No other difference between sexes was observed.

The propenyl label was slower eliminated compared to the phenyl label: especially in the high dose group longer half lives and higher AUC values were observed.

Individual concentrations measured at the different time points differed substantially. Data outlier corrections were performed for all data as the rate of uptake of the compound in each animal was idiosyncratic and disparate within the sampling groups. Furthermore ranges for T_{max} were reported in stead of a specific time point due to the variability of response among animal test groups.

Table 2.1.1-02: Pharmacokinetic parameters in plasma of male and female rats after single oral exposure to ^{14}C-S1812

	5 mg/kg bw [phenyl-^{14}C]S-1812		**500 mg/kg bw [phenyl-^{14}C]S-1812**	
	M	**F**	**M**	**F**
C_{max} (ppm)	0.255	0.212	21.04	21.15
T_{max} (h)	6-8	6-8	8	8
$T_{1/2}$ (h)$^{(A)}$	18	18	35	35
AUC (µg*h/g)	5.54	3.15	568.09	481.10
½ C_{max} AB (ppm)	0.07	0.11	9.49	10.22
½ T_{max} AB (h)	4-6	4-6	6	6
½ C_{max} EL (ppm)	0.07	0.16	11.73	6.13
½ T_{max} EL (h)	12-24	12-24	12-24	12-24
1/10 C_{max} EL (ppm)	0	0	2.15	1.38
1/10 T_{max} EL (h)	48	48	48	48
	5 mg/kg bw [propenyl-^{14}C]S-1812		**500 mg/kg bw [propenyl-^{14}C]S-1812**	
	M	**F**	**M**	**F**
C_{max} (ppm)	0.412	0.324	34.75	32.35
T_{max} (h)	6-12	6-12	12-24	12-24
$T_{1/2}$ (h)$^{(A)}$	60	52	80	91
AUC (µg*h/g)	23.33	18.38	3596.51	4116.33
½ C_{max} AB (ppm)	0.20	0.06	17.76	16.99
½ T_{max} AB (h)	3-4	3-4	6-8	6-8

½ C_{max} EL (ppm)	0.20	0.15	19.05	15.95
½ T_{max} EL (h)	24-48	24-48	72	72
1/10 C_{max} EL (ppm)	0.06	0.03	11.86	13.10
1/10 T_{max} EL (h)	96-120	96-120	120	120

AB = absorption phase
EL = elimination phase

Conclusions

Pharmacokinetic parameters of ^{14}C-S1812 were investigated after a single low (5 mg/kg bw) and high (500 mg/kg bw) oral dose of [phenyl-^{14}C]S-1812 or [propenyl-^{14}C]S-1812.

Peak [phenyl-^{14}C]S-1812 concentrations in blood were observed after 6-8 hours in both sexes. The half life of [phenyl-^{14}C]S-1812 was approximately 18 hours after a dose of 5 mg/kg bw and 35 hours after a dose of 500 mg/kg bw. C_{max} was 0.255 ppm (males) and 0.212 ppm (females) after the low dose and 21.04 ppm (males) and 21.15 ppm (females) after the high dose. The area under the curve was 5.54 µg*h/g (males) and 3.15 µg*h/g (females) after the low dose and 568.09 µg*h/g (males) and 481.10 µg*h/g (females) and after the high dose. Both C_{max} and AUC increased dose proportional. No difference between sexes was observed.

Peak [propenyl-^{14}C]S-1812 concentrations in blood were observed after 6-12 hours in both sexes after the low dose and 12-24 hours in both sexes after the high dose. The half-life of [propenyl-^{14}C]S-1812 was 60 hours (males) and 52 hours (females) after a dose of 5 mg/kg bw and 80 hours (males) and 91 hours (females) after a dose of 500 mg/kg bw. C_{max} was 0.412 ppm (males) and 0.324 ppm (females) after the low dose and 34.75 ppm (males) and 32.35 ppm (females) after the high dose. The area under the curve was 23.33 µg*h/g (males) and 18.38 µg*h/g (females) after the low dose and 3597 µg*h/g (males) and 4116 µg*h/g (females) after the high dose. C_{max} increased dose proportional, whereas AUC increased dose proportional in male rats and more than dose proportional in female rats. No other difference between sexes was observed.

The propenyl label was slower eliminated compared to the phenyl label: especially in the high dose group longer half lives and higher AUC values were observed.

Acceptability

The study is **acceptable.**

Remarks include variations between radioactivity levels in individual animals. Individual concentrations measured at the different time points differed substantially. Data outlier corrections were performed for all data as the rate of uptake of the compound in each animal was idiosyncratic and disparate within the sampling group. The three animals with the lowest deviation from the mean concentration were selected to generate the Representative Group mean (3 animals) per time point. However, the actual group mean (5

animals) demonstrated the same trend of the results. The number of animals per group was sufficient and the number of sampling points was sufficient, leading to the conclusion that this study is acceptable.

2.1.2 Study 2

Characteristics

reference	:	IIA 5.1.1/02, project No. 807W/1221E-1	exposure	:	single by gavage
type of study	:	Metabolism	doses	:	5.0 & 500 mg/kg bw for males and females; [^{14}C-pyridyl]S-1812 only 5.0 mg/kg bw.
year of execution	:	1999-2001	vehicle	:	Corn oil
test substances	:	^{14}C-S-1812 (Pyridalyl) (^{14}C labelled in the phenyl ring): lot no. RIS98015 (spec. act. 10.2 MBq/mg), chemical purity 98.1%, radiochemical purity 99.0% of . ^{14}C-S-1812 (^{14}C labelled in the propenyl group: lot. no RIS98018 (spec. act 4.12 MBq/mg), chemical purity 98.5%, radiochemical purity 98.7%. ^{14}C-S-1812 (^{14}C labelled in the pyridyl ring: lot. no RIS97020 (spec. act 9.05 MBq/mg), chemical purity 100%, radiochemical purity 99.6%. Unlabelled S-1812: lot no. 980302G, chemical purity 99.7%.	GLP statement	:	Yes
route	:	Oral (intubation).	guidelines	:	EPA guideline OPPTS 870.7485
species	:	Sprague Dawley (M 188-229 g; F 153-199 g)	acceptability	:	Acceptable
group size	:	4 male and 4 non pregnant female rats per dose group (40 rats in total)			

Study design

The metabolism of ^{14}C-S-1812 (pyridalyl) was investigated in 7 days collection intervals of urine, faeces, cage wash and expired volatiles (not for [^{14}C-pyridyl]S-1812). At 7 days after dosing blood, tissues and carcass were collected. For study design and collection intervals of urine and faeces, see Table 2.1.2-01.

Table 2.1.2-01 Collection intervals and pooled urine and faeces subsamples

Treatment	Specimen	Sampling times (h after dosing)	Pooled subsamples*
5 mg/kg bw [phenyl-^{14}C]S-1812	urine	6, 12, 24, 48, 72, 96, 120, 144, 168 h	6, 12, 24, 48-168 h
5 mg/kg bw [phenyl-^{14}C]S-1812	faeces	24, 48, 72, 96, 120, 144, 168 h	24, 48, 72-168 h
500 mg/kg bw [phenyl-^{14}C]S-1812	urine	6, 12, 24, 48, 72, 96, 120, 144, 168 h	0-72 h
500 mg/kg bw [phenyl-^{14}C]S-1812	faeces	24, 48, 72, 96, 120, 144, 168 h	24, 48, 72, 96-168 h
5 mg/kg bw [propenyl-^{14}C]S-1812	urine	6, 12, 24, 48, 72, 96, 120, 144, 168 h	M: 6, 12, 24, 48-168 h F: 6, 12, 24, 48, 72-168 h
5 mg/kg bw [propenyl-^{14}C]S-1812	faeces	24, 48, 72, 96, 120, 144, 168 h	24, 48, 72-168 h
500 mg/kg bw [propenyl-^{14}C]S-1812	urine	6, 12, 24, 48, 72, 96, 120, 144, 168 h	0-24, 48-168 h
500 mg/kg bw [propenyl-^{14}C]S-1812	faeces	24, 48, 72, 96, 120, 144, 168 h	24, 48, 72-168 h
5 mg/kg bw [pyridyl-^{14}C]S-1812	urine	6, 12, 24, 48, 72, 96, 120, 144, 168 h	0-48 h
5 mg/kg bw [pyridyl-^{14}C]S-1812	faeces	24, 48, 72, 96, 120, 144, 168 h	24, 48, 72-168 h

* $\geq$ 5% radioactivity per sex/time point: excreta of rats from that time point were pooled per sex; <5% radioactivity per sex/time point: excreta of rats from this time point and the next were pooled per sex.

Collection intervals for expired volatiles were 0-6, 6-12, 12-24 and once daily thereafter. Blood and tissues were collected at 7 days after dosing. Tissues collected were: adrenal gland, bone, bone marrow, brain, heart, lungs, muscle, pancreas, pituitary gland, salivary glands, spinal cord, spleen, thymus, thyroid, fat, hair/skin, liver, kidneys, caecum, small intestine, large intestine, stomach, gastro intestinal contents, plasma, red blood cells, whole blood, ovaries and uterus (females), testes (males) and residual carcass. Cage wash was performed at the end of each collection interval.

Radiocarbon in urine and expired carbon dioxide traps were directly analysed by direct LSC. Faeces, tissue and carcass radiocarbon was determined by combustion to carbon dioxide and LSC.
Metabolites were identified by a HPLC System (faecal extracts, urine metabolite separation), LC/MS was used for confirmation of metabolites in urine. Mass Spectrometry was performed to identify metabolites from pyridyl labelled urine. Thin-layer chromatography (TLC) was used for separation and quantitation of urine metabolites and confirmation of faecal metabolites.

Analysis of ^{14}C in excreta
After pooling, urine samples were directly used for TLC analysis, [pyridyl-^{14}C] urine samples were also analyzed after acid hydrolysis (HCl). Faecal samples were extracted prior to analysis. Faeces samples were homogenized with acetone, supernatant was radio assayed by LSC, residues were further extracted with methanol/water (3 x), methanol/0.1 N HCl, 3x methanol/0.1 NaOH and finally an enzyme hydrolysis (pronase hydrolysis) took place until >90% of the radiocarbon was recovered.
Analysis of ^{14}C in tissues and carcass: analyzed by combustion and LSC of trapped CO_2. Tissue was combusted in aliquots of 100-500 mg (when available, otherwise: halved and each half combusted). Duplicates were analyzed.
Urine metabolites were separated and quantified by HPLC and one dimensional TLC (and HPLC examination). HPLC was used for identification of faecal metabolites. Two dimensional TLC was used for confirmation and identification of faecal metabolites.

Pilot study
A pilot trapping study was conducted to determine a more efficient system for trapping volatile radioactivity generated during metabolism of [propenyl-^{14}C]S-1812. Four (2 male and 2 female) rats were treated with a single dose of 500 mg/kg bw [propenyl-^{14}C]S-1812. Urine, faeces and carcass were collected. Common carbon-dioxide trapping solutions were used as well as trapping agent known for their affinity for small weight aldehyde and alkaline compounds. The results indicated that the trapping media utilized accounted for no other radioactive compounds that the previously established $^{14}CO_2$.

Results

Doses were administered with a relative standard deviation of 1.2-7.0%.
Total recovery was 90.6-100.1% of administered radioactivity after administration of [phenyl-^{14}C]S-1812, 87.1-91.1% after administration of [propenyl-^{14}C]S-1812 and 95.9-100.6% after administration of [pyridyl-^{14}C]S-1812. Reasons for the relatively low recovery after administration of [propenyl-^{14}C]S-1812 could be that trapping of $^{14}CO_2$ might have been incomplete or cage wash did not efficiently remove all the radiocarbon (S-1812 could absorb to the glass), or complete homogenization of carcasses was not obtained (due to the lipophilic nature of S-1812).

After a single dose of [phenyl-^{14}C]S-1812 the majority of administered radioactivity was excreted in faeces from 0-168 hours (83.8-96.1% application rate (AR), see table 2.1.2-02), whilst radioactivity in urine accounted for 1.6-2.0% AR from 0-168 hours. Radioactivity in tissues ranged from 0.6-1.2%, in the carcass radioactivity ranged from 1.1-4.1%, whereas no radioactivity was found in expired air.

After a single dose of [propenyl-^{14}C]S-1812 the majority of administered radioactivity was excreted in faeces as well (54.9-58.8% AR in 0-168 hours, see table 2.1.2-02), radioactivity in urine accounted for 9.7-17.7% AR from 0-168 hours and radioactivity in expired air accounted for 10.8-11.6% from 0-168 hours. Radioactivity in tissues ranged from 1.5-2.1% and in the carcass radioactivity ranged from 3.9-7.9%.
Administered radioactivity of [pyridyl-^{14}C]S-1812 was mainly excreted in faeces from 0-168 hours (92.7-96.7% AR, see table 2.1.2-03), radioactivity in urine accounted for 2.0-2.1% AR from 0-168 hours. Radioactivity in tissues ranged from 0.4-0.6%, in the carcass radioactivity ranged from 0.7-1.3%. Expired air was not collected.

In general the majority of the administered radioactivity was excreted in the first 48 hours after administration.

Table 2.1.2-02 Recovery of radioactivity (% AR) in rats after single oral exposure to [phenyl-^{14}C]S-1812 and [propenyl-^{14}C]S-1812

		5 mg/kg bw [phenyl^{14}C]S-1812		500 mg/kg bw [phenyl^{14}C]S-1812		5 mg/kg bw [propenyl^{14}C]S-1812		500 mg/kg bw [propenyl^{14}C]S-1812	
sample		**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Faeces	0-24 h	64.4	52.6	51.2	29.2	43.4	32.6	36.0	30.3
	24-48 h	23.9	40.1	27.7	44.6	9.7	22.1	15.7	24.5
	48-72 h	2.3	2.1	5.0	6.5	0.8	1.3	1.6	1.5
	72-96 h	0.6	0.6	1.4	1.7	0.4	0.5	0.6	0.7
	96-120 h	0.3	0.3	0.9	0.8	0.3	0.3	0.6	0.4
	120-144 h	0.3	0.2	0.6	0.5	0.2	0.2	0.3	0.3
	144-168 h	0.2	0.2	0.5	0.5	0.1	0.2	0.4	1.1
	0-168 h	**92.0**	**96.1**	**87.3**	**83.8**	**54.9**	**57.2**	**55.2**	**58.8**
Urine including cage wash	0-6 h	0.3	0.1	0.1	0.0	1.3	2.0	0.3	0.5
	6-12 h	0.7	0.5	0.4	0.3	7.8	5.4	1.5	1.0
	12-24 h	0.6	0.6	0.7	0.7	4.4	5.1	4.5	3.6
	24-48 h	0.3	0.6	0.4	0.4	1.8	3.6	3.4	3.2
	48-72 h	0.1	0.1	0.1	0.1	0.5	0.6	0.7	0.6
	72-96 h	0.0	0.0	0.1	0.0	0.4	0.4	0.4	0.3
	96-120 h	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3
	120-144 h	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.1
	144-168 h	0.0	0.1	0.1	0.1	0.2	0.1	0.2	0.1
	0-168 h	**2.0**	**2.0**	**1.9**	**1.6**	**16.9**	**17.7**	**11.5**	**9.7**
Expired air	0-6 h	0.0	0.0	0.0	0.0	2.3	1.6	0.2	0.4
	6-12 h	0.0	0.0	0.0	0.0	3.9	3.1	0.6	0.8
	12-24 h	0.0	0.0	0.0	0.0	2.7	2.6	3.8	3.9
	24-48 h					1.4	2.2	3.7	3.7
	48-72 h					0.5	0.5	1.5	0.8
	72-96 h					0.4	0.4	0.5	0.5
	96-120 h					0.2	0.2	0.3	0.3
	120-144 h					0.1	0.1	0.2	0.2
	144-168 h					0.1	0.1	0.2	0.2
	0-168 h	**0.0**	**0.0**	**0.0**	**0.0**	**11.6**	**10.8**	**11.0**	**10.8**
Tissues	**0-168 h**	**0.6**	**0.6**	**1.2**	**1.1**	**1.6**	**1.5**	**2.1**	**2.1**
Carcass	**0-168 h**	**1.8**	**1.1**	**3.6**	**4.1**	**5.4**	**3.9**	**7.9**	**5.7**
Recovery	**168 h**	**96.4**	**100.1**	**94.0**	**90.6**	**90.4**	**91.1**	**87.7**	**87.1**

Table 2.1.2-03 Recovery of radioactivity (% AR) in rats after single oral exposure to [pyridyl-^{14}C]S-1812

		5 mg/kg bw [pyridyl^{14}C]S-1812	
Sample		**M**	**F**
Faeces	0-24 h	78.1	63.2
	24-48 h	16.0	26.8
	48-72 h	1.4	1.8
	72-96 h	0.4	0.4
	96-120 h	0.3	0.2
	120-144 h	0.2	0.2
	144-168 h	0.3	0.1
	0-168 h	**96.7**	**92.7**
Urine including cage wash	0-6 h	0.2	0.0
	6-12 h	1.0	0.6
	12-24 h	0.8	0.8
	24-48 h	0.2	0.7
	48-72 h	0.0	0.1
	72-96 h	0.0	0.0
	96-120 h	0.0	0.0
	120-144 h	0.0	0.0
	144-168 h	0.0	0.0
	0-168 h	**2.0**	**2.1**
Expired air*			
Tissues	**0-168 h**	**0.6**	**0.4**
Carcass	**0-168 h**	**1.3**	**0.7**
Recovery	**168 h**	**100.6**	**95.9**

* not collected.

Extraction of the faeces samples showed that the major part of radioactivity was present in the acetone extract (77-85% of the administered radioactivity) after dosing with [phenyl-^{14}C]S-1812 and 38-44% after dosing with [proprenyl-^{14}C]S-1812), while aqueous extracts contained 9-12% (low dose [phenyl-^{14}C]S-1812); 2-4% (high dose [phenyl-^{14}C]S-1812); 4-8% ([propenyl-^{14}C]S-1812); 5% ([pyridyl-^{14}C]S-1812). Post extraction solids (PES) contained 4-5% (low dose [phenyl-^{14}C]S-1812); 0.3% (high dose [phenyl-^{14}C]S-1812); 1-5% ([propenyl-^{14}C]S-1812); 2% ([pyridyl-^{14}C]S-1812). All other extractions contained <3% of the administered radioactivity. See Table 2.1.2.-04.

Table 2.1.2-04 Recovery of radioactivity (% of dose) in faeces after extraction after single exposure to ^{14}C-S1812

	5 mg/kg bw [phenyl-^{14}C]S-1812							
	0-24h		24-48h		72-168h		TOTAL	
	M	F	M	F	M	F	M	F
Average recovery (%AR)	69.5	53.1	23.8	38.2	3.4	2.4	96.7	93.7
Acetone extract	58.0	44.4	19.7	31.0	2.4	1.7	80.1	77.1
MeOH/H2O extract	6.5	6.4	2.1	4.6	0.6	0.5	9.2	11.5
MeOH/HCl extract	1.7	1.0	0.4	0.7	0.1	ND	2.1	1.7
PES	3.4	1.3	1.6	2.6	0.3	0.2	5.3	4.1

	500 mg/kg bw [phenyl-^{14}C]S-1812									
	0-24h		24-48h		48-72h		96-168h		TOTAL	
	M	F	M	F	M	F	M	F	M	F
Average recovery (%AR)	51.6	31.9	25.2	47.7	4.9	7.3	3.4	3.7	85.1	90.6
Acetone extract	50.1	30.8	23.3	44.1	4.4	6.5	2.8	3.1	80.5	84.5
MeOH/H2O extract	0.9	0.8	0.9	2.4	0.3	0.5	0.3	0.3	2.4	4.0
MeOH/HCl extract	0.1	0.1	0.2	0.4	0.0	0.1	0.0	0.1	0.4	0.7
MeOH/NaOH extract	0.3	0.1	0.4	0.4	0.1	0.1	0.1	0.1	0.8	0.7
Aqueous phosphate buffer	0.1	-	0.1	0.1	0.0	0.0	0.0	0.0	0.2	0.1
Protease hydrolysis	0.0	-	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1
Methanol (100%)	0.1	-	0.1	0.1	0.0	0.0	0.0	0.0	0.2	0.1
PES	0.1	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.3	0.3

	5 mg/kg bw [propenyl-^{14}C]S-1812							
	0-24h		24-48h		72-168h		TOTAL	
	M	F	M	F	M	F	M	F
Average recovery (%AR)	44.5	34.6	9.3	21.4	1.8	2.6	55.6	58.6
Acetone extract	32.1	26.8	5.0	12.6	0.8	1.5	37.9	40.9
MeOH/H2O extract	3.9	3.0	1.4	4.4	0.3	0.4	5.6	7.8
MeOH/HCl extract	0.9	0.6	0.3	1.1	0.1	0.1	1.3	1.8
MeOH/NaOH extract	1.1	0.6	0.2	0.9	0.1	0.1	1.4	1.6
Aqueous phosphate buffer	2.1	1.2	0.5	1.3	0.1	0.1	2.7	2.6
Protease hydrolysis	1.4	0.8	0.6	1.0	0.2	0.2	2.2	2.0
PES	3.0	1.6	1.3	1.1	0.3	0.3	4.6	3.0

Table 2.1.2-04 Recovery of radioactivity (% of dose) in faeces after extraction after single exposure to ^{14}C-S1812 (cont)

	500 mg/kg bw [propenyl-^{14}C]S-1812							
	0-24h		24-48h		72-168h		TOTAL	
	M	F	M	F	M	F	M	F
Average recovery (%AR)	34.9	27.9	12.9	22.5	2.4	4.4	50.2	54.8
Acetone extract	32.4	26.6	7.8	14.9	1.5	2.7	41.7	44.2
MeOH/H2O extract	1.5	0.8	2.2	3.1	0.4	0.8	4.1	4.7
MeOH/HCl extract	0.3	0.2	0.8	1.3	0.1	0.2	1.2	1.7
MeOH/NaOH extract	0.3	0.2	0.5	1.1	0.1	0.2	0.9	1.5
Aqueous phosphate buffer	0.4	0.1	1.2	1.6	0.3	0.3	1.9	2.0
Protease hydrolysis	0.1	0.0	0.4	0.5	0.1	0.2	0.6	0.7
PES	0.2	0.1	0.6	0.8	0.2	0.4	1.0	1.3
	5 mg/kg bw [pyridyl-^{14}C]S-1812							
	0-24h		24-48h		72-168h		TOTAL	
	M	F	M	F	M	F	M	F
Average recovery (%AR)	79.6	64.9	12.2	28.6	1.4	1.2	93.2	94.7
Acetone extract	71.9	59.3	11.0	24.8	1.1	1.0	84.0	85.1
MeOH/H2O extract	4.0	2.9	0.6	1.6	0.1	0.1	4.7	4.6
MeOH/HCl extract	0.6	0.4	0.1	0.4	0.1	0.0	0.8	0.8
MeOH/NaOH extract	0.9	0.6	0.1	0.3	0.0	0.0	1.0	0.9
Aqueous phosphate buffer	0.3	0.3	0.0	0.2	0.0	0.0	0.3	0.5
Protease hydrolysis+methanol	0.5	0.4	0.1	0.3	0.0	0.0	0.6	0.7
PES	1.3	1.0	0.3	1.0	0.1	0.1	1.7	2.1

In tissues only 0.6-2.1% of the administered radioactivity was recovered. In the carcass the recovery was somewhat higher, ranging from 0.7-7.9% AR, the highest concentrations were found after the higher doses and after dosing with the propenyl labelled S-1812.

Highest average radiocarbon concentrations (ppm of [^{14}C]S-1812 equivalents) in tissues after a single dose of ^{14}C-labeled S-1812 were found in fat, adrenal gland, pancreas, salivary glands, thyroid and hair/skin. In female animals relatively high radiocarbon concentrations were found in ovaries and uterus. After the higher doses (500 mg/kg bw) the recovered radiocarbon concentrations were higher compared to the lower doses. The average radiocarbon concentrations recovered in tissues was higher after dosing with [propenyl-^{14}C]S-1812 compared to [phenyl-^{14}C]S-1812 and [pyridyl-^{14}C]S-1812. No differences between males and females could be detected. See Table 2.1.2-05.

Table 2.1.2-05 Summary (highest concentrations) distribution of radioactivity in tissues and organs [ppm average radiocarbon concentrations and average % of administered dose] at 168 hours post dose of ^{14}C-labeled S-1812

	5 mg/kg bw [phenyl-^{14}C]S-1812				500 mg/kg bw [phenyl-^{14}C]S-1812				5 mg/kg bw [propenyl-^{14}C]S-1812				500 mg/kg bw [propenyl-^{14}C]S-1812			
	male		Female		male		female		male		female		male		female	
tissue	ppm	% AR	ppm	% AR	ppm	% AR	ppm	% AR	ppm	% AR	ppm	% AR	ppm	% AR	ppm	% AR
Adrenal gland	0.281	0.00	0.330	0.00	92	0.00	85	0.00	1.248	0.01	0.880	0.01	166	0.01	143	0.02
Pancreas	0.190	-	0.241	0.03	55	0.00	49	0.10	0.542	0.05	0.531	0.07	103	0.14	80	0.09
Salivary glands	0.065	0.01	0.099	0.01	36	0.00	34	0.00	0.320	0.02	0.355	0.02	64	0.05	59	0.04
Thyroid	0.102	0.00	0.072	0.00	48	0.00	34	0.00	0.647	0.00	0.423	0.00	64	0.00	61	0.00
Fat	0.873	-	0.809	-	186	-	173	-	1.682	-	1.660	-	293	-	277	-
Hair/skin	0.220	-	0.190	-	60	-	44	-	0.563	-	0.413	-	111	-	54	-
Ovaries	-	-	0.291	0.01	-	-	60	0.00	-	-	0.567	0.01	-	-	135	0.03
Uterus	-	-	0.074	0.00	-	-	14	0.00	-	-	0.314	0.03	-	-	36	0.02

Table 2.1.2-05 Summary (highest concentrations) distribution of radioactivity in tissues and organs [ppm average radiocarbon concentrations and average % of administered dose] at 168 hours post dose of ^{14}C-labeled S-1812 (cont)

	5 mg/kg bw [pyridyl-^{14}C]S-1812			
	Male		Female	
tissue	ppm	% AR	ppm	% AR
Adrenal gland	0.308	0.00	0.267	0.00
Pancreas	0.263	0.05	0.415	0.07
Salivary glands	0.100	0.01	0.101	0.01
Thyroid	0.014	0.00	0.00	0.00
Fat	0.715	-	0.806	-
Hair/skin	0.238	-	0.161	-
Ovaries	-	-	0.217	0.00
Uterus	-	-	0.138	0.01

Metabolite identification in faeces (table 2.1.2-06)

After dosing with 5 mg/kg bw [phenyl-^{14}C]S-1812 approximately 30% of the administered radioactivity was excreted as the parent compound (S-1812) in 0-48 h compared to approximately 50% after dosing with 500 mg/kg bw [phenyl-^{14}C]S-1812.

After dosing with (both low and high dose) [phenyl-^{14}C]S-1812, the main metabolite in faeces was identified as S-1812-DP (47.3 and 49.9% AR after 5 mg/kg bw and 24.6-28.7% AR after 500 mg/kg bw). Other metabolites identified included S-1812-Py-OH (0.9-2.2% AR), HPHM (0.9-1.2% AR), DCHM (2.4-3.3% AR) and polar metabolites (1.5-1.8% AR). All identified metabolites were confirmed by co-chromatography.

No differences in metabolite pattern between the sexes became apparent. After the higher dose relatively more parent compound (50%) and less S-1812-DP (30%) was excreted compared to the lower dose (30% and 50% respectively).

The parent compound and metabolites were excreted mainly in the first 48 hours after dosing. Only S-1812-DP was present for >1% at 48-168 hours after dosing.

After dosing with 5 mg/kg bw [propenyl-^{14}C]S-1812 approximately 30% of the administered radioactivity was excreted as the parent compound (S-1812) compared to approximately 40% after dosing with 500 mg/kg bw [propenyl-^{14}C]S-1812.

After dosing with (both low and high dose) [propenyl-^{14}C]S-1812, metabolites in faeces were identified as S-1812-Py-OH (3.4 and 4.1% AR after 5 mg/kg bw and 1.1-1.7% AR after 500 mg/kg bw), HPHM (2.5 and 3.9% AR after 5 mg/kg bw and 0.3-0.5% AR after 500 mg/kg bw), DCHM (3.4% AR after 5 mg/kg bw and 0.2-0.0% AR after 500 mg/kg bw) and polar metabolites (0.3-3.5% AR after 5 mg/kg bw and 0.8-1.8% AR after 500 mg/kg bw). S-1812-DP did not occur, since its structure does not include the propenyl moiety.

No differences in metabolite pattern between the sexes became apparent. After the higher dose relatively more parent compound (40%) was excreted compared to the lower dose (30%).

After dosing with 5 mg/kg bw [pyridyl-^{14}C]S-1812 approximately 33-39% of the administered radioactivity was excreted as the parent compound (S-1812).

After dosing with [pyridyl-^{14}C]S-1812, the main metabolite in faeces was identified as S-1812-DP (41.9 and 50.5% AR). 1.3% AR was excreted as S-1812-Py-OH. No other metabolites were identified. All identified metabolites were confirmed by co-chromatography.

The parent compound and metabolites were excreted mainly in the first 48 hours after dosing. Only S-1812-DP was present for >1% at 48-168 hours after dosing. No differences in metabolite pattern between the sexes became apparent, the metabolite pattern was comparable to that of [phenyl-^{14}C]S-1812.

Table 2.1.2-06 Metabolite identification (% AR) in faeces of male and female rats after single oral exposure to ^{14}C-S-1812

	% AR in faeces TOTAL										
	5 mg/kg bw [phenyl-^{14}C]S-1812		500 mg/kg bw [phenyl-^{14}C]S-1812		5 mg/kg bw [propenyl-^{14}C]S-1812		500 mg/kg bw [propenyl-^{14}C]S-1812		5 mg/kg bw [pyridyl-^{14}C]S-1812		
Fraction	M	F	M	F	M	F	M	F	M	F	**Identity**
Acetone	31.7	31.0	51.5	49.8	32.2	28.1	39.4	41.8	32.5	39.2	S-1812
	1.6	2.2	0.9	1.1	3.4	4.1	1.1	1.7	1.3	1.3	S-1812-Py-OH
	49.9	47.3	24.6	28.7					50.5	41.9	S-1812-DP
			0.9	1.2	2.5	3.9	0.3	0.5			HPHM
			3.3	2.4	3.4	3.4	0.2	0.0			DCHM
			1.5	1.8	0.3	3.5	1.8	1.7			Polar A[a]
					1.1	1.6	0.8	1.4			Polar B[a]
	12.6	13.3	0.3	0.3	0.9	4.1	1.8	1.4	3.7	6.0	Other metabolites[b]
total identified[c]	96.4	93.7	84.9	90.5	55.7	59.7	51.4	56.1	93.2	94.7	

[a] Retention times for peak A = sf-3.2 min and for peak B: 3.2-4.5 min. These retention times are approximate due to slight variation between analyses. For the phenyl labelled S-1812 peak A was appr. 5.5 min.
[b] All unidentified metabolites other than polar metabolites.
[c] Including %AR identified in all other extracts.

Table 2.1.2-06 Metabolite identification (% AR) in faeces of male and female rats after single oral exposure to ^{14}C-S-1812 (cont)

	% AR in faeces 0-24 h interval										
	5 mg/kg bw [phenyl^{14}C]S-1812		500 mg/kg bw [phenyl^{14}C]S-1812		5 mg/kg bw [propenyl^{14}C]S-1812		500 mg/kg bw [propenyl^{14}C]S-1812		5 mg/kg bw [pyridyl^{14}C]S-1812		
Fraction	M	F	M	F	M	F	M	F	M	F	**Identity**
Acetone	28.9	23.9	44.0	26.7	27.0	21.5	32.2	26.5	28.9	30.4	S-1812
	1.3	0.8	0.1	0.0	2.5	2.0	0.0	0.0	1.1	0.7	S-1812-Py-OH
	27.2	19.4	6.0	4.0					41.0	26.2	S-1812-DP
			0.0	0.0	0.9	0.6	0.0	0.0			HPHM
			0.0	0.0	1.0	0.8	0.0	0.0			DCHM
			0.0	0.0	0.1	0.3	0.0	0.0			Polar A[a]
					0.3	0.9	0.0	0.0			Polar B[a]
	0.6	0.3	0.0	0.1	0.3	0.7	0.2	0.1	0.9	2.0	Other metabolites[b]
Methanol: water	1.9	2.0	0.2	0.3	1.7	0.2	0.6	0.2	0.3	0.4	S-1812
	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	S-1812-Py-OH
	3.6	2.5	0.3	0.2					1.3	1.0	S-1812-DP
			0.0	0.0	0.2	0.2	0.0	0.0			HPHM
			0.0	0.0	1.3	0.6	0.0	0.0			DCHM
			0.4	0.3	0.1	1.2	0.6	0.3			Polar A[a]
					0.4	0.0	0.3	0.2			Polar B[a]
	0.9	1.8	0.0	0.0	0.1	0.8	0.0	0.1	2.4	1.5	Other metabolites[b]
All other extracts	1.7	1.0	0.6	0.2	5.5	3.2	1.1	0.5	2.4	1.7	Radiocarbon as % of dose
Unextracted	3.4	1.3	0.1	0.1	3.0	1.6	0.2	0.1	1.3	1.0	Radiocarbon as % of dose
total identified	69.6	53.1	51.7	31.9	44.5	34.6	35.2	28.0	79.6	64.9	

[a] Retention times for peak A = sf-3.2 min and for peak B: 3.2-4.5 min. These retention times are approximate due to slight variation between analyses. For the phenyl labelled S-1812 peak A was appr. 5.5 min.
[b] All unidentified metabolites other than polar metabolites.

Table 2.1.2-06 Metabolite identification (% AR) in faeces of male and female rats after single oral exposure to ^{14}C-S-1812 (cont)

	% AR in faeces 24-48 h interval										
	5 mg/kg bw [phenyl-^{14}C]S-1812		500 mg/kg bw [phenyl-^{14}C]S-1812		5 mg/kg bw [propenyl-^{14}C]S-1812		500 mg/kg bw [propenyl-^{14}C]S-1812		5 mg/kg bw [pyridyl-^{14}C]S-1812		
Fraction	M	F	M	F	M	F	M	F	M	F	**Identity**
Acetone	0.9	4.6	6.9	22.6	3.2	5.7	6.4	13.2	3.3	8.0	S-1812
	0.2	1.2	0.7	0.9	0.7	1.8	0.9	1.3	0.2	0.6	S-1812-Py-OH
	17.5	22.1	13.7	19.0					7.1	13.9	S-1812-DP
			0.2	0.2	0.6	1.6	0.3	0.0			HPHM
			1.8	1.3	0.3	1.1	0.2	0.0			DCHM
			0.0	0.1	0.1	0.0	0.0	0.0			Polar A[a]
					0.0	0.6	0.0	0.0			Polar B[a]
	1.1	2.6	0.0	0.0	0.4	1.8	0.0	0.7	0.4	2.3	Other metabolites[b]
Methanol: water	-	0.5	0.0	-	0.3	0.6	0.1	0.2	-		S-1812
	-	0.1	0.0	-	0.1	0.1	0.0	0.0	-		S-1812-Py-OH
	-	2.6	0.3	-					-		S-1812-DP
			0.0	-	0.4	0.7	0.0	0.0	-		HPHM
			0.0	-	0.3	0.8	0.0	0.0	-		DCHM
			0.5	-	0.0	1.8	1.2	1.4	-		Polar A[a]
				-	0.3	0.0	0.5	1.2	-		Polar B[a]
	2.1	1.3	0.1	-	0.0	0.4	0.4	0.4	0.6	1.6	Other metabolites[b]
All other extracts		0.7	0.9	1.1	1.6	4.3	2.9	4.1	0.3	1.2	Radiocarbon as % of dose
Unextracted	1.6	2.6	0.1	0.2	1.3	1.1	0.6	0.8	0.3	1.0	Radiocarbon as % of dose
total identified	23.4	38.2	25.2	47.8	9.3	22.4	13.5	23.3	12.2	28.6	

[a] Retention times for peak A = sf-3.2 min and for peak B: 3.2-4.5 min. These retention times are approximate due to slight variation between analyses. For the phenyl labelled S-1812 peak A was appr. 5.5 min.
[b] All unidentified metabolites other than polar metabolites.

Table 2.1.2-06 Metabolite identification (% AR) in faeces of male and female rats after single oral exposure to ^{14}C-S-1812 (cont)

	% AR in faeces 48-72 h interval										
	5 mg/kg bw [phenyl-^{14}C]S-1812		500 mg/kg bw [phenyl-^{14}C]S-1812		5 mg/kg bw [propenyl-^{14}C]S-1812		500 mg/kg bw [propenyl-^{14}C]S-1812		5 mg/kg bw [pyridyl-^{14}C]S-1812		
Fraction	M	F	M	F	M	F	M	F	M	F	**Identity**
Acetone			0.3	0.2							S-1812
			0.1	0.0							S-1812-Py-OH
			2.7	4.7							S-1812-DP
			0.2	0.3							HPHM
			0.9	0.9							DCHM
			0.0	0.4							Polar A[a]
											Polar B[a]
			0.2	0.0							Other metabolites[b]
Methanol: water			0.0								S-1812
			0.0								S-1812-Py-OH
			0.1								S-1812-DP
			0.0								HPHM
			0.0								DCHM
			0.2								Polar A[a]
											Polar B[a]
			0.0	0.5							Other metabolites[b]
All other extracts			0.1	0.2							Radiocarbon as % of dose
Unextracted			0.0	0.0							Radiocarbon as % of dose
total identified			4.8	7.2							

[a] Retention times for peak A = sf-3.2 min and for peak B: 3.2-4.5 min. These retention times are approximate due to slight variation between analyses. For the phenyl labelled S-1812 peak A was appr. 5.5 min.
[b] All unidentified metabolites other than polar metabolites.

Table 2.1.2-06 Metabolite identification (% AR) in faeces of male and female rats after single oral exposure to ^{14}C-S-1812 (cont)

	% AR in faeces 72-168 h interval										
	5 mg/kg bw [phenyl-^{14}C]S-1812		500 mg/kg bw [phenyl-^{14}C]S-1812		5 mg/kg bw [propenyl-^{14}C]S-1812		500 mg/kg bw [propenyl-^{14}C]S-1812		5 mg/kg bw [pyridyl-^{14}C]S-1812		
Fraction	M	F	M	F	M	F	M	F	M	F	**Identity**
Acetone	0.0	0.0	0.1	0.0	0.0	0.1	0.1	1.7	0.0	0.0	S-1812
	0.0	0.0	0.0	0.2	0.0	0.2	0.2	0.4	0.0	0.0	S-1812-Py-OH
	1.6	0.7	1.4	0.8					1.1	0.8	S-1812-DP
			0.5	0.7	0.2	0.7	0.0	0.5			HPHM
			0.6	0.2	0.5	0.1	0.0	0.0			DCHM
			0.2	1.0	0.0	0.1	0.0	0.0			Polar A[a]
					0.0	0.1	0.0	0.0			Polar B[a]
	0.8	1.0	0.0	0.2	0.1	0.2	1.2	0.1	0.0	0.2	Other metabolites[b]
Methanol: water			0.0		0.0	0.0					S-1812
			0.0		0.0	0.0					S-1812-Py-OH
			0.1								S-1812-DP
			0.0		0.2	0.1					HPHM
			0.0		0.0	0.0					DCHM
			0.2		0.0	0.1					Polar A[a]
					0.1	0.0					Polar B[a]
	0.6	0.5	0.0	0.3	0.0	0.2	0.4	0.8	0.1	0.1	Other metabolites[b]
All other extracts	0.1		0.1	0.2	0.5	0.5	0.6	0.9	0.1	0.0	Radiocarbon as % of dose
Unextracted	0.3	0.2	0.0	0.0	0.3	0.3	0.2	0.4	0.1	0.1	Radiocarbon as % of dose
total identified	3.4	2.4	3.2	3.6	1.9	2.7	2.7	4.8	1.4	1.2	

[a] Retention times for peak A = sf-3.2 min and for peak B: 3.2-4.5 min. These retention times are approximate due to slight variation between analyses. For the phenyl labelled S-1812 peak A was appr. 5.5 min.
[b] All unidentified metabolites other than polar metabolites.

Metabolite identification in urine (table 2.1.2-07)

After dosing with [phenyl-^{14}C]S-1812 the excretion in urine of radiocarbon was maximally 2% of the administered dose (1.6-2.0%). Cursory HPLC (system A) examination revealed that none of the metabolites exceeded 1% of the dose. S-1812 and S-1812-DP were identified in urine.

After dosing with [propenyl-^{14}C]S-1812 the excretion in urine of radiocarbon was higher (9.7-17.7% of the administered dose). In Table 2.1.2-07 a summary is given, in total 11 different compounds could be determined by HPLC and TLC. However, none of these compounds accounted for > 2% of the administered dose, therefore identification of the compounds has not been performed.

After dosing with [pyridyl-^{14}C]S-1812 the excretion in urine of radiocarbon was low (2.0-2.1% of the administered dose). The following metabolites were identified (and confirmed by HPLC and LC-MS): HPDO glucuronide and HPDO sulphate and HTFP.

Table 2.1.2-07 TLC quantitation of metabolites in (% AR) in urine of male and female rats after single oral exposure to ^{14}C-S-1812

	% AR in urine				
	5 mg/kg bw [propenyl-^{14}C]S-1812		500 mg/kg bw [propenyl-^{14}C]S-1812		
TLC zone[a]	M	F	M	F	Identity
0.92-0.93	0.1	0.4	0.7	0.5	
0.80-0.82	3.0	2.1	5.0	3.9	
0.73-0.76	1.3	2.2	0.9	1.1	
0.67-0.70	2.6	1.4	1.8	1.1	
0.62-0.64	0.2	1.0	1.8	1.6	
0.59	1.2	0.9	0.7	0.5	
0.46-0.47	2.0	0.9	0.4	0.7	
0.28-0.29	2.0	1.5			
0.17-0.18	0.6	2.0			
0.09	0.4	0.8			
Original	0.2	0.2			S-1812
Total	17.1	17.9	11.3	9.4	

[a] Zones determined using TLC system B

	% AR in urine (6-48 h)	
	5 mg/kg bw [pyridyl-^{14}C]S-1812	
Identity[a]	M	F
HTFP	1.3	1.1
HPDO	0.0	0.0
HPDO glucuronide	0.3	0.3
HPDO sulphate	0.4	0.6
Other	0.0	0.1
Total identified	2.0	2.1

[a] Determined using HPLC method C

Metabolites in expired air

Only [propenyl-^{14}C]S-1812 showed detectable radiocarbon in expired air (10.8-11.6% of the administered dose). (Radio)carbon dioxide was expired mainly 0-72 hours after dosing. Trapping might have been incomplete, only $^{14}CO_2$ was recovered in the alkaline trap. In other traps (XAD resin, charcoal or mercuric perchlorate) no radiocarbon was detected.

Metabolic pathway of S-1812 in rats

The formation of major metabolite (S-1812-DP) involves oxidative cleavage of the dichloropropenyl group, yielding S-1812-DP from the phenyl and pyridyl labels and $^{14}CO_2$ and minor polar metabolites from the propenyl label. Other minor path ways are: oxidation of the inter-ring methylene groups and hydroxylation. In the pyridyl label free HTFP and sulphate and glucuronide conjugates of HPDO are formed in small quantities in urine.

Conclusions

After oral dosing of ^{14}C-S-1812 to rats the main excretion route was the faeces (% of the administered radioactivity), mainly the parent compound (30-50% of the administered radioactivity) was found in faeces for all compounds and doses administered.

In tissues only 0.6-2.1% of the administered radioactivity was recovered. In the carcass the recovery was somewhat higher, ranging from 0.7-7.9% AR. Highest average radiocarbon concentrations in tissues after a single dose of ^{14}C-labeled S-1812 were found in fat, adrenal gland, pancreas, salivary glands, thyroid and hair/skin, ovaries and uterus (females only). After the higher doses (500 mg/kg bw) the recovered radiocarbon concentrations were higher compared to the lower doses. The average radiocarbon concentrations recovered in tissues was higher after dosing with [propenyl-^{14}C]S-1812 compared to [phenyl-^{14}C]S-1812 and [pyridyl-^{14}C]S-1812. No differences between males and females could be detected.

The metabolic pattern found after oral dosing of ^{14}C-S-1812 to rats did not show sex specific differences. After the higher dose of [phenyl-^{14}C]S-1812 relatively more parent compound and less S-1812-DP was detected when compared to the low dose. After the higher dose of [propenyl-^{14}C]S-1812 also more parent compound was observed, but the main metabolite (S-1812-DP) was not measured, since its structure does not include the propenyl moiety.

The major metabolite detected in faeces (after dosing with [phenyl-^{14}C]S-1812 and [pyridyl-^{14}C]S-1812) was S-1812-DP. Other metabolites detected in faeces were S-1812-Py-OH, HPHM and DCHM (last two were not detected after dosing with [pyridyl-^{14}C]S-1812). After dosing with [propenyl-^{14}C]S-1812 the main metabolite could not be detected as its structure does not include the propenyl moiety. Carbon dioxide in expired was the main metabolite detected after [propenyl-^{14}C]S-1812 (approximately 11% of the administered radioactivity). Other metabolites in faeces were identified as S-1812-Py-OH, HPHM, DCHM and polar metabolites.

After dosing with [phenyl-^{14}C]S-1812 the excretion in urine of radiocarbon was maximally 2% of the administered dose. None of the metabolites exceeded 1% of the dose, and were therefore not identified. After dosing with [pyridyl-^{14}C]S-1812 the excretion in urine of radiocarbon was maximally 2% of the administered dose. The following metabolites were identified after dosing with [pyridyl-^{14}C]S-1812: HPDO glucuronide and HPDO sulphate and HTFP. After dosing with [propenyl-^{14}C]S-1812 the excretion in urine of radiocarbon was higher (9.7-17.7% of the administered dose). Different compounds could be determined, but as none of these compounds accounted for > 2% of the administered dose, identification of the compounds has not been performed.
All metabolites were confirmed by co-chromatography.

The major metabolic pathway in the rat is probably cleavage of the dichloropropenyl group and of the methylene bridge between pyridyl and dichlorophenyl rings. A minor pathway may be oxidation of the pyridyl ring.

In rats given a single dose of phenyl and pyridyl labelled (14C)-S-1812, oral absorption was 3.2-6.8% in 168 hours, based on the amount radiolabel recovered from urine, carcass, expired air and tissues. In rats given a single dose of the propenyl labelled S-1812, oral absorption amounts to 28.3-35.5% at 168 hours, based on radiolabel recovered from urine, carcass, expired air and tissues. Actual absorption might have been higher because of the fact that excretion via entero-hepatic circulation was not taken into account.

Acceptability
The study is considered acceptable.

2.1.3 Study 3

Characteristics

reference	:	IIA 5.1.1/03, Project No. 986W-1	exposure	:	single by gavage
type of study	:	Metabolism	doses	:	5.0 & 500 mg/kg bw for males and females
year of execution	:	2001-2002	vehicle	:	Corn oil
test substances	:	^{14}C-S-1812 (pyridalyl) (^{14}C labelled in the phenyl ring): lot no. RIS98015 (spec. act. 10.2 MBq/mg), chemical purity 98.1%, radiochemical purity 99.0%. ^{14}C-S-1812 (^{14}C labelled in the propenyl group: lot. no RIS98018 (spec. act 4.12 MBq/mg), chemical purity 98.5%, radiochemical purity 98.7%. Unlabelled S-1812: lot no. 980302G, chemical purity 99.7%.	GLP statement	:	Yes, with an exception (a reference standard used (AMAC standard form H, Lot CJ52890) may not have been characterized according to GLP)
route	:	Oral (gavage)	guidelines	:	EPA 870.7485 (Tier 2) and Japanese MAFF (12 Nohsan No. 8147). GLP standards: EPA (40 CFR Part 160) and Japanese MAFF (11 Nohsan No. 6283).
species	:	Sprague Dawley rat (M: 184-230 g) (F: 160-230 g)	acceptability	:	Acceptable
group size	:	See table 2.1.3-01			

Study design

Total radioactive residue concentrations were determined in selected tissues after oral dosing with [phenyl-^{14}C]S-1812 (pyridalyl) and [propenyl-^{14}C]S-1812 at doses of 5.0 mg/kg bw and 500 mg/kg bw.

Four collection intervals were determined post treatment: ½ C_{max} (AB), C_{max}, ½ C_{max} (EL) and $^{1}/_{10}$ C_{max} (EL). At these time points per dose and sex 3 rats were sacrificed and tissues (see Table 2.1.3-01) were sampled and Total Radioactivity Residue (TRR) was determined. Composite tissues of liver, kidney, lung, whole blood and fat were extracted and metabolites were determined by HPLC.

TRR was determined by combustion followed by LSC, this was done for each individual animal.

Composite tissues (liver, kidney, lung, blood and fat): tissue samples from each rat within a dose group and sex were pooled by collection interval. These samples were used for extraction and metabolite identification.

Extraction: tissue sub-samples were homogenized with acetone, samples were centrifuged, supernatant was combined (for all sub-samples per dose/sex/interval). Radio assayed by LSC, residues were extracted (2x) with methanol/0.1 N HCl. The remaining Post Extraction Solids were combusted.

TLC was used for one-dimensional separation and quantification of metabolites from acetone extracts from some phenyl labelled composite tissues.

Two dimensional separation was used for separation and quantification of metabolites from propenyl, low dose, female liver, ½ C_{max} (EL) time point (acetone extract).

Because the extraction of composite tissues was not complete after dosing with [propenyl-^{14}C]S-1812 additional extractions were performed. Acid hydrolysis of extracts (only female liver, male kidney, male lung, female whole blood), using 6N HCl, supernatants were used for derivatization (pheylisothiocyanate) and chromatographic analysis. Residue + 1 N NaOH supernatants were used for derivatization and chromatographic analysis.

Table 2.1.3-01 Experimental groups for each dose level

				Collection interval (hours post dose)		
Dose group		**Dose**	**Number of rats**	**Interval**	**Male**	**Female**
A	Control	0 mg/kg bw	3M/3F	NA	NA	NA
B	Low dose phenyl-^{14}C	5 mg/kg bw	3M/3F	½ C_{max} (AB)	4	4
			3M/3F	C_{max}	8	8
			3M/3F	½ C_{max} (EL)	24	24
			3M/3F	$^{1}/_{10}$ C_{max} (EL)	48	48
C	High dose phenyl-^{14}C	500 mg/kg bw	3M/3F	½ C_{max} (AB)	6	6
			3M/3F	C_{max}	8	8
			3M/3F	½ C_{max} (EL)	24	24
			3M/3F	$^{1}/_{10}$ C_{max} (EL)	48	48
D	Low dose propenyl-^{14}C	5 mg/kg bw	3M/3F	½ C_{max} (AB)	4	4
			3M/3F	C_{max}	12	12
			3M/3F	½ C_{max} (EL)	48	48
			3M/3F	$^{1}/_{10}$ C_{max} (EL)	120	120
E	High dose propenyl -^{14}C	500 mg/kg bw	3M/3F	½ C_{max} (AB)	6	6
			3M/3F	C_{max}	12	12
			3M/3F	½ C_{max} (EL)	72	72

			3M/3F	$^{1}/_{10}$ C_{max} (EL)	120	120

Results

Animals received mean doses of 5.0 mg/kg bw [phenyl-^{14}C]S-1812 (low dose) and 506.7 and 507.4 mg/kg bw (high dose, males and females respectively). Animals received mean doses of 5.0 mg/kg bw and 5.1 mg /kg bw [propenyl-^{14}C]S-1812 (low dose, males and females respectively) and 508.0 and 511.6 mg/kg bw (high dose, males and females respectively).

In the control group no [^{14}C]S-1812 was observed in tissues after combustion.

Total radioactive residues in tissues

Total recovery in tissues differed between time points on which the tissues were collected. At ½ C_{max} (AB) the recovery was high (82.8-99.4%), thereafter the recovery decreased (see Table 2.1.3-02). The reason for the decrease is excretion of S-1812 in faeces, urine and air. These excreta have not been collected, as it was not the aim of this study.

Table 2.1.3-02 Total recovery of radioactivity (% AR) in rat tissues after single oral exposure to ^{14}C-labeled S-1812

	[phenyl-^{14}C]S-1812 5.0 mg/kg bw		[phenyl-^{14}C]S-1812 500 mg/kg bw		[propenyl-^{14}C]S-1812 5.0 mg/kg bw		[propenyl-^{14}C]S-1812 500 mg/kg bw	
	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)
½ C_{max} (AB)	99.4	87.7	82.0	82.1	82.8	87.0	87.7	88.7
C_{max}	81.4	91.4	74.9	76.4	62.7	66.5	52.3	72.8
½ C_{max} (EL)	28.5	33.7	41.7	40.2	21.1	12.8	11.2	9.7
$^{1}/_{10}$ C_{max} (EL)	17.3	11.3	29.8	18.2	8.3	6.1	9.1	5.4

Highest residue concentrations (>0.1% AR) were found in GIT contents, small intestine, carcass, stomach, caecum, large intestine, liver, fat, hair/skin, pancreas, muscle, heart and lungs (see Tables 2.1.3-03 to 2.1.3-06). The highest concentrations were observed at C_{max}, at ½ C_{max} (AB) or ½ C_{max} (EL) for most tissues. For most tissues the residue concentrations decreased after ½ C_{max} (EL). The following tissues showed an increase of residue concentrations over time, or did not show a decrease after ½ C_{max} (EL): bone marrow (phenyl high dose), ovaries (phenyl low and high dose), uterus (phenyl low dose), thyroid (phenyl low and high dose, propenyl low dose), pancreas (phenyl high dose, propenyl low dose), fat (phenyl low dose, phenyl high dose), hair/skin (phenyl low dose), salivary glands (phenyl high dose).

At the last time point measured ($^{1}/_{10}$ C_{max} (EL), 48 hours after dosing with [phenyl-^{14}C]S-1812 and 120 hours after dosing with [propenyl-^{14}C]S-1812) residue concentrations were still high in fat, thyroid, pancreas, ovaries, adrenals and GI tract and GI contents.

The residue levels in the carcass were generally somewhat higher for males than for females, except for the 500 mg/kg bw [propenyl-^{14}C]S-1812 group, where no differences were observed. Highest concentrations were generally seen at C_{max}, at $^{1}/_{10}$ C_{max} (EL), 48 hours after dosing with [phenyl-^{14}C]S-1812 and 120 hours

after dosing with [propenyl-^{14}C]S-1812), concentrations decreased (except for male rats after dosing with 500 mg/kg bw [phenyl-^{14}C]S-1812).

Composite Tissue analysis

In the composite tissues (liver, kidney, lung, blood and fat) the levels of radioactive residues found were comparable to the results described above. Highest radioactive residues were found in liver. In liver, lung and blood radioactive residues peaked at ½ C_{max} (AB) or at C_{max} and generally decreased thereafter. For kidneys after a single dose of [propenyl-^{14}C]S-1812 radioactive residues peaked at ½ C_{max} (AB) or at C_{max} and generally decreased thereafter, whereas after dosing with [phenyl-^{14}C]S-1812 the highest levels were observed at C_{max}, ½ C_{max} (EL) or $^{1}/_{10}$ C_{max} (EL).

In fat the radioactive residues increased over time after dosing with [phenyl-^{14}C]S-1812 and until ½ C_{max} (EL) after dosing with [propenyl-^{14}C]S-1812, at $^{1}/_{10}$ C_{max} (EL) a decrease was observed.

Extractability of radiocarbon from composite tissues (% recovery normalized to the total DPM recovered)

After a dose of 5.0 mg/kg bw [phenyl-^{14}C]S-1812 the extractable recovery in male rats varied from 68.9% (liver, ½ C_{max} (EL)) to 99.9% (fat). This equals 0.00% to 6.61% of the dose.

After a dose of 5.0 mg/kg bw [phenyl-^{14}C]S-1812 the extractable recovery in female rats varied from 77.3% (lung, $^{1}/_{10}$ C_{max} (EL)) to 99.9% (fat). This equals 0.01% to 4.87% of the dose.

After a dose of 500 mg/kg bw [phenyl-^{14}C]S-1812 the extractable recovery in male rats varied from 84.8% (liver, $^{1}/_{10}$ C_{max} (EL)) to 99.9% (fat). This equals 0.01% to 1.84% of the dose.

After a dose of 500 mg/kg bw [phenyl-^{14}C]S-1812 the extractable recovery in female rats varied from 90.6% (liver, $^{1}/_{10}$ C_{max} (EL)) to 99.9% (fat). This equals 0.01% to 3.43% of the dose.

Extractable recovery (% recovery normalized to the total DPM recovered) after dosing with [phenyl-^{14}C]S-1812 was high: fat >99%; kidney >90%; liver >80%; blood >80%; lung >75%.

After dosing with [phenyl-^{14}C]S-1812, the majority of the radiocarbon was detected in the acetone extract (60.5-99.9%), in MeOH/HCl extraction accounted for maximally 14.5%.

After a dose of 5.0 mg/kg bw [propenyl-^{14}C]S-1812 the extractable recovery in male rats varied from 40.7% (kidney, ½ C_{max} (EL)) to 98.2% (fat). This equals 0.00% to 1.04% of the dose.

After a dose of 5.0 mg/kg bw [propenyl-^{14}C]S-1812 the extractable recovery in female rats varied from 22.2% (blood, $^{1}/_{10}$ C_{max} (EL)) to 98.9% (fat). This equals 0.02% to 3.09% of the dose.

After a dose of 500 mg/kg bw [propenyl-^{14}C]S-1812 the extractable recovery in male rats varied from 0.00% (blood, $^{1}/_{10}$ C_{max} (EL)) to 98.7% (fat). This equals 0.02% to 2.17% of the dose.

After a dose of 500 mg/kg bw [propenyl-^{14}C]S-1812 the extractable recovery in female rats varied from 0.00% (blood, $^{1}/_{10}$ C_{max} (EL)) to 99.3% (fat). This equals 0.01% to 2.99% of the dose.

Extractable recovery after dosing with [propenyl-^{14}C]S-1812 was lower compared to [phenyl-^{14}C]S-1812: fat >95%; liver >39%; kidney >36%; lung >50%; blood >11% (high dose at $^{1}/_{10}$ C_{max} (EL): 0%).

After dosing with [propenyl-^{14}C]S-1812, the acetone extract accounted for the majority of the radiocarbon in liver, lung and fat (28-100%), MeOH/HCl extraction accounted for the majority of the radiocarbon in kidney and blood (0-55%).

Metabolite identification in composite tissues (liver, kidney, lung, blood and fat)
Metabolites were reported per time point. Metabolites were reported as percentage of the (acetone or other) extract per composite tissue, not as % over administered dose. Metabolites identified were S-1812-DP, S-1812-Ph-CH_2CO_2H, HPHM and polar metabolites.
S-1812, S1812-DP and polar metabolites (in fat only after dosing with [propenyl-^{14}C]S-1812) occurred in all composite tissues, S-1812-Ph-CH_2CO_2H and HPHM did not occur in fat.

<u>[phenyl-^{14}C]S-1812 (acetone extracts)</u>
In the liver S-1812 (% of extract) decreased over time (from maximally 94% of the extract to 8 % of the extract), whereas S-1812-Ph-CH_2CO_2H (% of extract) increased over time (from 3% to 84% of the extract). For S-1812 DP no clear time-concentration relation could be observed (present between 7-23% of the extract). For polar metabolites no clear time-concentration relation could be observed (present 0-8% of the extract). HPHM contributed to maximally 9 % of the extract.

In the kidney, the lung and fat the major compound was S-1812 on all time points. The % of extract ranged from 59-100% in the kidney, 70-98% in the lung and 95-100% in fat.
S-1812-DP contributed for less than 25% (1 exception, described below); S-1812-Ph-CH_2CO_2H for less than 15% (1 exception, described below); polar metabolites for less than 15%; HPHM for less than 5%.
In fat the only metabolite detected was S-1812-DP (0-5% of the extract).
In females (low dose [phenyl-^{14}C]S-1812) at $^{1}/_{10}$ C_{max} (EL) a higher % of extract of S-1812-DP in the kidney (26.3%) and a high level of S-1812-Ph-CH_2CO_2H in the lung (30.4%) was observed.

In blood S-1812 (% of extract) decreased over time (100 to 0 %), whereas S-1812-Ph-CH_2CO_2H (% of extract) and polar metabolites increased over time (9-100% and 0-85% respectively). In females (low dose) at $^{1}/_{10}$ C_{max} (EL) an increase was seen of S-1812-DP.

[propenyl-^{14}C]S-1812 (acetone or acetone/MeOH/HCl extracts)

In the liver S-1812 (% of extract) decreased over time (from maximally 73 to 0% of the extract), whereas S-1812-Ph-CH_2CO_2H (% of extract) and polar metabolites increased over time (maximally 69 and 41% of the extract respectively). HPHM was present for maximally 10%.

In the kidney S-1812 seemed to increase over time (maximum contribution 61%; minimal contribution 4%). Polar metabolites were the major compounds detected at all time points (maximum contribution 91%; minimal contribution 14%) and generally increased over time after the high dose in males. After the low dose in males and females an increase of S-1812-Ph-CH_2CO_2H over time was observed (maximum contribution: 20.1%), whereas a decrease was seen after the high dose.

In the lung S-1812 (31-94%) and polar metabolites (5-66%) were the most prominent compounds at all time points. S-1812-Ph-CH_2CO_2H contributed to maximally 8.5% of the extract.

In blood S-1812 and polar metabolites were the major compounds. S-1812 (% of extract) decreased over time in males, but increased over time in females. Polar metabolites increased over time in males, but decreased in females at the low dose. S-1812-Ph-CH_2CO_2H contributed to 0-19% of the extract, except for females at the low dose: 42% at $^1/_{10}$ C_{max} (EL).

In fat the major compound detected was S-1812 on all time points (53-100% of the extract). Only polar metabolites were detected (0-17%).

Table 2.1.3-03 Distribution of radioactivity in tissues and organs [ppm average radiocarbon concentrations and average % of dose] of 5 mg/kg bw [phenyl-^{14}C]S-1812

	5 mg/kg bw [phenyl-^{14}C]S-1812 ½ Cmax (AB)				5 mg/kg bw [phenyl-^{14}C]S-1812 Cmax (AB)				5 mg/kg bw [phenyl-^{14}C]S-1812 ½ Cmax (EL)				5 mg/kg bw [phenyl-^{14}C]S-1812 $^1/_{10}$ Cmax (EL)			
	male		female		male		female		male		female		male		female	
tissue	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***
Adrenals	**5.40**	**0.04**	2.52	0.03	5.18	0.03	**4.94**	**0.05**	4.04	0.03	2.33	0.02	2.61	0.02	2.57	0.02
Bone	**0.20**	**0.01**	0.14	0.01	0.15	0.01	**0.29**	**0.02**	0.15	0.01	0.08	0.00	0.09	0.01	0.13	0.01
Bone marrow	**0.39**	**0.00**	0.38	0.00	0.34	0.00	**0.43**	**0.00**	0.22	0.00	0.22	0.00	0.08	0.00	0.06	0.00
Brain	0.09	0.01	0.05	0.01	**0.09**	**0.02**	**0.10**	**0.02**	0.04	0.01	0.04	0.01	0.01	0.00	0.01	0.00
Heart	**1.60**	**0.15**	**1.53**	**0.13**	0.44	0.04	0.65	0.05	0.11	0.01	0.16	0.01	0.15	0.01	0.06	0.01
Lungs	**1.05**	**0.13**	**0.89**	**0.12**	1.53	0.18	0.72	0.08	0.16	0.02	0.41	0.05	0.25	0.03	0.12	0.01
Muscle	**0.45**	**0.18**	0.22	0.11	0.37	0.12	**0.34**	**0.11**	0.2	0.06	0.15	0.05	0.16	0.06	0.12	0.05
Ovaries	--	--	0.74	0.01	--	--	1.44	0.03	--	--	1.13	0.01	--	--	**2.06**	**0.03**
Pancreas	1.84	0.20	0.86	0.11	**1.60**	**0.23**	0.94	0.10	0.9	0.09	**1.57**	**0.21**	0.85	0.17	1.14	0.19
Pituitary gland	0.13	0.00	**0.22**	**0.00**	0.14	0.00	0.19	0.00	**0.15**	**0.00**	0.04	0.00	0.02	0.00	0.02	0.00
Whole blood	**0.30**	**0.07**	**0.16**	**0.04**	0.10	0.03	0.12	0.03	0.04	0.01	0.08	0.02	0.02	0.01	0.02	0.01
Plasma	**0.42**	**0.09**	**0.21**	**0.07**	0.15	0.04	0.18	0.04	0.05	0.01	0.11	0.02	0.02	0.00	0.04	0.00

	5 mg/kg bw [phenyl-^{14}C]S-1812 ½ Cmax (AB)				5 mg/kg bw [phenyl-^{14}C]S-1812 Cmax (AB)				5 mg/kg bw [phenyl-^{14}C]S-1812 ½ Cmax (EL)				5 mg/kg bw [phenyl-^{14}C]S-1812 $^{1}/_{10}$ Cmax (EL)			
	male		female		male		female		male		female		male		female	
tissue	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***
RBC	**0.13**	**0.02**	**0.07**	**0.02**	0.06	0.01	0.07	0.01	0.02	0.00	0.04	0.00	0.01	0.00	0.02	0.00
Salivary glands	0.60	0.04	0.38	0.02	0.69	0.04	0.68	0.05	**0.7**	**0.04**	**0.71**	**0.05**	0.72	0.03	0.67	0.04
Spinal cord	0.09	0.00	0.05	0.00	**0.09**	**0.00**	**0.11**	**0.00**	0.04	0.00	0.04	0.00	0.01	0.00	0.02	0.00
Spleen	**1.08**	**0.07**	**0.92**	**0.06**	0.14	0.01	0.30	0.02	0.07	0.00	0.10	0.01	0.05	0.00	0.07	0.00
Testes	**0.25**	**0.05**	--	--	0.09	0.02	--	--	0.04	0.01	--	--	0.06	0.01	--	--
Thymus	0.34	0.02	0.21	0.02	**0.70**	**0.05**	**2.69**	**0.10**	0.57	0.03	0.25	0.02	0.32	0.02	0.33	0.02
Thyroid	1.19	0.00	**3.27**	**0.01**	1.66	0.00	1.69	0.00	1.04	0.00	1.03	0.00	2.6	0.00	**1.72**	**0.00**
Uterus	--	--	0.19	0.01	--	--	0.31	0.02	--	--	0.24	0.01	--	--	**0.81**	**0.04**
Fat	2.63	0.29	0.82	0.08	3.06	0.35	1.97	0.26	4.01	0.58	2.60	0.39	**3.25**	**0.59**	**3.82**	**0.50**
Hair/skin	1.02	0.16	0.25	0.07	1.00	0.27	0.65	0.15	0.96	0.21	0.84	0.17	**0.91**	**0.27**	**0.90**	**0.28**
Liver	**6.04**	**6.03**	4.79	4.29	3.03	3.05	**7.22**	**6.07**	0.99	1.13	2.32	1.98	0.54	0.66	1.09	0.91
Kidneys	**1.01**	**0.22**	0.85	0.16	0.58	0.12	**0.98**	**0.18**	0.58	0.12	0.63	0.12	0.31	0.07	0.49	0.09
Caecum	6.81	1.34	5.55	0.94	**31.65**	**5.22**	**25.44**	**4.69**	7.91	1.63	9.97	1.96	1.35	0.29	2.73	0.35
Small intestine	**21.94**	**15.71**	**21.94**	**15.32**	8.64	5.72	13.92	10.11	2.84	2.44	6.00	3.93	1.04	0.84	1.32	0.86
Large intestine	2.00	0.34	1.42	0.24	**28.69**	**4.23**	**33.72**	**6.38**	1.46	0.22	3.03	0.43	0.57	0.10	1.15	0.16
Stomach	**18.59**	**5.03**	**19.14**	**3.93**	4.50	0.97	16.42	3.25	1.33	0.46	11.30	2.64	2.19	0.75	0.28	0.04
GIT contents	**45.40**	**55.59**	**59.34**	**56.08**	51.49	51.57	66.27	50.96	8.79	12.14	24.60	15.29	3.57	5.06	6.27	2.63
Carcass	**0.87**	**13.64**	0.41	5.78	0.61	9.03	**0.60**	**8.65**	0.57	9.27	0.46	6.33	0.51	8.26	0.37	5.08

* calculated as µg [^{14}C]S-1812/g tissue.

In bold: highest concentrations per sex.

Table 2.1.3-04 Distribution of radioactivity in tissues and organs [ppm average radiocarbon concentrations and average % of dose] of 500 mg/kg bw [phenyl-^{14}C]S-1812

	500 mg/kg bw [phenyl-^{14}C]S-1812 ½ Cmax (AB)				500 mg/kg bw [phenyl-^{14}C]S-1812 Cmax (AB)				500 mg/kg bw [phenyl-^{14}C]S-1812 ½ Cmax (EL)				500 mg/kg bw [phenyl-^{14}C]S-1812 $^{1}/_{10}$ Cmax (EL)			
	male		female		male		female		male		female		male		female	
tissue	**ppm***	**% AR***	**ppm***	**% AR***	**Ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***
Adrenals	190	0.01	367	0.02	532	0.02	631	0.05	727	0.04	**663**	**0.05**	**759**	**0.04**	344	0.01
Bone	10	0.01	12	0.01	**18**	**0.01**	**20**	**0.01**	12	0.01	17	0.01	6	0.00	25	0.01
Bone marrow	9	0.00	39	0.00	15	0.00	92	0.00	18	0.00	87	0.00	**31**	**0.00**	**122**	**0.00**
Brain	4	0.01	4	0.01	6	0.01	**9**	**0.02**	**9**	**0.02**	9	0.01	5	0.01	4	0.00
Heart	35	0.03	78	0.07	**62**	**0.05**	**98**	**0.07**	45	0.04	45	0.04	19	0.01	11	0.01
Lungs	**185**	**0.18**	**98**	**0.12**	111	0.13	102	0.11	99	0.12	87	0.10	40	0.05	28	0.03
Muscle	18	0.08	24	0.11	27	0.13	**48**	**0.20**	**46**	**0.21**	21	0.03	32	0.10	17	0.10
Ovaries	--	--	96	0.01	--	--	144	0.03	--	--	**215**	**0.03**	--	--	212	0.03
Pancreas	68	0.09	101	0.13	103	0.11	134	0.16	177	0.13	187	0.25	**190**	**0.14**	**247**	**0.45**
Pituitary gland	4	0.00	9	0.00	**9**	**0.00**	**50**	**0.00**	8	0.00	6	0.00	8	0.00	6	0.00
Whole blood	5	0.01	9	0.02	9	0.02	**9**	**0.02**	**11**	**0.03**	8	0.02	6	0.01	3	0.01
Plasma	8	0.02	12	0.02	12	0.03	**13**	**0.02**	**15**	**0.04**	12	0.02	8	0.01	5	0.01
RBC	3	0.00	4	0.01	3	0.01	5	0.01	6	0.01	**5**	**0.01**	**5**	**0.02**	3	0.01
Salivary glands	26	0.02	34	0.02	49	0.03	77	0.05	96	0.07	90	0.05	**109**	**0.06**	**86**	**0.06**
Spinal cord	3	0.00	3	0.00	5	0.00	**15**	**0.00**	**8**	**0.00**	7	0.00	6	0.00	5	0.00
Spleen	19	0.01	39	0.02	**27**	**0.02**	**40**	**0.02**	23	0.02	16	0.01	9	0.00	11	0.00
Testes	3	0.01	--	--	7	0.02	--	--	**9**	**0.02**	--	--	8	0.02	--	--
Thymus	15	0.01	27	0.02	30	0.01	89	0.04	74	0.05	**100**	**0.06**	**104**	**0.05**	60	0.03
Thyroid	187	0.00	178	0.00	163	0.00	203	0.00	256	0.01	**241**	**0.00**	**585**	**0.00**	235	0.00
Uterus	--	--	16	0.01	--	--	36	0.02	--	--	**83**	**0.04**	--	--	69	0.04
Fat	79	0.10	82	0.07	118	0.10	156	0.12	330	0.43	384	0.39	**548**	**0.70**	**520**	**0.75**
Hair/skin	72	0.19	37	0.16	50	0.12	46	0.12	145	0.28	**110**	**0.29**	**245**	**0.44**	96	0.20
Liver	206	1.77	**425**	**3.34**	**292**	**2.50**	467	3.25	220	2.04	239	1.93	108	0.71	86	0.67
Kidneys	34	0.06	46	0.08	55	0.11	**120**	**0.18**	**69**	**0.13**	60	0.10	61	0.10	36	0.07
Caecum	1689	3.64	**1989**	**3.90**	**3070**	**5.14**	1977	3.30	537	0.91	656	0.92	538	0.83	151	0.21
Small intestine	421	2.39	**814**	**5.54**	366	2.26	889	5.23	306	2.08	843	5.08	**582**	**3.11**	257	1.44
Large intestine	1020	1.66	321	0.46	**2338**	**4.30**	**678**	**0.93**	230	0.34	183	0.26	173	0.20	68	0.11
Stomach	**3086**	**8.06**	**2451**	**6.35**	2782	7.48	2524	5.50	1596	4.93	1484	2.45	220	0.33	863	1.73
GIT contents	**4072**	**50.37**	**4474**	**56.88**	3852	44.07	8467	47.63	1760	21.06	2381	19.69	2078	11.51	458	4.71
Carcass	**95**	**13.24**	33	4.70	61	8.18	**67**	**9.25**	58	8.68	61	8.31	87	11.30	52	7.49

* calculated as µg [^{14}C]S-1812/g tissue.

In bold: highest concentrations per sex.

Table 2.1.3-05 Distribution of radioactivity in tissues and organs [ppm average radiocarbon concentrations and average % of dose] of 5 mg/kg bw [propenyl-^{14}C]S-1812

	5 mg/kg bw [propenyl-^{14}C]S-1812 ½ Cmax (AB)				5 mg/kg bw [propenyl-^{14}C]S-1812 Cmax (AB)				5 mg/kg bw [propenyl-^{14}C]S-1812 ½ Cmax (EL)				5 mg/kg bw [propenyl-^{14}C]S-1812 $^{1}/_{10}$ Cmax (EL)			
	male		female		male		female		male		female		male		female	
tissue	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***	**ppm***	**% AR***
Adrenals	3.74	0.02	**2.15**	**0.02**	**3.66**	**0.04**	1.54	0.01	3.15	0.03	1.98	0.02	1.55	0.03	1.25	0.01
Bone	0.39	0.03	0.44	0.02	**0.60**	**0.04**	0.27	0.02	0.49	0.03	**0.42**	**0.03**	0.28	0.03	0.21	0.01
Bone marrow	0.78	0.00	**2.32**	**0.01**	**0.95**	**0.00**	0.86	0.00	0.79	0.00	1.21	0.01	0.66	0.00	0.40	0.00
Brain	0.30	0.05	**0.35**	**0.06**	**0.32**	**0.05**	0.19	0.03	0.23	0.03	0.21	0.03	0.18	0.04	0.16	0.03
Heart	**1.73**	**0.14**	**1.31**	**0.12**	0.61	0.05	0.41	0.03	0.41	0.04	0.38	0.03	0.29	0.08	0.24	0.03
Lungs	**1.07**	**0.13**	0.64	0.09	0.84	0.10	**0.69**	**0.10**	0.54	0.07	0.68	0.09	0.34	0.10	0.38	0.05
Muscle	**0.36**	**0.19**	0.26	0.09	0.33	0.15	0.17	0.07	0.42	0.13	**0.26**	**0.13**	0.27	0.16	0.23	0.11
Ovaries	--	--	0.50	0.01	--	--	0.68	0.01	--	--	**1.20**	**0.02**	--	--	0.76	0.01
Pancreas	**1.88**	**0.27**	1.17	0.08	1.31	0.18	0.71	0.06	1.22	0.09	0.68	0.09	0.71	0.18	**1.12**	**0.17**
Pituitary gland	0.25	0.00	0.22	0.00	**0.51**	**0.00**	**0.25**	**0.00**	0.25	0.00	0.24	0.00	0.22	0.00	0.19	0.00
Whole blood	**0.62**	**0.17**	**0.30**	**0.08**	0.35	0.08	0.22	0.06	0.29	0.06	0.26	0.07	0.2	0.10	0.13	0.03
Plasma	**0.48**	**0.12**	**0.40**	**0.08**	0.47	0.12	0.30	0.06	0.25	0.06	0.26	0.05	0.12	0.10	0.07	0.01
RBC	**0.38**	**0.07**	0.16	0.02	0.20	0.03	0.12	0.02	0.28	0.07	**0.20**	**0.05**	0.19	0.06	0.16	0.04
Salivary glands	0.68	0.04	0.68	0.05	**1.29**	**0.06**	0.70	0.05	0.71	0.04	**0.71**	**0.05**	0.46	0.05	0.37	0.03
Spinal cord	0.24	0.00	0.25	0.01	**0.37**	**0.01**	**0.34**	**0.01**	0.36	0.01	0.33	0.01	0.32	0.01	0.25	0.01
Spleen	**1.05**	**0.07**	**1.23**	**0.07**	0.92	0.07	0.44	0.03	0.66	0.04	0.65	0.04	0.38	0.06	0.31	0.02
Testes	0.17	0.04	--	--	**0.27**	**0.06**	--	--	0.22	0.05	--	--	0.16	0.05	--	--
Thymus	0.75	0.04	0.55	0.03	**0.91**	**0.05**	0.57	0.02	0.78	0.04	**0.99**	**0.06**	0.39	0.04	0.37	0.02
Thyroid	**2.05**	**0.00**	2.50	0.01	0.90	0.00	0.99	0.00	1.25	0.00	1.45	0.00	0.76	0.00	**0.41**	**0.08**
Uterus	--	--	**0.48**	**0.03**	--	--	0.43	0.02	--	--	0.47	0.02	--	--	0.45	0.02
Fat	1.09	0.08	0.64	0.06	2.00	0.21	0.98	0.14	**3.57**	**0.44**	**2.60**	**0.39**	3.61	0.24	1.98	0.23
Hair/skin	0.60	0.11	0.34	0.06	0.84	0.20	0.46	0.13	**1.12**	**0.22**	**1.23**	**0.22**	0.77	0.18	0.61	0.13
Liver	**4.84**	**4.31**	**3.55**	**3.05**	3.47	3.09	2.56	2.03	1.67	1.68	1.81	1.95	0.84	3.03	0.67	0.61
Kidneys	2.94	0.49	**2.30**	**0.41**	**2.67**	**0.51**	1.91	0.34	1.61	0.29	1.82	0.36	0.83	0.43	0.86	0.17
Caecum	13.28	2.34	4.92	0.89	**27.15**	**4.32**	**57.27**	**12.19**	2.3	0.55	0.98	0.19	0.96	2.40	0.36	0.06
Small intestine	**20.97**	**14.84**	**19.49**	**12.77**	4.44	3.09	3.78	2.72	1.77	1.39	1.23	1.08	0.61	6.44	0.54	0.37
Large intestine	9.27	1.29	1.72	0.23	**10.35**	**1.73**	**30.11**	**4.79**	1.52	0.26	0.86	0.14	0.44	1.09	0.42	0.06
Stomach	**22.55**	**5.06**	17.95	3.83	19.92	3.68	**19.02**	**6.24**	1.78	0.57	0.51	0.15	0.48	3.10	0.22	0.04
GIT contents	**48.44**	**43.82**	**62.48**	**57.75**	25.34	27.63	31.36	31.12	4.57	5.96	0.85	1.00	1.2	25.80	0.30	0.20
Carcass	0.64	9.10	**0.48**	**7.08**	**1.19**	**17.20**	0.42	6.22	0.59	8.98	0.43	6.54	0.36	11.76	0.23	3.59

* calculated as µg [^{14}C]S-1812/g tissue.

In bold: highest concentrations per sex.

Table 2.1.3-06 Distribution of radioactivity in tissues and organs [ppm average radiocarbon concentrations and average % of dose] of 500 mg/kg bw [propenyl-^{14}C]S-1812

	500 mg/kg bw [propenyl-^{14}C]S-1812 ½ Cmax (AB)				500 mg/kg bw [propenyl-^{14}C]S-1812 Cmax (AB)				500 mg/kg bw [propenyl-^{14}C]S-1812 ½ Cmax (EL)				500 mg/kg bw [propenyl-^{14}C]S-1812 $^{1}/_{10}$ Cmax (EL)			
	Male		female		male		female		male		female		male		female	
tissue	ppm*	% AR*	ppm*	% AR*	ppm*	% AR*	ppm*	% AR*	ppm*	% AR*	ppm*	% AR*	ppm*	% AR*	ppm*	% AR*
Adrenals	156	0.01	329	0.03	**410**	**0.02**	**414**	**0.04**	288	0.02	199	0.02	196	0.02	152	0.01
Bone	13	0.01	19	0.02	**31**	**0.02**	**30**	**0.02**	27	0.02	29	0.02	20	0.02	14	0.01
Bone marrow	26	0.00	53	0.00	**49**	**0.00**	**81**	**0.00**	40	0.00	66	0.00	26	0.00	22	0.00
Brain	6	0.01	8	0.01	**21**	**0.03**	**18**	**0.03**	13	0.02	15	0.03	11	0.02	9	0.02
Heart	40	0.03	**83**	**0.07**	**73**	**0.07**	74	0.06	23	0.02	27	0.02	32	0.03	15	0.02
Lungs	**70**	**0.08**	**68**	**0.08**	86	0.11	44	0.06	48	0.06	61	0.08	37	0.05	22	0.03
Muscle	14	0.06	26	0.13	**40**	**0.20**	**36**	**0.16**	34	0.15	21	0.14	19	0.09	12	0.07
Ovaries	--	--	104	0.02	--	--	**164**	**0.04**	--	--	141	0.02	--	--	93	0.02
Pancreas	55	0.05	77	0.06	136	0.15	114	0.10	**135**	**0.20**	**114**	**0.10**	91	0.19	59	0.05
Pituitary gland	3	0.00	14	0.00	25	0.00	19	0.00	**32**	**0.00**	**36**	**0.00**	15	0.00	15	0.00
Whole blood	11	0.03	12	0.03	16	0.03	16	0.04	**16**	**0.03**	**17**	**0.04**	15	0.03	9	0.02
Plasma	15	0.02	16	0.03	**22**	**0.04**	**21**	**0.03**	12	0.02	16	0.02	8	0.02	5	0.01
RBC	8	0.01	9	0.02	12	0.03	14	0.02	16	0.04	**15**	**0.03**	**15**	**0.05**	10	0.03
Salivary glands	26	0.01	55	0.04	**80**	**0.05**	**79**	**0.05**	73	0.05	68	0.05	44	0.03	33	0.02
Spinal cord	6	0.00	7	0.00	19	0.01	16	0.01	**22**	**0.01**	**22**	**0.01**	22	0.00	13	0.00
Spleen	20	0.01	**50**	**0.03**	**40**	**0.02**	38	0.02	32	0.02	40	0.02	25	0.02	18	0.01
Testes	5	0.01	--	--	**14**	**0.03**	--	--	13	0.03	--	--	11	0.03	--	--
Thymus	14	0.01	20	0.01	43	0.03	60	0.04	**43**	**0.03**	**52**	**0.05**	35	0.02	22	0.01
Thyroid	104	0.00	94	0.00	**146**	**0.00**	**125**	**0.00**	107	0.00	82	0.00	77	0.00	56	0.00
Uterus	--	--	28	0.01	--	--	**57**	**0.03**	--	--	37	0.02	--	--	35	0.01
Fat	57	0.05	68	0.08	156	0.18	154	0.20	**287**	**0.35**	**323**	**0.49**	258	0.26	222	0.34
Hair/skin	24	0.07	32	0.07	61	0.12	55	0.15	68	0.15	**125**	**0.33**	**86**	**0.19**	45	0.15
Liver	242	2.12	326	2.74	**326**	**2.44**	**401**	**3.05**	85	0.92	97	0.98	58	0.65	39	0.41
Kidneys	55	0.10	**92**	**0.17**	129	0.23	115	0.22	**119**	**0.25**	85	0.18	57	0.14	44	0.08
Caecum	**3445**	**4.89**	1238	2.50	807	1.37	**3629**	**7.55**	49	0.09	57	0.13	25	0.05	20	0.03
Small intestine	349	2.09	**857**	**5.88**	**358**	**2.44**	344	2.26	75	0.59	91	0.66	36	0.28	43	0.33
Large intestine	**1681**	**2.88**	589	1.00	243	0.34	**1771**	**3.00**	52	0.07	83	0.14	38	0.07	33	0.06
Stomach	1873	5.11	**2795**	**6.63**	**2459**	**7.33**	2225	5.85	78	0.28	23	0.05	16	0.05	13	0.04
GIT contents	**4870**	**64.76**	**5737**	**62.23**	2505	30.03	3694	42.59	61	0.85	42	0.31	20	0.30	11	0.12
Carcass	38	5.31	47	6.79	50	6.93	**50**	**7.22**	**43**	**6.96**	41	5.79	38	6.52	23	3.50

* calculated as µg [^{14}C]S-1812/g tissue.

In bold: highest concentrations per sex.

Conclusions

The metabolic pattern found after application of ^{14}C-S-1812 (5.0 and 500 mg/kg bw [phenyl-^{14}C]S-1812 and 5.0 and 500 mg/kg bw [propenyl-^{14}C]S-1812) to rats did not show dose dependent or sex specific differences. No difference was found between both radiolabels.

Highest residue concentrations (>0.1% AR) were found in GIT contents, small intestine, carcass, stomach, caecum, large intestine, liver, fat, hair/skin, pancreas, muscle, heart and lungs. The highest concentrations were observed at C_{max}, at ½ C_{max} (AB) or ½ C_{max} (EL) for most tissues. For most tissues the residue

concentrations decreased after ½ C_{max} (EL). The following tissues showed an increase of residue concentrations over time, or did not show a decrease after ½ C_{max} (EL): bone marrow, ovaries, uterus, thyroid, pancreas, fat, hair/skin, salivary glands.

Metabolites were reported per time point. Metabolites were reported as percentage of the (acetone or other) extract per composite tissue (liver, kidney, lung, blood and fat), not as % over administered dose. S-1812, S1812-DP and polar metabolites (in fat only after dosing with [propenyl-^{14}C]S-1812) occurred in all composite tissues, S-1812-Ph-CH_2CO_2H and HPHM did not occur in fat.

All metabolites were confirmed by TLC co-chromatography.

Acceptability

The study is considered acceptable. Extraction of composite tissues was not complete after dosing with [propenyl-^{14}C]S-1812. Several attempts were made to hydrolyze liver tissue and derivatization of the expected amino acids released. Thereafter phenylisothiocyanate reagent was used. These methods only revealed a very minor extent of ^{14}C-derivative formation. This result was expected when minimal ^{14}C-amino acids are present in proportion with the endogenous materials. Therefore no extra methods were developed, which was acceptable.

2.1.4 Study 4

Characteristics

reference	:	IIA 5.1.1/04, Project No. 985W-1	exposure	:	single by oral intubation
type of study	:	Metabolism	dose	:	5.0 mg/kg bw for males and females
year of execution	:	2001	vehicle	:	Corn oil
test substances	:	^{14}C-S-1812 (pyridalyl) (^{14}C labelled in the phenyl ring): lot no. RIS98015 (spec. act. 10.2 MBq/mg), chemical purity 98.1%, radiochemical purity 99.0% Unlabelled S-1812: lot no. 980302G, chemical purity 99.7%.	GLP statement	:	Yes
route	:	Oral (intubation).	guidelines	:	EPA guideline OPPTS 870.7485
species	:	Sprague Dawley (M 140-181 g; F 148-156 g) group 2: M 135-171 g; F 138-161 g	acceptability	:	Acceptable
group size	:	4 male and 4 non pregnant female rats			

Study design

After a single dose of 5 mg/kg bw [phenyl-^{14}C]S-1812 (pyridalyl) the metabolism and billiary excretion of ^{14}C-S-1812 was investigated in 2 days collection intervals of bile (cannulated via bile conduct), urine, faeces and cage wash. Urine and bile were collected in intervals: 0-6 hr; 6-12 hr; 12-24 hr; 24-48 hr after dosing, faeces were collected at 0-24 hr and 24-48 hr intervals, cage wash was collected at 48 hr. At 2 days after dosing gastro intestinal tract contents, liver, kidneys and carcass were collected.

Radiocarbon in urine and cage wash was analysed by direct liquid scintillation counting (LSC). Bile, faeces, tissue and carcass radiocarbon was determined by combustion to carbon dioxide and LSC. Duplicate samples were analysed.

Chromatographic analysis was performed directly on urine and bile composites and bile hydrolysates. Chromatographic analysis was performed on faeces composites after extraction with acetone. Supernatant was radio assayed by LSC. Residues were subsequently extracted with methanol/0.1 N HCl. The remaining Post Extraction Solids (PES) were combusted.

A part of the bile underwent glucuronidase and sulphatase treatment prior to HPLC analysis.

Analysis of ^{14}C in tissues and carcasses was performed by combustion and LSC. Tissues were analysed in duplicate (aliquots of 100-500 mg), in case of high relative standard deviation (>20%) a second set of duplicates was combusted. If the RSD was <20% this set was used, if not all 4 samples were used for analysis. Of the carcasses two samples per animal were combusted (appr. 250 mg).

Metabolites were identified in urine, bile, faeces and liver (pooled per gender per collection interval) by a HPLC System (faecal extracts, urine metabolite separation), co-chromatography was used for confirmation of metabolites in two separate chromatographic systems. Kidneys were not combusted or analyzed due to the negligible radiocarbon content.

In the study report, the absorption rate was determined using data from the current study and the metabolism study (IIA 5.1.1/02). The absorption ratio was calculated as follows:
Absorption ratio (%) = oral dosage (100%) – unabsorbed S-1812 in faeces (% of dose)
The unabsorbed S-1812 in faeces was corrected for the metabolism process using data from the current study (CR).

Results

Mean doses administered were 5.1 mg/kg bw (± 0.9) for male rats and 5.2 mg/kg bw (± 0.1) for female rats in group 1 (initial group) and 5.1 mg/kg bw (± 0.1) for male and female rats in group 2 (contingency group). Two males and three females of the initial group were replaced by animals from the contingency group due to insufficient urine or bile production or because of low material balance (<90%).

Table 2.1.4-01 Recovery of radioactivity (% AR) in rats after single oral exposure to [phenyl-^{14}C]S-1812

		Average recovered 5 mg/kg bw [phenyl^{14}C]S-1812		Composite sample 5 mg/kg bw [phenyl^{14}C]S-1812	
Sample		**M**	**F**	**M**	**F**
Faeces	0-24 h	54.6	48.7	50.5	40.3
	24-48 h	20.9	6.1	13.8	3.8
	0-48 h	**75.5**	**54.8**		
Urine	0-6 h	0.1	NS	0.1	NS
	6-12 h	0.1	0.2	0.1	0.3
	12-24 h	0.4	0.3	0.3	0.3
	24-48 h	0.2	0.0	0.2	0.1
	0-48 h	**0.8**	**0.5**		
Cage wash	**0-48 h**	**0.9**	**1.3**		
Bile	0-6 h	2.6	2.6	1.4	2.5
	6-12 h	2.1	2.0	2.2	2.1
	12-24 h	2.0	1.8	2.0	1.7
	24-48 h	1.5	1.5	1.2	0.6
	0-48 h	**8.2**	**7.9**		
GI tract contents	**48 h**	**3.2**	**17.5**		
Liver	**48 h**	**0.3**	**0.5**	**0.3**	**0.3**
Kidneys	**48 h**	**0.0**	**0.0**		
Carcass	**48 h**	**5.2**	**10.5**		
Recovery	**48 h**	**94.1**	**93.0**		

NS = no sample available.

Mean total recovery was 94.1% (range from 88.9% to 94.5%) for males and 93.0% for females (range from 90.4-94.8%).

After a single dose of [phenyl-^{14}C]S-1812 the majority of administered radioactivity was excreted in faeces from 0-48 hours (54.8-75.5% AR, see table 2.1.4-01), the majority has been excreted in the first 24 hours (48.7-54.6% AR). Radioactivity in urine accounted for 0.9-1.3% AR from 0-48 hours.

Radioactivity excreted in bile was approximately 8% (% AR) in 48 hours (males ranged from 4.7-10% and females ranged from 3.4-8.8%).

Radioactivity in liver and kidneys was <1% AR in the first 48 hours after dosing. GIT contents accounted for 3.2-17.5% AR. In the carcasses average radioactivity ranged from 5.2-10.5% AR.

Composite samples

A comparison of the total radioactivity recovered in composite samples and the samples used for radio analysis was made. The recovered radioactivity was comparable for both excreta for each time point and for the liver.

Extractability of faeces samples

Extraction of the faeces samples showed that the major part of radioactivity was present in the acetone extract (51.0-71.1% of the administered radioactivity and 89.5-106.3% of the available radiocarbon in the sample). MeOH/HCl extraction accounted for 1.2-2.2% AR (<6% of the available radiocarbon in the sample). PES accounted for 3.0-4.2% AR.

Extraction of the liver samples showed that the major part of radioactivity was present in the acetone extract (79.3-81.3% of the available radiocarbon in the sample). MeOH/HCl extraction accounted for 11.6-14.4% of the available radiocarbon in the sample. PES accounted for 6.1-9.4% of the available radiocarbon in the sample. No percentage of the administered dose was provided.

Metabolite identification (table 2.1.4-02)

Faeces

After dosing with 5 mg/kg bw [phenyl-^{14}C]S-1812 in the acetone extracts of faeces the only detectable compound was S-1812. A very small amount of S-1812-DP was detected in male rats at 48 hours (amounts not known, not reported).

In the acidified methanol extractions S-1812 was detected and S-1812-DP was detected in small amounts (amounts unknown, not reported).

Urine

Excretion of radiocarbon in urine was <1%, therefore only cursory HPLC was conducted. None of the bands detected exceeded 0.5% of the dose. No identification of the compounds was made.

Bile

In bile no S-1812 was detected, only S-1812-DP and S-1812-DP glucuronide. In females only S-1812-DP glucuronide was detected.

Liver

After dosing with 5 mg/kg bw [phenyl-^{14}C]S-1812 <1% of the dose was detected as radiocarbon in the liver. Although only a small percentage of the dose was detected in the liver, the liver samples were analysed for metabolites. The major compound identified was S-1812-Ph-CH_2COOH (34-45% of the extract), also S-1812-DP (4.4-11.3%) and S-1812 (10.3%) were identified in the liver.

Table 2.1.4-02 Metabolite identification (% AR) in liver and bile of male and female rats after single oral exposure to ^{14}C-S-1812

Excreta/ tissue	Metabolite	5 mg/kg bw [phenyl^{14}C]S-1812									
		0-6 hours		6-12 hours		12-24 hours		24-48 hours		Total	
		M	F	M	F	M	F	M	F	M	F
Bile*	S-1812-DP	0.1	0.0	0.2	0.0	0.1	0.0	0.2	0.0	0.6	0.0
	S-1812-DP glucuronide	2.3	2.6	1.7	2.0	1.6	1.8	1.2	1.5	6.8	7.9
	Other***	0.2	0.0	0.2	0.0	0.3	0.0	0.1	0.0	0.8	0.0
	TOTAL	2.6	2.6	2.1	2.0	2.0	1.8	1.5	1.5	8.2	7.9
Liver**	S-1812									10.3	ND
	HPHM									2.8	0.9
	S-1812-DP									11.3	4.4
	Unknown 1									11.3	20.0
	S-1812-Ph CH_2COOH									33.9	44.6
	Unknown2									7.6	9.6
	Other metabolites									22.8	20.5
	TOTAL									100	100

* % of the administered dose.
** % in extract.
*** possibly a small amount of sulphate conjugate is also present.
ND: not detected

Metabolic pathway of S-1812 in rats

The formation of major metabolite (S-1812-DP) involves oxidative cleavage of the dichloropropenyl group, yielding S-1812-DP from the phenyl label. This product is then conjugated as the glucuronide and found in the bile. In liver S-1812-Ph-CH_2COOH was detected (formed after oxidation of the propenyl side chain).

Absorption rate of S-1812 in rats

In the study report, a calculation of the absorption rate in rats was made based on results from this study and another ADME study (IIA 5.1.1/02, see 2.1.2). As no parent compound was detected in bile, only S-1812 found in faeces is considered material not absorbed into the systemic circulation through the GI tract. Therefore all other %AR in faeces is considered to be potentially systemic available.

The CR (correction for metabolism process) was derived from the results in Table 2.1.4-03 (% of dose recovered/% of dose extracted as S-1812). For males the CR was 1.10 (77.5/70.2) and 1.08 (55.2/51.0) for females.

In the study IIA 5.1.1/02 after a single dose of 5 mg/kg bw [phenyl-^{14}C]S-1812 in males 31.7 %AR and in females 31.0% AR was recovered as S-1812 in faeces (see Table 2.1.2-06).

In the study report the absorption rate was determined to be 65.1% (100-1.10x31.7) for male rats and 66.5% (100-1.08x31.0) for female rats.

For the absorption ratio data from the study IIA 5.1.1/02 were used, covering a period of 168 hours, whereas in the current study a collection period of only 48 hours was used. The collection period of 48 hours is acceptable as most radiocarbon was excreted in that period.

Table 2.1.4-03 Extractable radiocarbon in faeces and S-1812 concentration as percent of dose

	Average % ^{14}C in faeces	Total % of dose extracted	% of dose extracted as S-1812	%PES*	% of dose recovered
Males					
0-24 hours	54.6	50.1	48.9	1.0	51.1
24-48 hours	20.9	23.2	21.3	3.2	26.4
TOTAL	**75.5**	**73.3**	**70.2**	**4.2**	**77.5**
Females					
0-24 hours	48.7	45.8	44.9	1.2	47.0
24-48 hours	6.1	6.4	6.1	1.8	8.2
TOTAL	**54.8**	**52.2**	**51.0**	**3.0**	**55.2**

* Post extracted solids.

Conclusions

After oral dosing of ^{14}C-S-1812 to rats the main excretion route was the faeces (>70% of the administered dose was found in the faeces and the GI tract), mainly the parent compound was found in faeces. In bile approximately 8% of the administered dose was recovered over a period of 48 hours. In bile no S-1812 was detected, only S-1812-DP (males) and S-1812-DP glucuronide (males and females).

The carcass contained 5-10% of the radiocarbon administered.

Urine (and cage wash), liver and kidneys contained <2% of the radiocarbon administered.

The major compound identified in the liver was S-1812-Ph-CH_2COOH (34-45% of the extract), also S-1812-DP (4.4-11.3% of the extract) and S-1812 (10.3% of the extract) were identified in the liver.

The metabolic pattern found after oral dosing of ^{14}C-S-1812 to rats did not show clear sex specific differences. All metabolites were confirmed by co-chromatography.

The formation of major metabolite (S-1812-DP) involves oxidative cleavage of the dichloropropenyl group, yielding S-1812-DP from the phenyl label. This product is then conjugated as the glucuronide and found in the bile. In liver S-1812-Ph CH_2COOH was detected (formed after oxidation of the propenyl side chain).

Acceptability

The study is considered acceptable.

2.1.5 Study 5

Characteristics

reference	:	IIA 5.1.3/01, Project No. SUM-009	exposure	:	1, 6, 10 or 14 days, once daily by oral intubation
type of study	:	distribution and metabolism	Dose	:	5.0 mg/kg bw/day for males and females
year of execution	:	2000-2001	vehicle	:	Corn oil
test substances	:	^{14}C-S-1812 (pyridalyl) (^{14}C labelled in the phenyl ring): lot no. RIS98015 (spec. act. 10.2	GLP statement	:	Yes

		MBq/mg), chemical purity 98.1%, radiochemical purity 99.0% Unlabelled S-1812: lot no. 980302G, chemical purity 99.7%.			
route	:	Oral (intubation).	guidelines	:	Japanese MAFF; EPA OPPTS 870.7485 guidances.
species	:	Sprague Dawley (M 227-252 g; F 150-195 g)	acceptability	:	Acceptable
Group size	:	3 male and 3 non pregnant female rats per group; 7 groups in total			

Study design

The tissue distribution of a single dose and repeated doses (6, 10 and 14 days daily dosing) of [phenyl-^{14}C]S-1812 (pyridalyl) was investigated as well as excretion and the metabolism after 14 days daily dosing. Animals were divided over 7 groups of 3 male and 3 females each, for duration of treatment and analytical item see Table 2.1.5-01.

In total 25 male and 31 female animals were treated, maximally 14 days (14 animals were not used for analysis). The reason was an insufficient number of evaluable animals as one male and one female were dead by mistake of dosing and 3 males and 8 females were found erroneously dosed at the time of sacrifice.

Table 2.1.5-01 Experimental groups

Group no.	No. of animals	Days of treatment	Sacrifice (days after first dose)	Average daily dose of [phenyl-^{14}C]S-1812 (mg/kg bw)	Analytical item
A	3 M & 3 F	1	1	5	^{14}C-tissue distribution*
B	3 M & 3 F	6	6	5	^{14}C-tissue distribution*
C	3 M & 3 F	10	10	5	^{14}C-tissue distribution*
D	3 M & 3 F	14	14	5	^{14}C-tissue distribution* metabolite analysis in tissues
E	3 M & 3 F	14	17	5	^{14}C-tissue distribution*
F	3 M & 3 F	14	20	5	^{14}C-tissue distribution*
G	3 M & 3 F	14	27	5	^{14}C-tissue distribution* ^{14}C-excretion** metabolite analysis in faeces and urine

*): adrenals, blood, plasma, red blood cells, brain, fat (brown), fat (perirenal), fat (testicular), heart, kidneys, liver, lungs, muscle, skin, spleen, testes, thymus, thyroids, ovaries and uterus.
**): urinary and faecal excretion

Radiocarbon in whole blood, blood cells, plasma and tissues were analyzed by combustion radio analysis (duplicate samples were analysed).

Urine and faeces were collected in group G only. Radiocarbon in urine and cage wash was directly analysed by direct liquid scintillation counting (LSC). Urine and cage wash samples of Days 19-27 were mixed and then radio assayed. Urine samples of Day 0-3 and 11-14 were pooled for each sex and used for analysis of metabolites. Faeces were homogenized with water and duplicate homogenates were analyzed by combustion radio analysis. Homogenized faeces of Days 0-3 and Days 11-14 (group G) were pooled for each sex and

used for metabolite analysis. Extraction with acetone and methanol/water was performed. Each extract was radio assayed by LSC, the residues were analyzed by combustion radio analysis.

Urine and faecal extracts of group G were analyzed by TLC to quantify metabolite profiles. Metabolites were identified by TLC and/or HPLC co-chromatography.

Whole blood, fat (perirenal), kidney, liver and lung of the rats of group D were pooled for sex. Metabolites were extracted 3 times with acetone and 3 times with methanol/water. Duplicate aliquots of the extracts were radio assayed by LSC. The residues were analyzed by combustion radio analysis.

Results

Mean doses administered were 4.7 mg/kg bw (± 0.27) for male and 5.4 mg/kg bw (± 0.08) for female rats in group A; in group B mean doses ranged from 4.9-5.3 mg/kg bw (males) and 5.2-5.4 mg/kg bw (females); in group C mean doses ranged from 5.1-5.4 mg/kg bw (males) and 5.2-5.4 mg/kg bw (females); in group D mean doses ranged from 5.0-5.6 mg/kg bw (males) and 5.1-5.6 mg/kg bw (females); in group E mean doses ranged from 5.1-5.3 mg/kg bw (males) and 5.1-5.5 mg/kg bw (females); in group F mean doses ranged from 5.0-5.6 mg/kg bw (males) and 5.0-5.4 mg/kg bw (females); in group G mean doses ranged from 4.7-5.7 mg/kg bw (males) and 5.2-5.6 mg/kg bw (females).

Four males and nine females of the initial treated groups were replaced by animals from the additional treated groups due to dead by erroneous dosing or “suspected” erroneous dosing of the animals. No data available.

Tissue distribution

Blood and tissues were collected and radio assayed at days 1, 6, 10, 14, 17 (3 days after last dose), 20 (6 days after last dose) and 27 (13 days after last dose). ^{14}C-concentrations are shown in Table 2.1.5-02. No difference between sexes occurred. Highest ^{14}C-concentrations were observed in fat (brown, perirenal and testicular), liver, skin, thyroids, adrenals, kidneys, thymus and ovaries. In fat the highest concentrations ranged from 37.87 ppm – 57.50 ppm, whereas the maximum concentration in the other tissues was 5.60 ppm.
In blood and in some tissues ^{14}C-concentrations reached steady-state on Day 6 or Day 10 (after a proportional increase). In the adrenals (females only), brain (males only), brown fat, perirenal fat, testicular fat (males only), heart, liver (males only), muscle, spleen, thymus, thyroids (males only) maximum ^{14}C-concentrations were reached on Day 14 or later (perirenal fat and skin in females).
In blood and most tissues the accumulation ratio ranged from 3-6 (Day 14/Day 1), higher accumulation ratios were seen in the heart (males: 8); lungs (females: 8); spleen (males: 9); skin (males: 11, females: 9); thymus (males: 17); brown fat (males: 11); perirenal fat (males: 23, females: 17); peritesticular fat (males: 23).
Generally a biphasic decrease was observed (except for blood, perirenal and testicular fat, skin, thymus and uterus), with a biological half-life of 1-5 days (α phase) and 4-24 days (β phase). In blood and plasma the

biological half-life ranged from 1-2 days, in perirenal and testicular fat the biological half-life ranged from 10-15 days, in the skin, thymus and uterus the half-life ranged from 4-10 days. See Table 2.1.5-03.

Table 2.1.5-02 Distribution of radioactivity in tissues and organs [µg S-1812 equivalent/g tissue] of ^{14}C-labeled S-1812

	MALES						
Tissue	**Day 1**	**Day 6**	**Day 10**	**Day 14**	**Day 17**	**Day 20**	**Day 27**
Adrenals	0.26	0.90	1.65	1.55	1.23	0.66	0.38
Blood	0.02	0.09	0.06	0.10	0.03	0.01	<0.01
Plasma	0.03	0.15	0.09	0.13	0.04	0.02	<0.01
Red blood cells	<0.01	0.04	0.03	0.03	<0.01	<0.01	<0.01
Brain	0.03	0.07	0.09	0.11	0.02	0.02	0.01
Fat (brown)	5.42	42.21	51.77	57.50	24.58	10.06	5.08
Fat (perirenal)	1.87	23.35	42.06	43.11	37.87	30.17	19.45
Fat (testicular)	1.73	19.30	31.58	40.12	34.44	29.39	21.56
Heart	0.05	0.26	0.31	0.43	0.11	0.15	0.04
Kidneys	0.25	1.33	1.07	1.34	0.43	0.47	0.21
Liver	1.12	4.32	4.35	5.60	1.35	0.77	0.25
Lungs	0.12	0.42	0.85	0.70	0.30	0.13	0.09
Muscle	0.04	0.16	0.23	0.26	0.14	0.10	0.07
Skin	0.38	2.61	5.95	4.23	3.12	3.09	1.59
Spleen	0.03	0.14	0.23	0.24	0.09	0.09	0.04
Testes	0.04	0.11	0.12	0.12	0.05	0.07	0.03
Thymus	0.09	0.37	1.38	1.42	0.20	0.99	0.18
Thyroids	0.60	2.02	3.32	3.76	0.79	0.95	0.32

Table 2.1.5-02 Distribution of radioactivity in tissues and organs [µg S-1812 equivalent/g tissue] of ^{14}C-labeled S-1812, continued

	FEMALES						
Tissue	**Day 1**	**Day 6**	**Day 10**	**Day 14**	**Day 17**	**Day 20**	**Day 27**
Adrenals	0.33	1.77	1.89	2.00	1.05	0.63	0.41
Blood	0.03	0.07	0.12	0.11	0.02	<0.01	<0.01
Plasma	0.05	0.10	0.18	0.16	0.03	0.01	<0.01
Red blood cells	<0.01	0.02	0.04	0.04	<0.01	<0.01	<0.01
Brain	0.02	0.05	0.07	0.07	0.02	<0.01	<0.01
Fat (brown)	7.00	38.88	31.94	33.70	20.07	6.87	4.80
Fat (perirenal)	1.96	16.92	26.69	32.36	38.37	25.88	19.89
Heart	0.07	0.23	0.25	0.30	0.11	0.08	0.04
Kidneys	0.19	0.56	0.80	0.90	0.31	0.27	0.14
Liver	1.02	2.71	3.91	3.74	0.91	0.51	0.24
Lungs	0.09	0.39	0.38	0.74	0.41	0.11	0.05
Muscle	0.04	0.17	0.17	0.26	0.13	0.10	0.05
Ovaries	0.51	1.13	1.48	1.42	0.84	0.56	0.62
Skin	0.31	1.95	2.48	2.68	3.48	3.24	1.07
Spleen	0.04	0.13	0.15	0.18	0.09	0.06	0.04
Thymus	0.08	0.30	0.25	0.39	0.34	0.21	0.06
Thyroids	0.40	1.13	1.92	1.83	1.28	0.71	0.26
Uterus	0.03	0.12	0.14	0.13	0.05	0.08	0.05

Table 2.1.5-03 ^{14}C -Accumulation ratios and biological half-life times in tissues during and after repeated dosing with [phenyl-^{14}C]S-1812

MALES						
		Biological half-time (days)				
Tissue	**Accumulation ratio (Day 14/Day 1)**	**α phase**	**Calculation days**		**β phase**	**Calculation days**
Adrenals	6	5	D 14-20		9	D 20-27
Blood	4	2	D 14-20			
Plasma	4	2	D 14-20			
Red blood cells	ND	ND				
Brain	3	1	D 14-17		9	D 17-27
Fat (brown)	11	2	D 14-20		7	D 20-27
Fat (perirenal)	23	10	D 17-27			
Fat (testicular)	23	15	D 17-27			
Heart	8	2	D 14-17		7	D 17-27
Kidneys	5	2	D 14-17		10	D 17-27
Liver	5	1	D 14-17		4	D 17-27
Lungs	6	2	D 14-20		13	D 20-27
Muscle	6	4	D 14-17		9	D 17-27
Skin	11	9	D 14-27			
Spleen	9	2	D 14-17		10	D 17-27
Testes	3	2	D 14-17		14	D 17-27
Thymus	17	4	D 14-27			
Thyroids	6	1	D 14-17		8	D 17-27

Table 2.1.5-03 ^{14}C -Accumulation ratios and biological half-life times in tissues during and after repeated dosing with [phenyl-^{14}C]S-1812, continued

FEMALES						
		Biological half-time (days)				
Tissue	**Accumulation ratio (Day 14/Day 1)**	**α phase**	**Calculation days**		**β phase**	**Calculation days**
Adrenals	6	3	D 14-17		7	D 17-27
Blood	3	1	D 14-17			
Plasma	3	2	D 14-20			
Red blood cells	ND	ND				
Brain	3	2	D 14-17			
Fat (brown)	5	3	D 14-20		14	D 20-27
Fat (perirenal)	17	11	D 17-27			
Heart	4	2	D 14-17		7	D 17-27
Kidneys	5	2	D 14-17		9	D 17-27
Liver	4	1	D 14-17		5	D 17-27
Lungs	8	2	D 14-20		6	D 20-27
Muscle	6	3	D 14-17		7	D 17-27
Ovaries	3	4	D 14-17		24	D 17-27
Skin	9	6	D 17-27			
Spleen	5	3	D 14-17		8	D 17-27
Thymus	5	5	D 14-27			
Thyroids	5	4	D 14-20		5	D 20-27
Uterus	5	10	D 14-27			

Excretion

Faeces and urine were collected daily during the dosing period of 14 days in group F. ^{14}C-concentrations have been reported as percentage of the total dose given, see Table 2.1.5-04. No sex-related differences were observed. Most ^{14}C was excreted in faeces (91.5-94.5% of the total dose within 27 days). In urine only 2.0-4.4% of the total dose was excreted over a period of 27 days. Two days after the last dose (Day 16) most ^{14}C was excreted (89.2 and 92.8% in faeces and 2.0 and 4.2% in urine). In faeces on Day 27 still ^{14}C was detected.

In total 96-97% of the total dose was excreted in faeces and urine.

Table 2.1.5-04 Cumulative recovery of radioactivity (% total dose) after repeated (14 days) oral exposure to 5 mg/kg bw [phenyl-^{14}C]S-1812

	Average recovered radiocarbon in urine		Average recovered radiocarbon in faeces	
Day	**M**	**F**	**M**	**F**
1	0.2	0.1	5.0	4.0
2	0.3	0.2	10.5	11.0
3	0.5	0.3	16.2	18.1
4	0.8	0.4	22.0	23.8
5	1.0	0.6	28.2	29.3
6	1.4	0.7	33.4	36.2
7	1.7	0.9	39.7	42.9
8	2.0	1.0	45.8	48.2
9	2.3	1.2	53.0	54.2
10	2.8	1.3	58.9	63.0
11	3.1	1.5	65.3	70.0
12	3.5	1.6	72.3	77.0
13	3.8	1.8	80.0	83.7
14	4.1	1.9	85.7	90.5
15	4.2	1.9	88.7	92.4
16	4.2	2.0	89.2	92.8
17	4.2	2.0	89.7	93.1
19	4.3	2.0	90.3	93.5
21	4.3	2.0	90.7	93.8
23	4.3	2.0	91.0	94.1
25	4.3	2.0	91.3	94.3
27	4.4	2.0	91.5	94.5

Metabolism

Metabolites were analyzed in tissues (group D) after 14 days dosing. In perirenal fat and the lungs the major compound detected was S-1812. In perirenal fat also S-1812-DP was detected in substantial concentrations. In blood, kidneys and liver the most prominent compound was M3 (S-1812-Ph-CH_2-COOH). Other metabolites identified were S-1812-Py-OH and HPHM. See Table 2.1.5-05.

Table 2.1.5-05 Metabolite identification (µg equivalent of S-1812/g wet tissues) in tissues after repeated oral exposure to ^{14}C-S-1812 (group D)

	Amounts of metabolite (µg equivalent of S-1812/g wet tissues)									
	Whole blood		**Fat (perirenal)**		**Kidneys**		**Liver**		**Lungs**	
	M	F	M	F	M	F	M	F	M	F
S-1812	0.03	0.03	38.96	31.11	0.24	0.151	1.67	0.23	0.43	0.46
S-1812 DP	0.01	0.01	1.87	0.46	0.14	0.03	0.23	0.14	0.03	<0.01
M1	0.00	<0.00	0.59	0.41	<0.05	<0.02	0.06	<0.06	<0.01	<0.01
M2	0.00	0.01	<0.36	0.39	<0.04	0.04	0.08	<0.05	0.06	0.02
M3	0.05	0.05	0.73	<0.36	0.45	0.29	2.12	1.30	0.03	0.04
OTHERS	0.01	0.02	0.97	0.00	0.35	0.14	0.69	1.53	0.04	0.14
UNEXTR.	0.00	0.00	0.00	0.00	0.15	0.24	0.74	0.54	0.10	0.08
TOTAL	**0.10**	**0.11**	**43.11**	**32.36**	**1.34**	**0.90**	**5.60**	**3.74**	**0.70**	**0.74**

M1: S-1812-Py-OH
M2: HPHM
M3: S-1812-Ph-CH_2-COOH

Metabolites were analyzed in faeces and urine in pooled samples of 0-3 days and 11-14 days (group G), see Table 2.1.5-06. No sex related differences were observed. In faeces S-1812-DP was detected as the major metabolite: 6.05-6.68% of the total dose in the sample of 0-3 days increasing to 9.50-9.85% of the total dose in the sample of 11-14 days. S-1812 excretion in the faeces sample of 0-3 days was higher (6.97-7.85% of total dose) than in the sample of 11-14 days (4.84-5.88% of the total dose). Other metabolites identified in faeces were S-1812-Py-OH, HPHM, S-1812-Ph-CH_2-COOH.

In urine S-1812 was not detected, only polar metabolites <1% of the administered dose.

Table 2.1.5-06 Metabolite identification (% AR) in faeces and urine after repeated oral exposure to ^{14}C-S-1812 (group G)

		5 mg/kg bw [phenyl^{14}C]S-1812							
		0-3 days				11-14 days			
		M		F		M		F	
Excreta/ tissue	**Metabolite**	%AR	% of total metabolites	%AR	% of total metabolites	%AR	% of total metabolites	%AR	% of total metabolites
Faeces	S-1812	6.97		7.85		4.84		5.88	
	S-1812-DP	6.05	62.1	6.68	63.6	9.85	59.4	9.50	63.2
	M1	0.14	1.5	0.15	1.5	0.07	0.5	0.29	2.0
	M2	0.29	3.0	0.36	3.4	0.57	3.5	0.46	3.1
	M3	0.27	2.8	0.31	3.0	0.66	4.0	0.53	3.5
	Other	1.18	12.1	1.40	13.3	2.25	13.6	2.06	13.7
	Unextractable	1.28	13.2	1.30	12.4	2.18	13.2	1.76	11.7
	SUBTOTAL	16.18		18.05		20.44		20.49	
Urine	Polar	0.49	5.0	0.25	2.4	0.89	5.4	0.34	2.3
	Other metabolites	0.04	0.4	0.05	0.5	0.09	0.6	0.10	0.6
	SUBTOTAL	0.53		0.30		0.99		0.44	
	TOTAL	16.71		18.36		21.42		20.93	

M1: S-1812-Py-OH
M2: HPHM
M3: S-1812-Ph-CH_2-COOH

Conclusions

After oral repeated oral dosing of 5 mg/kg bw [phenyl-^{14}C]S-1812 to rats the main excretion route was the faeces (>90% of the administered dose was found in the faeces). Excretion in urine account for 2.0-4.4% AR within 27 days. Parent compound was found in faeces, 4-7% AR. The main metabolite found in faeces was S-1812-DP (6-10% AR). Other minor metabolites were S-1812-Py-OH and HPHM.

These results were in line with the results after a single dose of 5 mg/kg bw [phenyl-^{14}C]S-1812 (study 02, Table 2.1.5-06).

Highest ^{14}C-concentrations were observed in fat (brown, perirenal and testicular), liver, skin, thyroids, adrenals, kidneys, thymus and ovaries. In perirenal and testicular fat the highest accumulation ratio's (Day 14/Day 1) were found (accumulation ratio: 17-23). In other tissues accumulation ratios were maximally 11 (skin and brown fat).

Generally a biphasic decrease was observed (except for blood, perirenal and testicular fat, skin, thymus and uterus), with a biological half-life of 1-5 days (α phase) and 4-24 days (β phase). In perirenal and testicular fat the biological half-life was long and ranged from 10-15 days, in blood and plasma the biological half-life ranged from 1-2 days, in the skin, thymus and uterus the half-life ranged from 4-10 days.

In perirenal fat and the lungs the major compound detected (after 14 days dosing) was S-1812. In perirenal fat also S-1812 DP was detected in substantial concentrations. In blood, kidneys and liver the most prominent compound was S-1812-Ph-CH_2-COOH. Other metabolites identified were S-1812-Py-OH and HPHM.

In faeces S-1812-DP was detected as the major metabolite. S-1812 excretion in the faeces sample of 0-3 days was higher than in the sample of 11-14 days. Other metabolites identified in faeces were S-1812-Py-OH, HPHM, S-1812-Ph-CH_2-COOH. In urine S-1812 was not detected, only polar metabolites <1% of the administered dose. Comparing these results to the results from metabolism study 02 (Table 2.1.5-06) we can conclude that the ratio's between parent and main metabolite (S-1812-DP) were comparable between 14 days dosing and a single dose of 5 mg/kg bw [phenyl-^{14}C]S-1812: 52-61% of recovered radioactivity S-1812-DP and 30-33% parent after single dosing and 50-52% metabolite and 33% parent after repeated dosing. After 3 days of dosing relatively less S-1812-DP was detected (37%) compared to the parent compound (43%).

Acceptability

The study is considered acceptable.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 Study 1

Characteristics

reference	:	IIA 5.2.1/01 Report No. 6311-217	exposure	:	Once by gavage
type of study	:	Acute oral toxicity study	doses	:	5000 mg/kg bw (both sexes)
year of execution	:	1999	vehicle	:	None
test substance	:	S-1812 (Pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 401 (1987)
species	:	Rat, Crl:CD (SD)	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	> 5000 mg/kg bw (male and female)

Study design

The study was performed in accordance with OECD 401 (1987).

Results

Mortality: No mortality occurred.

Symptoms of toxicity: No treatment related finding were noted.

Body weight: One female showed weight loss during the first week (-3.5%). The mean body weights shown by the other animals over study period was considered to be similar to that expected of normal untreated animals of the same age and strain.

Pathology: One female showed an enlarged kidney during pathology. No further abnormalities were seen. The pathologist did not consider this to be treatment related.

Acceptability

This study is considered acceptable.

Conclusions

The acute oral LD_{50} of S-1812 was found to be greater than 5000 mg/kg bw in male and female rats.

3.1.2 Human data

No data available.

3.1.3 Other data

No data available.

3.2 Acute toxicity - dermal route

3.2.1 Animal data

3.2.1.1 Study 1

Characteristics

reference	:	IIA 5.2.2/01 Project No. 6311-218	exposure	:	24 hours on a skin area of 24 cm^2 (occlusive exposure).
type of study	:	Acute dermal toxicity study	doses	:	5000 mg/kg bw (both sexes)
year of execution	:	1999	vehicle	:	None
test substance	:	S-1812 (Pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	Dermal	guideline	:	In accordance with OECD 402
species	:	Rat, Crl: CD(SD)	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	> 5000 mg/kg bw

Study design

The study was performed in accordance with OECD 402 (1987).

Results

Mortality: No mortality was observed.

Symptoms of toxicity: No treatment related signs were observed.

Body weight: One female showed weight loss during the first week (-1.3%). The mean body weights shown by the other animals over study period was considered to be similar to that expected of normal untreated animals of the same age and strain.

Pathology: No abnormalities were observed.

Acceptability

The study is considered acceptable.

Conclusions

The acute dermal LD_{50} of S-1812 in rats was found to be greater than 5000 mg/kg bw in both males and females.

3.2.2 Human data

No data.

3.2.3 Other data

No data.

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

3.3.1.1 Study 1

Characteristics

reference	:	IIA 5.2.3/01 Project No SMO-568	exposure	:	4 hours; nose-only
type of study	:	Acute inhalation toxicity study	doses	:	8.3 mg/l (nominal concentrations), 2.01 mg/l (actual concentrations) ; MMAD 2.7 µm ; GSD 1.98 aerosol
year of execution	:	2001	vehicle	:	None
test substance	:	S-1812 (Pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	Inhalation	guideline	:	In accordance with OECD 403 (1981)
species	:	Rat, Crl: CD(SD)	acceptability	:	Acceptable (not for classification and labelling)
group size	:	5/sex/dose	**LC_{50}**	:	> 2.01 mg/l (male and female)

Study design

The study was performed in accordance with OECD 403 (1981).

Results

Mortality: No mortality occurred.

Symptoms of toxicity: All animals showed decreased breathing rate and exaggerate breathing during exposure. After exposure all animals showed these clinical signs for 2 hours and 2 days after exposure respectively. Lethargy, whole body cold, and wet fur were observed for all animals after exposure until 2 hours following exposure. Brown staining around snout was observed in one male rat following exposure until 2 hours post exposure.

Body weight: Mean body weight gain of both sexes decreased after the first week following exposure, and increased thereafter. The mean body weight gain increased more compared to the control animals

Water consumption: No treatment related effects.

Pathology: In some male control rats severe congestion towards the lower periphery on the lobes of the lungs was observed. In one male test rat small dark foci were noted. This finding was also noted in one control male animal. In the remaining test animals no abnormalities were found. It can be concluded that no treatment-related effects were found.

Acceptability

It is not clear from the study report that maximum effort was taken to attain higher test substance concentration than 2.01 mg/L. In absence of data it is concluded that the maximum concentration tested is not the highest attainable concentration. However, the study is considered acceptable.

Conclusions

The acute inhalation LC_{50} of S-1812 in rats was found to be greater than 2.01 mg/l for male and female rats.

3.3.2 Human data

No data.

3.3.3 Other data

No data.

3.4 Skin corrosion/irritation

3.4.1 Animal data

3.4.1.1 Study 1

Characteristics

reference	:	IIA 5.2.4/01, Project No. 6311-219	exposure	:	4 hours, semi-occlusive, application area 6.25 cm^2
type of study	:	Skin irritation study	doses	:	0.5 ml
year of execution	:	1999	vehicle	:	None
test substance	:	S-1812 (Pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes

route	:	Dermal	guideline	:	In accordance with OECD 404 (1992)
species	:	Rabbit, New Zealand White	acceptability	:	Acceptable
group size	:	2 males, 4 females	**Effect**	:	Not skin irritating

Study design

The study was performed in accordance with OECD 404 (1992).

Results

The results are summarised in tables 3.4.1.1-1 and 3.4.1.1-2.

Table 3.4.1.1-1 Individual irritation scores

Scores observed after	1 hour	24 hours	48 hours	72 hours
Erythema	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0
Oedema	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0

Table 3.4.1.1-2 Mean value irritation scores

Animal	mean 24-72 hrs	
	erythema	oedema
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0

Acceptability

The study is considered acceptable. The amount of animals tested could have been lower (n=3).

Conclusions

S-1812 was found to be non-irritating to rabbit skin.

3.4.2 Human data

No data.

3.4.3 Other data

No data.

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

3.5.1.1 Study 1

Characteristics

reference	:	IIA 5.2.5/01 Project No 6311-220	exposure	:	Single instillation
type of study	:	Acute eye irritation study	doses	:	0.1 mL
year of execution	:	1999	vehicle	:	None
test substance	:	S-1812 (Pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	Ocular	guideline	:	In accordance with OECD 405 (1981)
species	:	Rabbit, New Zealand White	acceptability	:	Acceptable
group size	:	6 males	**Effect**	:	Not eye irritating

Study design

The study was performed in accordance with OECD 405 (1981).

Results

The results are summarised in tables 3.5.1.1-1 and 3.5.1.1-2.

Table 3.5.1.1-1

Scores observed after	**1 hour**	**24 hours**	**48 hours**	**72 hours**
Cornea/opacity	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0
Iris	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0
Conjunctiva redness	0,1,1,1,1,1	1,1,1,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0
Conjunctiva chemosis	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0
Conjunctiva discharge	0,1*,0,0,0,1*	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0

** clear discharge*

Table 3.5.1.1-2 Mean value irritation scores

Animal	mean 24-72 hrs			
	Corneal opacity	Iris	Conjunctiva redness	Conjunctiva chemosis
1	0	0	0.3	0
2	0	0	0.3	0
3	0	0	0.3	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0

Acceptability

The study is acceptable, although less animals could have been used (n=3).

Conclusions

S-1812 has been found to be not eye irritating in rabbits.

3.5.2 Human data

No data.

3.5.3 Other data

No data.

3.6 Respiratory sensitisation

3.6.1 Animal data

No data.

3.6.2 Human data

No data.

3.6.3 Other data

No data.

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 Study 1

Characteristics

reference	:	IIA 5.2.6/01 Project No 3650	exposure	:	Intradermal and topical induction, topical challenge (occlusive, 24h).
type of study	:	Skin sensitisation study (GPMT)	doses	:	2% intradermal induction 100% topical induction 10% challenge
year of execution	:	2001-2002	vehicle	:	Corn oil (intradermal induction), acetone (topical induction)
test substance	:	S-1812 (Pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	Dermal	guideline	:	In accordance with OECD 406 (1992)
species	:	Guinea-pig, Hartley	acceptability	:	Acceptable
group size	:	10 controls (females) 20 test animals (females)	**Effect**	:	Skin sensitising

Study design

The study was performed in accordance with OECD 406 (1992) and conducted according to Magnusson and Kligman. α-hexylcinnamaldehyde (HCA) was used as the positive control.

Dose levels were based on the results of a range-finding study using 0.1, 0.2, 0.5, 1, 2, and 5% for intradermal injections and 10, 25, 50 and 100% for topical applications. Intradermal injection with 2% induced slight erythema and no or slight oedema. Topical application with 100% induced slight erythema and no or slight oedema.
Intradermal induction was performed with 2% test substance in corn oil. Topical induction was initiated after intradermal induction with treatment of 100% concentration of test substance 7 days after the first induction. Fourteen days later, challenge was performed with dermal application of 10% test substance in acetone.

Results

After intradermal induction with 2% S-1812 and after topical induction with 100% S-1812, no observation of irritation was made. After topical challenge with 10% S-1812, slightly patch erythema in 8/20 and 16/20 females was observed after 24 hours and 48 hours respectively. Moderate erythema was observed in 2 females only after 24 hours. Slight oedema was observed in 5/20 and 4/20 females after 24 and 48 hours respectively. Topical challenge in control animals did not induce any dermal reaction.

Acceptability

The study is considered acceptable.

Conclusions

In this study, S-1812 has been found to be skin sensitising.

3.7.2 Human data

No data.

3.7.3 Other data

No data.

3.8 Germ cell mutagenicity

3.8.1 In vitro data

3.8.1.1 Study 1 – bacterial gene mutaton

Type of study: Ames test, preincubation method, with independent repeat assay

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
B: *S. typh.*	point mut.	-	-	rat liver	Phenobarbital	DRF:all strains:	IIA 5.4.1/01

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
TA 98 TA 100 TA 1535 TA 1537 B : *E.coli* WP2uvrA	point mut. point mut. point mut. point mut. point mut.	- - - - -	- - - - -		and 5,6-benzoflavone	4.88 to 5000 µg/plate Exp. 1 and 2: -S9: 9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate +S9: 39.1, 78.1, 156, 313, 625, 1250 µg/plate Solvent: DMSO Positive controls included	Study no. 3376
Test substance: S 1812 (pyridalyl), 2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy] propyl ether, pale yellow liquid, batch nr. PS-98041G, purity: 93.7% Active ingredient was tested Precipitation observed at dose level: with S9: ≥ 1250 µg/plate, without S9: ≥ 313 µg/plate Toxicity: no GLP statement: yes According to OECD 471: yes							

Study design

S-1812 was evaluated for its mutagenic potential by the reverse mutation test with four strains of *S. typhimurium* (TA100, TA98, TA1535 and TA1537) and one strain of *E. coli* (Wp2uvrA). The test was conducted by the preincubation method in the presence and absence of a rat liver drug-metabolizing enzyme system (S9 mix). The dose levels were based on a dose-range finding study. Precipitation was observed at dose levels of 1250 µg a.s./plate in the presence of S9 and at dose levels of 313 µg a.s./plate in the absence of S9 mix. No cytotoxicity was observed. The positive controls were sodium azide, 9-aminoacridine, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide and 2-aminoanthracene. To test a dose-dependent increase in the number of revertant colonies a linear regression analysis was applied.

Results

S-1812 did not show any dose-dependent increase in the number of revertant colonies with or without S9 mix in any of the strains. Positive control chemicals showed marked increased in the numbers of revertant colonies.

Table 2 Reverse mutation test of S-1812
(Main assay I)

Chemical (μg/plate)	S9mix	Revertant colonies/plate (Mean ± S.D)											
		Base exchange type						Frameshift type					
		TA100		TA1535		WP2*uvr*A		TA98		TA1537			
0	-	105		16		17		24		9			
		111		7		15		19		3			
		104	(107 ± 3.8)	7	(10 ± 5.2)	20	(17 ± 2.5)	16	(20 ± 4.0)	7	(6 ± 3.1)		
9.77	-	111		2		9		21		8			
		133		4		14		17		6			
		146	(130 ± 17.7)	14	(7 ± 6.4)	25	(16 ± 8.2)	9	(16 ± 6.1)	5	(6 ± 1.5)		
19.5	-	125		2		13		19		5			
		112		5		14		20		7			
		108	(115 ± 8.9)	12	(6 ± 5.1)	15	(14 ± 1.0)	14	(18 ± 3.2)	8	(7 ± 1.5)		
39.1	-	110		4		16		24		13			
		101		15		18		19		6			
		129	(113 ± 14.3)	4	(8 ± 6.4)	18	(17 ± 1.2)	19	(21 ± 2.9)	3	(7 ± 5.1)		
78.1	-	116		12		20		21		12			
		100		7		18		21		8			
		122	(113 ± 11.4)	5	(8 ± 3.6)	19	(19 ± 1.0)	20	(21 ± 0.6)	12	(11 ± 2.3)		
156 #	-	109		8		13		16		8			
		115		5		15		24		6			
		124	(116 ± 7.5)	8	(7 ± 1.7)	5	(11 ± 5.3)	17	(19 ± 4.4)	5	(6 ± 1.5)		
313 #	-	133		13		20		31		4			
		122		5		18		14		8			
		110	(122 ± 11.5)	8	(9 ± 4.0)	12	(17 ± 4.2)	12	(19 ± 10.4)	16	(9 ± 6.1)		
PC	-	827		307		159		416		799			
		806		299		122		357		987			
		800	(811 ± 14.2)	311	(306 ± 6.1)	136	(139 ± 18.7)	388	(387 ± 29.5)	853	(880 ± 96.8)		
0	+	108		7		21		40		15			
		99		9		27		37		17			
		111	(106 ± 6.2)	8	(8 ± 1.0)	24	(24 ± 3.0)	38	(38 ± 1.5)	13	(15 ± 2.0)		
39.1	+	104		9		15		36		8			
		125		8		31		33		5			
		126	(118 ± 12.4)	8	(8 ± 0.6)	28	(25 ± 8.5)	30	(33 ± 3.0)	9	(7 ± 2.1)		
78.1	+	114		5		27		19		6			
		115		5		28		25		6			
		136	(122 ± 12.4)	7	(6 ± 1.2)	30	(28 ± 1.5)	29	(24 ± 5.0)	13	(8 ± 4.0)		
156	+	116		11		18		21		17			
		125		13		30		25		8			
		99	(113 ± 13.2)	8	(11 ± 2.5)	24	(24 ± 6.0)	25	(24 ± 2.3)	3	(9 ± 7.1)		
313	+	108		18		17		20		13			
		109		8		27		25		15			
		103	(107 ± 3.2)	8	(11 ± 5.8)	31	(25 ± 7.2)	25	(23 ± 2.9)	7	(12 ± 4.2)		
625	+	115		7		24		26		19			
		135		6		30		24		14			
		99	(116 ± 18.0)	7	(7 ± 0.6)	27	(27 ± 3.0)	41	(30 ± 9.3)	9	(14 ± 5.0)		
1250 #	+	117		11		32		36		17			
		122		10		25		38		5			
		115	(118 ± 3.6)	6	(9 ± 2.6)	27	(28 ± 3.6)	28	(34 ± 5.3)	12	(11 ± 6.0)		
PC	+	666		157		441		295		117			
		706		183		417		283		138			
		648	(673 ± 29.7)	183	(174 ± 15.0)	472	(443 ± 27.6)	290	(289 ± 6.0)	113	(123 ± 13.4)		

Vehicle control : Dimethyl sulfoxide
PC(positive controls) (μg/plate) :

	Without S9mix	With S9mix
TA100	2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (0.01)	2-aminoanthracene (1)
TA1535	Sodium azide (0.5)	2-aminoanthracene (2)
WP2*uvr*A	2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (0.01)	2-aminoanthracene (10)
TA98	2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (0.1)	2-aminoanthracene (0.5)
TA1537	9-aminoacridine (80)	2-aminoanthracene (2)

: Precipitates of test chemical were observed.
All doses of the test chemical were adjusted to take its purity into account.

Table 3 Reverse mutation test of S-1812
(Main assay II)

Chemical (μg/plate)	S9mix	Revertant colonies/plate (Mean ± S.D)											
		Base exchange type						Frameshift type					
		TA100		TA1535		WP2*uvr*A		TA98		TA1537	
0	-	89 113 115	(106 ± 14.5)	7 6 10	(8 ± 2.1)	20 27 27	(25 ± 4.0)	18 17 15	(17 ± 1.5)	6 7 5	(6 ± 1.0)
9.77	-	103 109 99	(104 ± 5.0)	12 5 9	(9 ± 3.5)	17 29 21	(22 ± 6.1)	15 25 21	(20 ± 5.0)	2 10 6	(6 ± 4.0)
19.5	-	125 93 115	(111 ± 16.4)	10 7 11	(9 ± 2.1)	32 20 18	(23 ± 7.6)	9 19 12	(13 ± 5.1)	6 7 9	(7 ± 1.5)
39.1	-	111 112 100	(108 ± 6.7)	6 8 9	(8 ± 1.5)	24 16 27	(22 ± 5.7)	20 29 25	(25 ± 4.5)	7 5 10	(7 ± 2.5)
78.1	-	101 99 100	(100 ± 1.0)	9 15 13	(12 ± 3.1)	25 17 33	(25 ± 8.0)	7 17 17	(14 ± 5.8)	7 9 8	(8 ± 1.0)
156 #	-	121 124 99	(115 ± 13.7)	7 6 7	(7 ± 0.6)	24 26 16	(22 ± 5.3)	17 28 20	(22 ± 5.7)	3 4 14	(7 ± 6.1)
313 #	-	97 103 81	(94 ± 11.4)	8 2 8	(6 ± 3.5)	29 24 31	(28 ± 3.6)	16 16 14	(15 ± 1.2)	6 10 9	(8 ± 2.1)
PC	-	847 812 875	(845 ± 31.6)	295 294 307	(299 ± 7.2)	170 165 145	(160 ± 13.2)	263 298 282	(281 ± 17.5)	645 599 698	(647 ± 49.5)
0	+	101 87 85	(91 ± 8.7)	8 5 9	(7 ± 2.1)	32 38 38	(36 ± 3.5)	24 29 25	(26 ± 2.6)	17 9 15	(14 ± 4.2)
39.1	+	78 90 91	(86 ± 7.2)	6 7 7	(7 ± 0.6)	40 37 32	(36 ± 4.0)	30 27 40	(32 ± 6.8)	12 9 13	(11 ± 2.1)
78.1	+	115 93 97	(102 ± 11.7)	9 6 9	(8 ± 1.7)	33 36 36	(35 ± 1.7)	28 38 30	(32 ± 5.3)	14 15 14	(14 ± 0.6)
156	+	92 104 89	(95 ± 7.9)	8 4 12	(8 ± 4.0)	25 26 28	(26 ± 1.5)	16 36 29	(27 ± 10.1)	15 12 4	(10 ± 5.7)
313	+	103 117 102	(107 ± 8.4)	13 5 9	(9 ± 4.0)	43 37 37	(39 ± 3.5)	29 16 26	(24 ± 6.8)	7 13 7	(9 ± 3.5)
625	+	90 109 96	(98 ± 9.7)	13 4 6	(8 ± 4.7)	32 27 32	(30 ± 2.9)	25 21 30	(25 ± 4.5)	5 9 12	(9 ± 3.5)
1250 #	+	113 101 114	(109 ± 7.2)	6 7 6	(6 ± 0.6)	24 36 28	(29 ± 6.1)	41 36 25	(34 ± 8.2)	13 15 6	(11 ± 4.7)
PC	+	696 839 744	(760 ± 72.8)	175 221 205	(200 ± 23.4)	509 471 449	(476 ± 30.4)	286 307 261	(285 ± 23.0)	189 172 158	(173 ± 15.5)

Vehicle control : Dimethyl sulfoxide
PC(positive controls) (μg/plate) :

	Without S9mix	With S9mix
TA100	2-(furyl)-3-(5-nitro-2-furyl)acrylamide(0.01)	2-aminoanthracene (1)
TA1535	Sodium azide (0.5)	2-aminoanthracene (2)
WP2*uvr*A	2-(furyl)-3-(5-nitro-2-furyl)acrylamide(0.01)	2-aminoanthracene (10)
TA98	2-(furyl)-3-(5-nitro-2-furyl)acrylamide(0.1)	2-aminoanthracene (0.5)
TA1537	9-aminoacridine(80)	2-aminoanthracene (2)

: Precipitates of test chemical were observed.
All doses of the test chemical were adjusted to take its purity into account.

Acceptability

All acceptability criteria are fulfilled.

Conclusions

S-1812 (pyridalyl) is not mutagenic in the Ames test.

3.8.1.2 Study 2- chromosome aberration

Type of study: mammalian cells *in vitro*, cytogenetic assay, with independent repeat assay

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster lung (CHL/IU) cells	chromosome aberration	-	+	rat liver	Phenobarbital and 5,6-benzoflavone	DRF 1, - and +S9: 19.5 to 5000 µg/ml DRF 2, +S9: 1.25 to 40 µg/ml Exp 1: -S9 (treatment 6 hr and recovery 18 hr): 20, 40, 80 µg/ml +S9 (treatment 6 hr and recovery 18 hr): 15, 20, 25 µg/ml Exp 2: -S9 (treatment and harvest 24 hr): 625, 938, 1250 µg/ml -S9 (treatment and harvest 48 hr): 39.1, 78.1, 156 µg/ml Exp 3: +S9 (treatment 6 hr and recovery 18 hr): 15, 20, 25 µg/ml Solvent: DMSO Positive Controls included	IIA 5.4.3/02 Project No. 6311-215

Test substance: S 1812 (pyridalyl) Technical, Batch nr. PS-98041G, viscous transparent light yellow liquid, purity: 93.7%

Cytotoxicity observed at dose level: Without S9-mix, treatment 6 hr and recovery 18 hr: no
With S9-mix, treatment 6 hr and recovery 18 hr: ≥ 20 µg/ml
Without S9-mix, treatment and harvest 24 hr: ≥938 µg/ml
Without S9-mix, treatment and harvest 48 hr: ≥78.1 µg/ml

Precipitation observed at dose level: ≥ 78.1 µg/ml
GLP statement: yes
According to OECD 473: yes

Study design:

An *in vitro* chromosomal aberration test of pyridalyl was performed using Chinese hamster lung (CHL/IU) cells. The dose levels were based on a preliminary study. In the preliminary study exposure of CHL cells (-S9) to pyridalyl for 24 or 48 hours but not 6 hours caused moderate cytotoxicity which may have been dose-dependent. However, dose-dependency is difficult to judge as evidence of precipitation was seen in 7 of 9 doses. The positive controls in the study were mitomycin C (-S9) and cylcophosmphamide (+S9).

In the presence of S9, the first test with concentrations of 19.5 – 5000 µg/mL was too toxic to evaluate the 50% growth inhibition concentration. The additional test revealed that the 50% growth inhibition concentration was approximately 20 µg/mL.

Results:

In the short-term assay in the absence of S9, no increase in the incidence of chromosomally aberrant cells was observed. In the presence of S9, marginal increases in structural aberrations and polyploidy were observed. No increase in incidence of chromosomal aberration was seen in the 24 – and 48-hour continuous treatments in the absence of S9. A confirmatory 6-hour assay in the presence of S9 found marginal increases in structural aberrations (maximal induction was to 9.5%).

Group	Dose	Rel growth	N	Structural Aberrations								Polypl
				No. of Aberrations							Cells	
	(µg/mL)	(%)		gap	ctb	cte	csb	cse	mul	Tot	(%)	(%)
6-Hour Exposure, without S9												
Control	0	100	200	2	5	1	0	0	0	6	3.0	0.0
pyridalyl	20	102.9	200	1	1	0	0	0	0	1	0.5	1.0
	40	104.4	200	2	2	1	0	0	0	3	1.5	0.0
	80	103.0	200	3	3	1	0	0	0	4	1.5	0.5
MMC	0.06	101.1	200	13	50	61	0	1	0	112	37.0+	0.0
6-Hour Exposure, with S9												
Control	0	100	200	3	3	1	0	0	0	4	2.0	1.0
pyridalyl	15	79.1	200	1	5	6	0	1	0	12	5.0±	0.5
	20	51.0	200	4	11	11	0	0	1	32	7.5±	4.5
	25	38.4	200	2	12	1	0	1	1	24	6.0±	6.5±
CP	10	70.9	200	7	73	85	0	0	0	158	50.5+	0.0
24-Hour Exposure, without S9												
Control	0	100	200	1	4	0	0	0	0	4	2.0	0.5
pyridalyl	625p	71.1	200	2	4	0	0	0	0	4	1.5	0.0
	938p	63.5	200	1	3	1	0	0	0	4	2.0	0.5
	1250p	44.5	200	3	6	2	0	0	0	8	3.5	0.0
MMC	0.02	86.3	200	8	46	27	0	0	0	73	27.5+	0.5
48-Hour Exposure, without S9												
Control	0	100	200	1	2	0	0	0	0	2	1.0	0.5
pyridalyl	39.1	82.0	200	1	3	0	0	0	0	3	1.5	1.0
	78.1	59.9	200	0	3	0	0	0	0	3	1.5	0.0
	156p	45.8	200	1	3	0	0	0	0	3	1.5	0.0
MMC	0.02	96.4	200	7	59	46	0	1	1	116	36.0+	0.0
6-Hour Exposure, with S9 (Confirmatory Assay)												
Control	0	100	200	2	4	1	0	0	0	5	2.0	0.0
pyridalyl	15	82.3	200	8	9	6	0	1	0	16	5.0±	3.0
	20	51.6	200	6	14	13	0	0	1	37	8.0±	1.0
	25	38.0	200	10	7	15	0	0	2	42	9.5±	4.5
CP	10	66.3	200	14	103	197	0	0	1	310	71.0+	0.0

+ Positive, ± Marginal as determined by criteria of Ishidate, p Precipitation observed at the end of treatment

Rel. growth: % of controls (cytotoxicity);
ctb: chromatid break;
csb: chromosome break;
Tot: total aberrations excluding gaps;
cte: chromatid exchange;
cse: chromosome exchange;
mul: multiple aberrations (scored as 10 aberrations);
Polypl: polyploidy

Acceptability

All acceptability criteria are fulfilled.

Conclusions

S-1812 has a weak potential to induce chromosomal aberrations against CHL/IU cells.

3.8.1.3 Study 3 – mammalian gene mutation

Type of study: mammalian cells *in vitro*, gene mutation test, with independent repeat assay

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Mouse lymphoma (L5178Y/ TK+/-)) cells	gene mutations (TK)	-	-	rat liver	Phenobarbital and 5,6-benzoflavone	DRF: 0.15 to 150 µg/ml Exp 1: (-S9) 3.13, 6.25, 12.5, 25, 50 and 100 µg/ml (+S9) 2.5, 5, 7.5, 10, 12.5 and 15 µg/ml Exp 2: (-S9) 3.13, 6.25, 12.5, 25, 37.5 and 50 µg/ml (+S9) 2.5, 5, 7.5, 10 and 12.5 µg/ml Solvent: DMSO Positive controls included	IIA 5.4.3/01 Study No. M0991
Test substance: S 1812 (pyridalyl), Batch nr. TH95031601-HI, purity: 98.6% Cytotoxicity observed at dose level: 6.25 µg/ml and above (-S9) and 7.5 µg/ml and above (+S9) Precipitation observed at dose level: 150 µg/ml GLP statement: no According to OECD 476: yes							

Study design

Pyridalyl was evaluated for its mutagenic potential in an *in vitro* mammalian cell gene mutation test using L5178Y/TK$^{+/-}$ mouse lymphoma cells. The test was conducted in the presence and absence of a drug-metabolizing enzyme system (S9 mix). The cytotoxicity test was conducted at doses ranging from 0.15 to 150 µg/mL in the presence and absence of S9 mix. Dose-dependent cytotoxicity was seen.

The main assay was conducted at doses ranging from 3.13 to 100 µg/mL in the absence of S9 mix and from 2.50 to 15.0 µg/mL in the presence of S9 mix. Positive controls were methyl methanesulphonate (without S9) and 20-methylcholanthrene (with S9).

Results

In the absence of S9 mix, the mutant frequencies increased more than twice over the solvent control only at highly toxic doses with less than 10% relative total growth (RTG) which were thus excluded from evaluation. In the presence of S9 mix, the mutant frequencies up to 10.0 µg/mL were less than 2 times the solvent control. Doses higher than 10.0 µg/mL could not be assessed due to poor growth during expression.

Based on these results a second main assay was performed at slightly lower doses: 3.13 to 50 µg/mL in the absence of S9 mix and from 2.50 to 12.5 µg/mL in the presence of S9 mix. In the absence of S9, mutant frequencies never exceeded double the solvent control. In the presence of S9, mutant frequency exceeded twice the solvent control at 10.0 µg/mL, but this dose was excluded due to excessive toxicity (RTG < 10%). Pyridalyl did not cause an increase in forward-mutations equal to or greater than twice the solvent control at any dose that did not also cause excessive toxicity. No reproducible statistically significant dose-dependent increases in mutant frequencies were observed with or without S9 mix. Positive control chemicals showed marked increases in the mutant frequency under the same test conditions. Based on these results, it is concluded that pyridalyl is not mutagenic under the test conditions.

Acceptability

According to the guidelines at least 4 dose levels in duplo or 8 dose levels should be tested. In this study only 4 to 6 dose levels were tested, although since sufficient cytotoxicity was observed. The study was not conducted under GLP. There is no description of the stability of the test material during storage or in the solvent used. Overall the study is considered supplementary. However, the results of the study are in concordance with the second mammalian gene mutation study (see 3.8.1.4).

Conclusions

S-1812 is not mutagenic in L5178Y cells *in vitro*, either in the absence or presence of S9-mix.

3.8.1.4 Study 4 – Mammalian gene mutation

Type of study: mammalian cells *in vitro*, gene mutation test, with independent repeat assay

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster ovary (CHO) cells	Gene mutations (HGPRT)	-	-	rat liver	Arochlor 1254	DRF 1 (- and +S9): 9.85 to 5000 µg/ml DRF 2 -S9: 0.59 to 300 µg/ml +S9: 0.074 to 37.5 µg/ml Exp 1: (-S9) 9.4, 18.8, 37.5, 75, 150 and 300 µg/ml (+S9) 2, 4, 5, 6, 7 and 8 µg/ml Exp 2: (-S9) 9.4, 18.8, 37.5, 75, 150 and 300 µg/ml (+S9) 2, 4, 5, 6, 7, 8 and 10 µg/ml Solvent: DMSO Positive controls included	IIA 5.4.3/02 Project No. 6311-215
Test substance: S 1812 (pyridalyl) Technical, Batch nr. PS-98041G, viscous transparent light yellow liquid, purity: 93.7% Cytotoxicity observed in dose range finding test at dose level: no dose up to 5000 µg/ml (-S9) and 4.7 µg/ml (+S9) and above Cytotoxicity observed in mutation tests at dose level: Without S9-mix: no With S9-mix: 5 µg/ml and above Precipitation observed at dose level: 157 µg/ml and above GLP statement: yes According to OECD 476: yes							

Study design:

The mutation test at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in V79 Chinese hamster lung cells was undertaken to investigate the mutagenic activity of pyridalyl. Cytotoxicity assays were performed to determine concentrations for the mutation assay. Cell growth was not inhibited by treatment with pyridalyl up to 5000 µg/mL in the absence of metabolic activation (S9). In the presence of S9, cytotoxicity was tested from 0.0740 µg/mL to 37.5 µg/mL and found to be highly cytotoxic above 4.70 µg/mL.

Mutagenicity assays were conducted in duplicate and confirmatory assays were conducted with the same doses and protocols. In the absence of S9, mutagenicity was tested up to 300 µg/mL (twice the solubility limit).

Results:

Neither main assay nor confirmatory assay met criteria for a positive response. In the presence of S9, mutagenicity was tested up to 8.00 µg/mL (10.0 µg/mL in confirmatory assay). Neither main assay nor confirmatory assay met criteria for a positive response.

In the absence of metabolic activation, one replicate at 18.8 µg/mL was elevated ($P \leq 0.01$ or 0.05) in both main and confirmatory assay, but neither induced a mutant frequency that exceeded 15×10^{-6}. In addition, there was no dose dependence since higher more cytotoxic concentrations were not significantly elevated.

In the presence of metabolic activation, sporadic, significant increases in the mutant frequency were observed but none of the mutant frequencies exceeded 15 x 10^{-6} and no dose response was observed. In the confirmatory assay, none of the analyzed treatments induced a mutant frequency that was significantly elevated and none exceeded a mutant frequency of 15 x 10^{-6}.

These results suggest pyridalyl has no mutagenic activity under these test conditions.

MUTATION ASSAY WITHOUT METABOLIC ACTIVATION - TRIAL 1

SAMPLE NAME: S-1812 Technical SPONSOR: Sumitomo Chemical Co., Ltd. GT Study No. 19996-0-435 OECD ASSAY NO. 6311-215
TEST DATE: April 16, 1999 VEHICLE: DMSO
Selective Agent: 4 µg/ml 6-thioguanine Cells seeded for analysis: 200/dish for C.E.
Expression Time: 7 days 2x10^5/dish for mutants

NONACTIVATION TEST CONDITION	SURVIVAL TO TREATMENT		RELATIVE POPULATION GROWTH (% OF VC)	MUTANT COLONIES DISH NUMBER												TOTAL MUTANT COLONIES	ABSOLUTE C.E. ± S.D. (%)	MUTANT FREQUENCY IN 10^{-6} UNITS [a]
	MEAN COLONY NUMBERS ± S.D.	% VEHICLE CONTROL		1	2	3	4	5	6	7	8	9	10	11	12			
Vehicle Control	253.7 ± 14.4	113.6	83.6	0	0	0	0	0	1	0	0	1	1	1	1	5	96.5 ± 2.2	2.2
Vehicle Control	193.0 ± 13.0	86.4	115.5	0	1	0	0	0	0	0	1	2	1	0	0	5	86.5 ± 1.0	2.4
Positive Control (50 µg/ml BrdU) [b]	130.3 ± 4.0	58.4	130.6	25	24	19	22	18	19	16	19	24	18	22	22	248	96.8 ± 8.0	106.7**
Test Article (µg/ml)																		
9.40	176.7 ± 7.0	79.1	207.4	1	0	0	0	0	0	0	0	1	0	0	0	2	93.7 ± 7.3	0.9
9.40	163.0 ± 15.7	73.0	298.2	1	0	0	0	0	0	0	0	0	0	0	0	1	103.0 ± 2.6	0.4
18.8	148.3 ± 5.0	66.4	341.7	4	2	2	3	0	2	4	1	1	2	0	1	22	118.3 ± 2.0	7.7*
18.8	182.7 ± 15.0	81.8	458.1	0	0	1	0	1	1	1	2	0	0	1	1	8	102.8 ± 10.1	3.2
37.5	140.0 ± 11.0	62.7	363.6	0	1	0	3	1	0	1	0	0	1	0	2	9	105.5 ± 1.3	3.6
37.5	159.3 ± 2.5	71.3	260.0	1	0	0	1	0	0	0	0	0	0	0	0	2	101.3 ± 8.3	0.8
75.0	195.0 ± 13.0	87.3	117.7	0	0	0	0	0	0	0	1	1	0	0	0	2	119.2 ± 8.3	0.7
75.0	145.7 ± 15.3	65.2	132.2	1	2	2	0	0	1	0	1	4	0	2	0	13	92.7 ± 7.1	5.8
150	158.3 ± 20.0	70.9	104.7	0	0	1	0	2	1	0	0	0	0	1	1	6	99.7 ± 3.1	2.5
150	153.3 ± 7.1	68.7	87.1	1	2	0	0	0	1	2	1	2	0	1	0	10	98.8 ± 5.8	4.2
300+	151.3 ± 3.1	67.8	121.2	0	2	0	0	0	1	1	2	1	0	1	2	10	87.7 ± 7.0	4.8
300+	151.7 ± 20.3	67.9	186.0	1	1	0	0	1	1	1	0	1	0	1	1	8	104.2 ± 1.8	3.2

[a] Mutant Frequency = Total mutant colonies/(No. of dishes x 2 x 10^5 x absolute C.E.).
[b] BrdU = 5-Bromo-2'-deoxyuridine.
* Significant increase: Kastenbaum Bowman test $p \leq 0.01$ but mutant frequency < 15 x 10^{-6}.
** Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq$ 15 x 10^{-6}.
+Precipitate observed

MUTATION ASSAY WITHOUT METABOLIC ACTIVATION - TRIAL 2

SAMPLE NAME: S-1812 Technical SPONSOR: Sumitomo Chemical Co., Ltd. GT Study No. 19996-0-435 OECD ASSAY NO. 6311-215
TEST DATE: May 14, 1999 VEHICLE: DMSO
Selective Agent: 4 µg/ml 6-thioguanine Cells seeded for analysis: 200/dish for C.E., $2x10^5$/dish for mutants Expression Time: 7 days

NONACTIVATION TEST CONDITION	SURVIVAL TO TREATMENT		RELATIVE POPULATION GROWTH (% OF VC)	MUTANT COLONIES DISH NUMBER												TOTAL MUTANT COLONIES	ABSOLUTE C.E. ± S.D. (%)	MUTANT FREQUENCY IN 10^{-6} UNITS [a]
	MEAN COLONY NUMBERS ± S.D.	% VEHICLE CONTROL		1	2	3	4	5	6	7	8	9	10	11	12			
Vehicle Control	233.7 ± 25.9	119.8	95.2	0	0	0	1	0	1	0	1	0	1	0	0	4	110.0 ± 12.7	1.5
Vehicle Control	156.3 ± 6.8	80.2	104.8	0	2	0	1	1	0	0	1	0	1	1	0	7	102.3 ± 3.2	2.9
Positive Control (50 µg/ml BrdU) [b]	184.3 ± 16.2	94.5	61.3	10	17	17	14	32	20	18	23	24	18	21	14	228	125.3 ± 5.0	75.8**
Test Article (µg/ml)																		
9.40	182.0 ± 11.1	93.3	87.6	0	1	0	0	0	0	0	0	0	2	1	1	5	89.8 ± 9.6	2.3
9.40	234.0 ± 9.5	120.0	92.1	1	0	0	0	2	0	0	0	0	0	1	0	4	84.8 ± 5.1	2.0
18.8	161.0 ± 12.3	82.6	86.5	2	0	0	2	1	1	0	3	0	2	1	1	13	85.0 ± 2.8	6.4*
18.8	171.7 ± 6.0	88.0	79.1	0	0	1	0	1	2	0	1	0	1	2	0	8	97.2 ± 3.6	3.4
37.5	234.3 ± 15.0	120.2	60.9	0	1	2	1	0	0	0	0	1	0	0	0	5	104.0 ± 14.1	2.0
37.5	197.7 ± 12.5	101.4	54.5	0	0	0	1	0	0	0	0	0	0	0	0	1	95.2 ± 8.8	0.4
75.0	208.7 ± 7.5	107.0	89.1	1	0	1	2	0	0	1	0	0	0	3	0	8	104.5 ± 4.8	3.2
75.0	173.0 ± 12.8	88.7	53.2	1	2	2	1	0	2	0	1	0	1	1	1	12	101.5 ± 5.5	4.9
150	181.7 ± 7.1	93.2	62.9	3	0	0	1	1	1	1	0	0	0	0	2	9	109.3 ± 2.8	3.4
150	180.7 ± 9.9	92.6	61.8	0	0	0	0	0	0	0	0	0	1	0	0	1	118.2 ± 5.5	0.4
300+	152.3 ± 26.7	78.1	96.0	1	0	0	1	1	0	0	3	1	3	2	1	13	130.8 ± 4.6	4.1
300+	159.7 ± 11.0	81.9	68.0	0	0	0	1	1	1	2	0	1	0	1	0	7	101.8 ± 3.0	2.9

[a] Mutant Frequency = Total mutant colonies/(No. of dishes x 2 x 10^5 x absolute C.E.).
[b] BrdU = 5-Bromo-2'-deoxyuridine.
* Significant increase: Kastenbaum Bowman test $p \leq 0.05$ but mutant frequency $< 15 \times 10^{-6}$.
** Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10^{-6}$.
+Precipitate observed

MUTATION ASSAY WITH METABOLIC ACTIVATION - TRIAL 1

SAMPLE NAME: S-1812 Technical SPONSOR: Sumitomo Chemical Co., Ltd. GT Study No. 19996-0-435 OECD ASSAY NO. 6311-215
TEST DATE: April 28, 1999 VEHICLE: DMSO
Selective Agent: 4 µg/ml 6-thioguanine Cells seeded for analysis: 200/dish for C.E., $2x10^5$/dish for mutants Expression Time: 7 days

NONACTIVATION TEST CONDITION	SURVIVAL TO TREATMENT		RELATIVE POPULATION GROWTH (% OF VC)	MUTANT COLONIES DISH NUMBER												TOTAL MUTANT COLONIES	ABSOLUTE C.E ± S.D. (%)	MUTANT FREQUENCY IN 10^{-6} UNITS
	MEAN COLONY NUMBERS ± S.D.	% VEHICLE CONTROL		1	2	3	4	5	6	7	8	9	10	11	12			
Vehicle Control	72.0 ± 3.6	90.2	94.0	0	0	0	0	1	0	0	0	1	0	0	1	3	98.0 ± 2.8	1.3
Vehicle Control	87.7 ± 6.1	109.8	106.0	1	0	0	0	0	0	0	0	0	0	0	0	1	119.7 ± 6.0	0.3
Positive Control (5 µg/ml MCA) [b]	57.0 ± 2.0	71.4	84.8	33	32	40	28	36	32	27	32	33	28	34	31	386	96.0 ± 5.0	167.5**
Test Article (µg/ml)																		
2.00	0.0 ± 0.0	0.0	75.1	2	1	1	1	2	0	0	1	2	2	1	3	16	123.8 ± 1.6	5.4‡
2.00	0.0 ± 0.0	0.0	82.3	0	0	0	0	0	1	0	0	0	0	0	0	1	98.7 ± 0.3	0.4
4.00	0.0 ± 0.0	0.0	32.6	1	1	0	2	3	1	2	0	1	0	0	1	12	99.2 ± 8.5	5.0‡
4.00	0.0 ± 0.0	0.0	36.0	0	1	0	1	1	0	0	0	1	2	1	2	9	96.5 ± 13.5	3.9*
5.00	70.0 ± 3.0	87.7	44.1	1	0	0	0	0	1	0	0	0	2	0	0	4	125.5 ± 9.6	1.3
5.00	20.0 ± 2.0	25.1	63.1	0	0	1	0	0	0	0	2	0	0	0	0	3	109.2 ± 7.8	1.1
6.00	17.7 ± 2.1	22.1	30.1	1	1	1	0	0	0	0	1	0	1	0	0	5	97.7 ± 5.2	2.1
6.00	24.3 ± 3.1	30.5	26.0	0	2	0	0	0	1	0	0	1	0	0	0	4	98.5 ± 3.1	1.7
7.00	10.0 ± 4.4	12.5	18.2	1	0	0	0	1	0	2	0	0	0	0	0	4	105.7 ± 6.4	1.6
7.00	14.3 ± 0.6	18.0	12.4	0	0	0	0	0	0	0	0	0	0	0	0	0	92.5 ± 6.6	0.0
8.00	5.7 ± 0.6	7.1	10.8	0	0	0	1	0	0	0	1	0	1	1	0	4	101.7 ± 7.8	1.6
8.00	6.7 ± 2.1	8.4	9.1	1	0	1	1	2	1	0	2	1	2	2	1	14	98.5 ± 11.3	5.9‡

[a] Mutant Frequency = Total mutant colonies/(No. of dishes x 2 x 10^5 x absolute C.E.).
[b] MCA = methylcholanthrene
* Significant increase: Kastenbaum Bowman test $p \leq 0.05$ but mutant frequency $< 15 \times 10^{-6}$.
** Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10^{-6}$.
‡Significant increase: Kastenbaum Bowman test $p \leq 0.01$ but mutant frequency $< 15 \times 10^{-6}$.

TABLE 8
MUTATION ASSAY WITH METABOLIC ACTIVATION - TRIAL 2

SAMPLE NAME: S-1812 Technical SPONSOR: Sumitomo Chemical Co., Ltd. GT Study No. 19996-0-435 OECD ASSAY NO. 6311-215
TEST DATE: May 14, 1999 VEHICLE: DMSO
Selective Agent: 4 µg/ml 6-thioguanine Cells seeded for analysis: 200/dish for C.E., $2x10^5$/dish for mutants Expression Time: 7 days

NONACTIVATION TEST CONDITION	SURVIVAL TO TREATMENT		RELATIVE POPULATION GROWTH (% OF VC)	MUTANT COLONIES DISH NUMBER												TOTAL MUTANT COLONIES	ABSOLUTE C.E. ± S.D. (%)	MUTANT FREQUENCY IN 10^6 UNITS [a]
	MEAN COLONY NUMBERS ± S.D.	% VEHICLE CONTROL		1	2	3	4	5	6	7	8	9	10	11	12			
Vehicle Control	244.0 ± 11.5	110.5	87.6	1	1	1	2	1	1	0	2	0	1	3	1	14	116.2 ± 7.1	5.0
Vehicle Control	197.7 ± 15.0	89.5	106.4	1	1	1	0	2	0	1	1	0	0	1	1	9	94.3 ± 5.7	4.0
Positive Control [b] (5 µg/ml MCA) [b]	231.7 ± 37.2	104.9	105.2	26	23	20	32	20	23	30	25	18	21	28	18	284	94.7 ± 5.3	125.0**
Test Article (µg/ml)																		
2.00	160.7 ± 3.8	72.8	108.5	0	1	0	0	0	1	0	1	0	1	0	0	4	99.7 ± 6.2	1.7
2.00	164.3 ± 6.7	74.4	140.5	1	0	0	0	0	0	0	0	0	0	0	0	1	87.7 ± 2.6	0.5
4.00	125.0 ± 7.9	56.6	104.9	0	0	0	1	0	0	1	1	1	0	0	0	4	81.0 ± 5.8	2.1
4.00	139.7 ± 7.1	63.2	119.0	2	1	1	3	2	0	1	0	0	0	2	1	13	78.7 ± 6.1	6.9
5.00	92.3 ± 7.6	41.8	107.7	0	0	0	0	0	1	0	0	0	1	0	0	2	79.7 ± 0.6	1.0
5.00	115.3 ± 12.7	52.2	69.3	0	0	1	0	0	1	0	0	0	0	0	0	2	73.5 ± 11.1	1.1
6.00	122.3 ± 7.8	55.4	76.7	1	2	2	2	2	1	1	4	0	2	0	1	18	94.0 ± 8.3	8.0
6.00	98.3 ± 3.5	44.5	87.4	1	0	1	0	0	1	2	1	0	2	0	2	10	97.8 ± 9.1	4.3
7.00	107.7 ± 4.0	48.8	87.3	0	0	0	0	0	0	0	0	0	0	1	1	2	93.3 ± 5.2	0.9
7.00	61.0 ± 1.0	27.6	22.4	0	1	1	1	2	0	2	1	0	0	0	1	9	78.3 ± 6.3	4.8
8.00	38.7 ± 5.0	17.5	16.6	0	2	1	0	0	0	0	1	1	2	1	0	8	81.5 ± 3.9	4.1
8.00	38.3 ± 1.2	17.4	10.3	3	0	2	1	0	0	0	0	2	0	1	1	10	93.5 ± 7.2	4.5
10.0	3.7 ± 1.2	1.7	1.4	1	0	0	0	1	0	0	0	0	1	0	0	3	91.8 ± 10.4	1.4
10.0	4.0 ± 3.0	1.8	1.0	2	0	0	1	0	0	0	0	1	0	0	1	5	85.3 ± 5.8	2.4

[a] Mutant Frequency = Total mutant colonies/(No. of dishes x 2 x 10^5 x absolute C.E.).
[b] MCA = methylcholanthrene
**Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10^{-6}$.

Acceptability

All acceptability criteria are fulfilled.

Conclusions

S-1812 is not mutagenic in CHO cells *in vitro*, either in the absence or presence of S9-mix.

3.8.2 Animal data

3.8.2.1 Study 1 – in vivo micronucleus test

Type of study: mouse micronucleus test

Indicator cells	Endpoint	Result	Dose range	Reference
mouse, Crj:CD-1 (ICR) 24 hour treatment: 5/male/dose (vehicle and positive control, low, mid and high dose), 48 hour treatment: 5/male/dose (vehicle control and high dose)	micronuclei (bone marrow)	-	DRF(male and female) en main (male): 500, 1000 and 2000 mg/kg Route: Oral Solvent: Corn oil Positive controls included	IIA 5.4.4/01 Study No. 3421

Indicator cells	Endpoint	Result	Dose range	Reference
Test substance: S 1812 (pyridalyl), (2-[3-[2,6-dichloro-4-(3,3-dichloroprop-2-enyloxy)phenoxy]propoxy]-5-(trifluoromethyl)pyridine, batch nr. PS-98041G, purity: 93.7% Cytotoxicity observed in dose range finding test at dose level: 1000 and 2000 mg/kg Cytotoxicity observed in main test at dose level: 2000 mg/kg GLP statement: yes According to OECD 474: yes				

Study design

The bone marrow micronucleus test was performed with pyridalyl in CD-1 male mice. Dose levels of 500, 1000, and 2000 mg/kg bw (based on a preliminary range finding test) were administered to 5 male mice/group via gavage. Bone marrow smears were prepared 24 (all groups) and 48 (top dose only) hours after administration. The positive control was cyclophosphamide.

Results

In confirmation of the initial toxicity test, no mice died as a result of administration and only treatment-related clinical sign was soft stool at 2000 mg/kg bw within one day of treatment.

There was no dose-related increase in micronuclei as a result of treatment with pyridalyl. The positive control, cyclophosphamide, produced an appropriate response to validate the sensitivity of the model.

There was no decrease in the PCE/NCE ratio with pyridalyl or with the positive control.

Treatment	Dose (mg/kg)	Sampling time (hr)	Micronucleated PCE (%, mean ± SD)	PCE ratio (%, mean ± SD)
Control	0	24	0.14 ± 0.07	52.2 ± 8.8
pyridalyl	500		0.19 ± 0.14	51.6 ± 7.2
	1000		0.14 ± 0.12	53.7 ± 4.4
	2000		0.19 ± 0.09	52.7 ± 3.3
Cyclophosphamide	10		3.76 ± 0.93**	54.6 ± 7.6
Control	0	48	0.18 ± 0.08	49.0 ± 4.3
pyridalyl	2000		0.12 ± 0.08	51.0 ± 2.6

Micronuclei – 2000 polychromatic erythrocytes were examined from each animal
Ratio = PCE/(PCE+NCE), 1000 erythrocytes were examined from each animal

**p ≤ 0.05, **p ≤ 0.01. PCE= polychromatic erythrocytes; NCE= normochromatic erythrocytes*

Acceptability

All acceptability criteria are fulfilled. Although the study itself did not show if the bone marrow was reached results from the ADME studies indicate that pyridalyl reaches the bone marrow.

Conclusions

S-1812 has no potential to induce micronuclei in mouse bone marrow cells.

3.8.2.2 Study 2 – UDS

Type of study: UDS in mammalian cells *in vivo*

Indicator cells	Endpoint	Result	Dose range	Reference
Sprague Dawley rats(Crl: CD (SD)IGS BR) 4/male/dose (vehicle and positive control, low, mid and high dose),	UDS (Rat liver hepatocytes)	-	DRF: 200, 500, 800, 1500 and 2000 mg/kg Main: (harvest times 2 to 4 and 15 to 16 hours): 500, 1000 and 2000 mg/kg Solvent: Corn oil Positive controls included	IIA 5.4.5/01 Study No. 6311-214
Test substance: S 1812 (pyridalyl) Technical, Batch nr. PS-98041G, viscous transparent light yellow liquid, purity: 93.7% Cytotoxicity observed at dose level: none GLP statement: yes According to OECD 486: yes				

Study design

The unscheduled DNA synthesis (UDS) test was performed with pyridalyl in Sprague Dawley male rats. Dose levels of 500, 1000, and 2000 mg/kg bw (based on a preliminary range finding test) were administered to 4 male rats/group via gavage. The hepatocytes collected 2 to 4 and 15 to 16 hours after administration were cultured with labelled thymidine for 4 hours to assess UDS.

Results

No significant increases in net nuclear grain count or average percent of cells containing five or more net nuclear grains was seen at the 2- to 4-hour time point. At the 15- to 16-hour timepoint, the average percent of cells containing five or more net nuclear grains was slightly elevated in the 1000 mg/kg bw group. However, there were no other indications of increased UDS and the top dose group was not elevated, thus this response was judged to be irrelevant.

Acceptability

All acceptability criteria are fulfilled.

Conclusions

S-1812 is evaluated as negative in the *in vivo* unscheduled DNA synthesis in rat primary hepatocyte cultures at two time points.

3.8.3 Human data

No data.

3.8.4 Other data

No data.

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 Study 1 – 2 year carcinogenicity rat

Characteristics

reference	:	IIA 5.5.2/01 Study no: IET 99-0011	exposure	:	Main: 104 weeks, diet Satellite: 52 weeks, diet
type of study	:	104-week combined toxicity/carcinogenicity study	doses	:	0, 30, 100, 500 and 1000 mg/kg food[1]
year of execution	:	1999-2001	vehicle	:	None
test substance	:	S-1812 (pyridalyl), lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	Oral	guideline	:	OECD 453
species	:	Sprague-Dawley rats, Crj:CD (SD)	acceptability	:	acceptable
group size	:	Main: 50/sex/dose Satellite: 20/sex/dose	**NOAEL**	:	3.4 mg/kg bw/day in males, 4.1 mg/kg bw/day in females

[1] Equal to 0, 1.01-1.15, 3.40-3.87, 17.1-19.5 and 34.3-39.4 mg/kg bw/day in males and 0, 1.23-1.46, 4.10-4.81, 21.1-24.7 and 42.8-49.1 mg/kg bw/day in females.

Study design

The study was performed in accordance with OECD guideline 453. Dose levels were based on a 13-week oral toxicity study in rats (IIA 5.3.2/02). In this study a NOAEL of 100 mg/kg food was established, based on decreased body weight gain, decreased food consumption, increased liver weights and histopathological effects found in the liver at 1000 mg/kg food.

In the main study 50 animals/sex/dose were given S-1812 for 104 weeks. In the satellite study, 20 animals/sex/dose were given S-1812 for 52 weeks. Mortality, clinical signs, body weight, food consumption were monitored in all animals during the treatment period. Ten animals/sex/dose were subjected to functional observations at 12 months. Urinalysis, haematological and clinical biochemistry examinations were performed at 3 months, 6 months and 12 months in 10 animals/sex/dose of the satellite group and at 18 and 24 months in 10 animals/sex/dose of the main group. Ophthalmological examinations were performed on all animals in the control and high dose groups at 12 months (satellite group) and 24 months (main group). Organ weights were determined on 10 animals/sex/dose after 12 and 24 months. All animals were subjected to necropsy, and their tissues were examined histopathologically.

Results

The results are summarised in table 3.9.1.1-1 and -2.

Table 3.9.1.1-1

	Males					Females				
Diet concentration	**0**	**30**	**100**	**500**	**1000**	**0**	**30**	**100**	**500**	**1000**
Mortality (main groups)	22/50	21/50	20/50	13/50	25/50	33/50	31/50	23/50	29/50	22/50
Clinical observations										
Auricle; thickened area	1	1	0	0	0	0	1	1	2	6*
Motor activity, initial 10 minutes, week 49 (% control)	100	95	111	108	120*	100	111	103	117	124**
Weight gain, weeks 0 – 16 (g) Main group	380	390	376	343**	343**	165	165	162	146**	148**
Food consumption, week 5 (g/rat/day) Main group	22.6	22.7	22.1	21.3*	21.0**	24.2	23.3	23.5	21.9**	21.8**
Food efficiency, Average (weeks 0 – 13) (%)	18.4	18.7	18.3	17.4	17.5	11.1	11.1	10.9	10.2	10.4
Substance intake (mg /kg bw/day)	-	1.01	3.40	17.1	34.3	-	1.23	4.10	21.1	42.8
Haematology										
Haematocrit (%) week 52	38.9	36.9	38.7	37.0	35.2**	34.5	35.0	35.2	35.8	35.9
Hb (g/dL) week 52	14.3	13.5	14.2	13.5	13.1*	12.8	12.8	13.1	13.4	13.3
RBC (10^6/cmm) week 52	7.58	7.20	7.54	6.95	6.85*	6.36	6.25	6.59	6.68	6.57
Blood chemistry										
BUN (mg/dL) week 14	12.3	12.2	13.3	12.7	13.6	12.9	14.0	13.3	15.7**	15.7**
BUN (mg/dL) week 26	12.0	12.2	12.6	13.3	13.7	11.8	13.1	13.4	14.0	15.6**
T. bilirubin (mg/dL) week 14	0.16	0.17	0.19**	0.17	0.18**	0.19	0.20	0.19	0.19	0.18
Histopathology (main groups)										
Mammary Gland: n	-	-	-	-	-	50	40	41	43	50
Hyperplasia	-	-	-	-	-	36	29	21	25	34
Adenoma	-	-	-	-	-	2	3	3	3	2
Fibroadenoma	-	-	-	-	-	17	12	21	18	16
Adenocarcinoma	-	-	-	-	-	5	10	9	12	10
Spleen n	50	50	50	50	50	50	50	50	50	50
Deposition, brown pigment	9	9	4	10	18**	22	20	24	27	31
Auricle n	1	1	0	0	0	2	1	1	2	6

Table 3.9.1.1-2 Summary of the results

Dose (mg/kg food)	**0**		**30**		**100**		**500**		**1000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality - main study - satellite study	22/50 0/20	33/50 1/20	21/50 1/20	31/50 0/20	20/50 0/20	23/50 0/20	13/50 0/20	29/50 0/20	25/50 1/20	22/50 2/20	
Clinical signs - Thickened integument auricle	1/50	0/50	1/50	1/50	0/50	1/50	0/50	2/50	0/50	6/50	
Functional observations - motor activity (1 hr, % compared to control)	100	100	110	117	101	107	110	130	124	138	
Body weight gain							dc	dc	dc	dc	
Body weight (104 w)							dc	d	dc	d	
Food consumption							dc	dc	dc	dc	
Ophthalmoscopy	No treatment-related findings										
Haematology Week 52:											

Dose (mg/kg food)	**0**		**30**		**100**		**500**		**1000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
- haematocrit									dc		
- haemoglobin									dc		
- erythrocyte count									dc		
- prothrombin time									ic		
Week 14, 26 , 78, 104	No treatment related findings										
Urinalysis	No treatment-related findings										
Clinical chemistry											
Week 14:											
- blood urea nitrogen								ic		ic	
- total bilirubin					ic				ic		
Week 26:											
- blood urea nitrogen										ic	
Week 104	No treatment-related findings										
Organ weights											
Week 104:											
- brain									ic[r]		
- heart					dc[a]		dc[a]		dc[a]		
- liver							dc[a]		dc[a]		
- lung									dc[a]		
- testes									ic[r]		
Pathology											
Macroscopy											
Mammary gland hypertrophy											
- week 52	0/20	4/18	0/19	3/20	0/20	3/20	0/20	2/20	0/19	0/18	
- week 104	0/28	4/17	0/29	5/19	0/30	4/27	1/37	5/20	2/25	4/28	
- KIE	1/22	5/33	1/21	14/31**	0/20	9/23*	1/13	11/30*	3/25	8/22	
- total (main study)	1/50	9/50	1/50	19/50*	0/50	13/50	2/50	16/50	5/50	12/50	
Auricle, thick area											
- total (main study)	1/50	0/50	1/50	1/50	0/50	1/50	0/50	2/50	0/50	6/50*	
Liver, spots											
- total (main study)	6/50	6/50	2/50	6/50	2/50	3/50	3/50	3/50	2/50	12/50	
microscopy											
non-neoplastic lesions											
Liver, peliosis											
- week 52	1/20	3/18	0/19	0/20	1/20	0/20	0/20	0/20	0/19	0/18	
- week 104	3/26	0/17	1/29	0/19	0/30	2/27	6/37	2/20	2/25	7/28*	
- KIE	1/22	3/33	1/21	2/31	1/20	0/23	1/13	2/30	0/25	2/22	
- total (main study)	4/50	3/50	2/50	2/50	1/50	2/50	7/50	4/50	2/50	9/50	
Spleen, brown pigment deposition											
- week 52	4/20	4/18	1/19	4/20	2/20	5/20	4/20	3/20	3/19	11/18*	
- week 104	2/28	2/17	0/29	3/19	0/30	9/27	3/37	10/20*	5/25	16/28*	
- KIE	7/22	20/33	9/21	17/31	4/20	15/23	7/13	17/30	13/25	15/22	
- total (main study)	9/50	22/50	9/50	20/50	4/50	24/50	10/50	27/50	18/50*	31/50	
neoplastic lesions											
Mammary gland adenomas											
- week 52		0/18		0/5		0/4		1/2		0/18	
- week 104		0/17		0/9		0/18		1/14		1/28	
- KIE		2/33		3/31		3/23		2/29		1/22	
- Total (main study)		2/50		3/40		3/41		4/43		2/50	

Dose (mg/kg food)	0		30		100		500		1000		dr
	m	f	m	f	m	f	m	f	m	f	
Mammary gland fibroadenomas											
- week 52		0/18		1/5		0/4		0/2		0/18	
- week 104		9/17		5/9		15/18		10/14		11/28	
- KIE		8/33		7/31		6/23		8/29		5/22	
- Total (main study)		17/50		12/40		21/41		18/43		16/50	
Mammary gland, adenocarcinoma											
- week 52		0/18		2/5		1/4		0/2		0/18	
- week 104		3/17		3/9		4/18		6/14		4/28	
- KIE		2/33		7/31		5/23		6/29		6/22*	
- total (main study)		5/50		10/40		9/41		12/43		10/50	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative
KIE killed in extremis or found dead
* statistically significant

Acceptability

The study is considered acceptable.

Conclusions

Animals were given 0, 30 100, 500, and 1000 mg/kg food for up to 104 weeks. No treatment-related mortality was observed. No treatment-related clinical signs were observed, except an increased incidence of thickened area in the integument in the auricle in females at 1000 mg/kg food. Further changes in clinical signs were not dose related or not consistent throughout the study period. At functional observations an increase in motor activity was observed in males and females at 1000 mg/kg food and females at 500 mg/kg food. Decreased body weight gains were noted in females at 500 and 1000 mg/kg food (85 and 86% of controls in week 100, and comparable changes throughout the study period). In addition, changes in body weight gain were noted in males at 500 and 1000 mg/kg food mainly in the first 24 weeks of the study (e.g. 90 and 89% of controls in week 13). At termination of the study, decreased body weights were noted at 500 and 1000 mg/kg food in males (86 and 82% of controls) and females (80 and 84% of controls). A decrease in food consumption was noted at 500 and 1000 mg/kg food during the first 2-3 months of the study in both sexes.

No test substance related changes in ophthalmoscopy and urinalysis were observed. Statistically significant changes in urinalysis were not dose-related or were not consistent throughout the study period or related to other findings.

At haematology, males at 1000 mg/kg food showed statistically significant decreases in haematocrit, haemoglobin and erythrocyte count in week 52 of the study (90, 92 and 90% of controls, respectively). In addition, in week 52, an increased prothrombin time was noted in males at the same dose level. The changes in males at 1000 mg/kg food in week 52 were not apparent in any other time point (week 14, 26, 78 and 104 weeks). Further statistically significant changes in haematology were not considered toxicologically relevant, since findings were only slight, not dose-related, or did not occur consistently throughout the study period.

At clinical biochemistry an increase in total bilirubin was noted in males at 100 and 1000 mg/kg food (119 and 113% of controls) in week 14. No change in total bilirubin was noted at 500 mg/kg food, and the change at 100 mg/kg food was therefore not considered toxicologically significant. An increase in blood urea nitrogen was noted in females at 500 and 1000 mg/kg food in week 14 (122% of controls), at 1000 mg/kg food in week 26 (132% of controls). Several other statistical significant changes were noted at 100 and 500 mg/kg food at separate time points throughout the study period: in week 78 an increase in blood urea nitrogen in females at 500 mg/kg food, an increase in albumin and A/G ratio in males at 500 mg/kg food and a decrease in triglycerides in females at 500 mg/kg food, in week 52 an increase in total bilirubin in males at 500 mg/kg food and in week 14 an increase in creatinine in males at 100 mg/kg food. These statistically significant changes at 100 and 500 mg/kg food were not considered toxicologically relevant since there was no dose-response.

No changes in organ weight were noted at interim necropsy. At study termination, a decrease in absolute liver weight was noted in males at 500 and 1000 mg/kg food (75 and 72% of controls). Absolute heart weight was decreased in males at 100, 500 and 1000 mg/kg food (90, 89 and 86% of controls) and absolute lung weight was decreased at 1000 mg/kg food (88% of control). Both relative brain weight (122% of controls) and relative testes weight (130% of controls) were increased in males at 1000 mg/kg food. Both changes in absolute and relative organ weights were considered to be due to the decreased body weight.

Necropsy revealed an increased incidence of hypertrophy of the mammary gland at 30, 100 and 500 mg/kg food in the females killed in extremis or found dead during the study. As no dose-response was observed, these findings were not considered to be related to treatment. At 1000 mg/kg food, an increased incidence of thickened area in the auricles and spots in the liver were observed in females.

In females at 1000 mg/kg food, a significantly increased incidence of adenocarcinoma of the mammary gland in the animals killed in extremis or found dead was noted. A similar increase, but not statistically significant, was noted in all other treatment groups when compared to controls, without a dose response. An increased incidence of adenocarcinoma of the mammary gland was not apparent in the animals killed at 52 of 104 weeks. Historical control data (2 studies, SD (Crj:CD rats, same lab, years 1997-2001 (study carried out from 1999-2001)) indicated that in the present study, the incidence of adenocarcinoma of the mammary gland in control animals was rather low. The incidence of adenocarcinoma of the mammary gland in the historical control studies was 20% (10/50) and 20.4% (10/49). Since no dose-response was noted and since the observed incidence in treatment groups was equivalent to the incidence in historical control data, the observed increase in adenocarcinoma of the mammary gland was not considered toxicologically relevant. No further changes in neoplastic lesions were considered related to treatment.

Non-neoplastic observations included histopathological changes in the spleen and liver. Findings in liver included an increased incidence in peliosis in females at 1000 mg/kg food. Findings in spleen included an increased incidence of brown pigment deposition in males at 1000 mg/kg food and females at 500 and 1000 mg/kg food.

Based on increased motor activity, reduced body weight (gain), reduced food consumption and brown pigment deposition in the spleen at 500 and 1000 mg/kg food, the NOAEL is established at 100 mg/kg food (equal to 3.4 mg/kg bw/day in males and 4.1 mg/kg bw/day in females). No oncogenic potential of the test substance in rats was observed.

3.9.1.2 Study 2 – 78 week carcinogenicity study, mouse

Characteristics

reference	:	IIA 5.5.3/01 Study no: IET 99-0012	exposure	:	Main: 78 weeks, diet Satellite: 52 weeks, diet
type of study	:	78-week carcinogenicity study	doses	:	0, 15, 50, 1000 and 2500 mg/kg food[1]
year of execution	:	1999-2001	vehicle	:	None
test substance	:	S-1812 (pyridalyl), lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	Oral	guideline	:	OECD 451
species	:	ICR mice, Crj:CD-1	acceptability	:	acceptable
group size	:	Main: 52/sex/dose Satellite: 12/sex/dose	**NOAEL**	:	5.0 mg/kg bw/day in males, 4.8 mg/kg bw/day in females

[1] Equal to 0, 1.53-1.57, 5.04-5.40, 99-103 and 267-270 mg/kg bw/day in males and 0, 1.46-1.50, 4.78-4.87, 99-104 and 264-264 mg/kg bw/day in females.

Study design

The study was performed in accordance with OECD guideline 451. Dose levels were based on a 13-week oral toxicity study in mice (IIA 5.3.2/02). In this study a NOAEL of 70 mg/kg food was established, based on increased albumin and total cholesterol in males at 700 mg/kg food and histopathological effects found in the liver at 3500 mg/kg food and higher.

In the main study 52 animals/sex/dose were given S-1812 for 78 weeks. In the satellite study, 12 animals/sex/dose were given S-1812 for 52 weeks. Mortality, clinical signs, body weight, food consumption were monitored in all animals during the treatment period. Haematological examinations and organ weights analysis were performed on all surviving animals/sex/dose after 12 (satellite group) and 18 months of treatment. All animals were subjected to necropsy, and their tissues were examined histopathologically.

Results

The results are summarised in table 3.9.1.2-1 and -2.

Table 3.9.1.2-1:

	Males					Females				
Diet concentration (ppm)	**0**	**15**	**50**	**1000**	**2500**	**0**	**15**	**50**	**1000**	**2500**
Mortality (main groups)	18/52	15/52	20/52	20/52	16/52	14/52	11/52	11/52	12/52	7/52
Clinical observations										
Tactile hair: loss Main group	2	0	3	0	8*					
Satellite group						7	4	5	3	2*
Weight gain, weeks 0 – 52 (g) Main group	22.5	21.6	22.2	18.7**	15.3**	28.2	27.8	27.5	24.2*	18.5**
Food consumption, week 10 (g/mouse/day) Main group	5.0	5.1	5.0	4.9	4.7**	4.5	4.4	4.3	4.4	4.1
FCR, 0 – 13	3.5	3.2	3.5	3.1	2.9	3.4	3.1	3.5	3.0	2.6
Substance intake (mg /kg bw/day)		1.57	5.04	103	267		1.46	4.78	99	264
Histopathology										
Lung n	52	52	52	52	52	52	52	52	52	52
- adenoma	7	5	9	8	5	6	3	4	6	10
- adenocarcinoma	6	3	8	11	7	4	4	4	6	7
- adenoma & adenocarcinoma	13	7	17	17	11	9	7	8	12	17

Level of statistical significance: * 0.05≥ p > 0.01, ** 0.01≥ p in comparison with control
- irrelevant value

Table 3.9.1.2-2: Summary of the results

Dose (mg/kg food)	**0**		**15**		**50**		**1000**		**2500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality											
- main study	18/52	14/52	15/52	11/52	20/52	11/52	20/52	12/52	16/52	7/52	
- satellite study	3/12	1/12	2/12	1/12	2/12	0/12	1/12	0/12	0/12	1/12	
Clinical signs											
- Tactile hair loss of perinasal region	2/52	14/52	0/52	8/52	3/52	17/52	0/52	18/52	8/52*	18/52	
Body weight gain							dc	dc	dc	dc	m,f
Body weight (78 w)							d	d	dc	dc	m,f
Food consumption (78 w)									dc		
Haematology											
Week 52:	No treatment-related findings										
Week 78:											
- lymphocyte count									dc		
Organ weights											
Week 52:											
- liver									.	ic^{r}, i^{a}	
- kidney										ic^{r}, i^{a}	
- brain									ic^{r}		
- lung									ic^{r}		
- epididymes									ic^{r}		
Week 78:											
- testes									ic^{r}		
- heart									dc^{a}		
- lung									ic^{a}		
- brain									ic^{r}	ic^{r}	
- spleen										dc^{a}	

Dose (mg/kg food)	0		15		50		1000		2500		dr
	m	f	m	f	m	f	m	f	m	f	
Pathology											
Macroscopy	No treatment-related findings										
microscopy *non-neoplastic lesions*	No treatment-related findings										
neoplastic lesions Lung, adenoma											
- week 52	1/9	1/11	1/10	1/11	0/10	1/12	0/11	1/12	0/12	1/11	
- week 78	5/33	5/38	3/37	2/41	6/32	3/41	4/32	6/39	3/36	8/45	
- KIE	2/19	1/14	2/15	1/11	3/20	1/11	4/20	0/13	2/16	2/7	
- total (main study)	7/52	6/52	5/52	3/52	9/52	4/52	8/52	6/52	5/52	10/52	
Lung, adenocarcinoma											
- week 52	0/9	0/11	0/10	0/11	0/10	1/12	0/11	1/12	1/12	0/11	
- week 78	4/33	3/38	3/37	4/41	7/32	4/41	7/32	3/39	6/36	5/45	
- KIE	2/19	1/14	0/15	0/11	1/20	0/11	4/20	3/13	1/16	2/7	
- total (main study)	6/52	4/52	3/52	4/52	8/52	4/52	11/52	6/52	7/52	7/52	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative
KIE killed in extremis or found dead
* statistically significant

Acceptability

The study is considered acceptable.

Conclusions

Animals were given 0, 15, 50, 1000 and 2500 mg/kg food for up to 78 weeks. No treatment-related mortality was observed. No treatment-related clinical signs were observed, except an increased incidence of tactile hair loss of perinasal region in males at 2500 mg/kg food. Further changes in clinical signs were not dose related or not consistent throughout the study period.

Statistically significant lower body weights (83 and 82% of controls at week 78), and decreased body weight gain (61 and 68% of controls at week 78) at 2500 mg/kg food in both sexes, throughout the study period. At 1000 mg/kg food, lower body weights (97 and 90% of controls in week 78) and decreases in body weight gain (85 and 81% of controls) were also noted in both sexes. A slight decrease in food consumption was noted in males of the main group at 2500 mg/kg food (92% of controls), throughout the study period.

At haematology, males at 2500 mg/kg food showed statistically significant decrease in lymphocyte count at week 78. No further treatment-related changes in haematology were observed.

Changes in organ weight were noted at interim and terminal necropsy. At 52 weeks, females showed increased absolute and relative liver weights at 2500 mg/kg food (absolute 117% and relative 130% of controls). Absolute and relative kidney weights were also increased in females at 2500 mg/kg food (128 and 115% of controls, respectively). Changes in liver and kidney weight were not observed in week 78, and were not accompanied by histopathological changes. However, since absolute weights were increased more than 10%, these findings were considered toxicologically relevant.

Males at 2500 mg/kg food showed in week 52 increased relative weights of brain, lung and epididymes, but these changes were considered to be due to the decrease in body weight.
In week 78, males at 2500 mg/kg food showed a decrease in absolute weight of the heart (87% of controls), and an increase in the relative weight of the brain (117% of controls). Both changes were considered to be due to the decrease in body weight. In addition, males showed a slight increase in absolute lung weight (103% of controls). This slight change was not considered toxicologically significant. In addition, males showed in week 78, an increased relative weight of testes (135% of controls), however, this change was considered to be due to low control values (175 mg absolute weight versus 215-249 mg in historical control data). Females at 2500 mg/kg food showed in week 78 an increase in relative brain weight (117% of controls) and a decrease in absolute spleen weight (57% of controls), most probably due to the decrease in body weight.
Necropsy revealed no toxicologically relevant changes. Statistically significant changes were noted, but these included a decreased incidence of changes at 2500 mg/kg food when compared to controls (e.g. decreased incidence of hypertrophy of seminal vesicle or coagulating gland in males at 2500 mg/kg food, a decrease of ovary cysts in females at 2500 mg/kg food, or a decrease in hair loss in both sexes at 2500 mg/kg food), or changes which were not dose-related (e.g. spleen enlargement at 15, 50 and 1000 mg/kg food in females, thymus enlargement at 15, 50 and 2500 mg/kg food, with higher incidences at 15 and 50 than at 2500 mg/kg food).
At histopathology, an increased incidence of lung tumours (adenoma and adenocarcinoma) was noted in females at 2500 mg/kg food. The historical control data is consisted of 9 studies (1992-2001) carried out in same strain in the same lab and the total number of female mice is 462. Detail incidence of long tumours are as follows; adenoma is 13.85% (3.8 – 26.8%), adenocarcinoma is 9.31% (2 – 15.4%) and adenoma plus adenocarcinoma is 23.2% (15.4 – 42.3%). The historical control data indicate (range 8-22 lung tumours (adenoma and adenocarcinoma) of 50-52 animals, 15-42%), that the observed incidence at 2500 mg/kg food (17/52, 33%) was well within the historical control range. Furthermore, no changes in lungs were observed in males. It is therefore, concluded that the increased incidence of lung tumours in females is considered to be incidental and not related to treatment.
No treatment-related changes in non-neoplastic observations were observed.

Based on the lower body weights and body weight gains at 1000 and 2500 mg/kg food, the NOAEL is established at 50 mg/kg food (equal to 5.0 mg/kg bw/day in males and 4.8 mg/kg bw/day in females). There is no evidence of an oncogenic potential of the test substance in mice.

3.9.2 Human data

No data.

3.9.3 *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

No data.

3.9.4 Other data (e.g. studies on mechanism of action)

No data.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Study 1 – 2-generation study

Characteristics

reference	:	IIA5.6.1/02 Study No. 99-0077	exposure	:	Diet
type of study	:	2-generation reproduction study	doses	:	0, 40, 200 and 1000 mg/kg food[1]
year of execution	:	2000-2002	vehicle	:	None
test substance	:	S-1812 (pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 416 (1983 and 2001)
species	:	Rats, Sprague-Dawley, Crj:CD (SD)	acceptability	:	Acceptable
group size	:	24/sex/dose	**NOAEL**par	:	2.8 mg/kg bw/day
			NOAELdev	:	3.6 mg/kg bw/day
			reproductive effects	:	≥68.7 mg/kg bw/day

[1] Equal to 0, 2.80, 13.8 and 68.7 mg/kg bw/day for males and 0, 3.11, 15.7 and 79.1 mg/kg bw/day for females

Study design

The study was performed in accordance with OECD guideline 416 (1983 and 2001).

Dose levels were based on a 90-day oral toxicity study in rats (IIA 5.3.2/02). In this study decreased body weight gain, decreased food consumption, increased liver weights and histopathological effects in the liver were found at doses of 1000 and 2000 mg/kg food.

In addition, dose levels were based on a range finding two-generation reproduction study (IIA 5.6.1/01). In this range finding study, rats (8/sex/group) were fed S-1812 at 0, 100, 500 or 1000 mg/kg food. No effects on body weight, food consumption, macroscopy and fertility were noted at 100 mg/kg food. At 500 mg/kg food, a decrease in body weight gain and food consumption was noted in males and an increase in lung weight was noted in females. At 1000 mg/kg food, decreased body weight gain of P males and F1 females and decreased food consumption in P males was noted. A decrease in F1 pup weight was noted at 1000 mg/kg food. At necropsy, significant increases in lung weight in P females and single cell necrosis of hepatocytes was observed in both P and F1 females. No effect on fertility was noted at either dose level.

Results

The results of the study are summarised in table 3.10.1.1-1 to -3.

Table 3.10.1.1-1,

	Males				Females			
Diet concentration (ppm)	**0**	**40**	**200**	**1000**	**0**	**40**	**200**	**1000**
Parental animals								
Pyridalyl intake (mg/kg/day)								
P Premating (wks 0 – 10)		2.80	13.78	68.7		3.11	15.7	79.1
P gestation/lactation						4.32	22.0	111.2
F1 Premating (wks 0 – 10)		3.4	16.96	83.7		3.62	18.3	91.4
F1 gestation/lactation						4.63	23.2	117.2
Body weight (g),								
P animals, week 9	490	478	461*	439***	264	272	258	249*
F1 animals, week 10	511	501	492	461**	284	284	272	275
Food consumption (g/rat/day)								
P animals, week 5	26.5	25.6	24.1**	23.4***	17.2	17.4	16.7	16.8
F1 animals, week 5	29.0	28.5	28.0	26.2*	18.3	18.6	18.5	18.7

Level of statistical significance: * $0.05 \geq p > 0.01$, ** $0.01 \geq p > 0.001$, *** $p \geq 0.0001$ in comparison with control

	Males				Females			
Diet concentration (ppm)	**0**	**40**	**200**	**1000**	**0**	**40**	**200**	**1000**
Reproductive Performance								
P Mating index (%)	100	100	100	100	100	100	100	100
P Fertility Index					91.7	95.8	91.7	100
Gestation length (days)					22.2	22.1	22.0	22.0
P Litter size					14.4	14.9	14.4	13.5
F1 Mating index (%)	95.7	100	100	100	95.7	100	100	100
F1 Fertility Index					95.5	91.7	95.8	100
Gestation length (days)					22.4	22.1	22.0*	22.0*
F1 Litter size					12.7	14.4	14.0	15.3
Pup Growth and Development								
F1 Bodyweight, day 0 (g)	6.4	6.2	6.4	6.3	6.1	5.9	6.0	5.9
F1 Bodyweight, day 21 (g)	58.5	56.8	53.5***	52.9***	55.9	54.6	51.3***	50.5***
F1 Preputial sep" (days)	41.9	42.1	42.5	41.5				
F1 Vaginal opening (days)					29.0	29.8	31.0***	31.1***
F2 Bodyweight, day 0 (g)	6.8	6.4	6.4	6.1**	6.3	6.1	6.1	5.9
F2 Bodyweight, day 21 (g)	63.8	61.8	59.2**	57.9***	60.4	59.3	56.4**	55.8***
F2 Vaginal opening (days)					29.6	29.5	30.3	31.3***
Organ Weights								
P Terminal body wt (g)	564	560	540	514**	299	302	295	289
P Testis weight (mg)	1716	1666	1741	1711				
P Ovary weight (mg)					55.1	56.7	58.8	61.2**
F1 Terminal bodywt (g)	601	602	585	562	315	314	303	313
F1 Testis weight (mg)	1754	1800	1849*	1889**				
F1 Ovary weight (mg)					57.0	57.4	64.0*	69.5***
F1 weanlings; bodywt (g)	84	80	75***	73***	76	74	69***	66***
F1 weanlings: thymus (mg)	312	301	261**	251***	311	282	257**	239***
Histopathology (Incidence/no. rats observed)								
Ovarian interstitial cell vacuolation								
P					1/23	1/24	0/24	6/24
F1					1/23	0/23	0/24	8/24*
Thyroid "increased small follicles"								
P	1/24	2/24	2/24	2/24	1/23	2/24	0/24	7/24*
F1	3/24	2/24	2/24	3/24	1/23	3/23	2/24	7/24*

Level of statistical significance: * $0.05 \geq p > 0.01$, ** $0.01 \geq p > 0.001$, *** $p \geq 0.001$ in comparison with control

Table 3.10.1.1-2

	Males				Females			
Dose (mg/kg food)	**0**	**40**	**200**	**1000**	**0**	**40**	**200**	**1000**
F0 animals								
Body weight								
- end of pre-mating period	507	496	479*	455***	269	280	265	255
- final weight	564	560	540	514**	299	302	295	289
Body weight gain								
- pre-mating period	368	357	340*	316***	151	161	146	136*
- final weight gain	425	421	401	375***	180	184	177	171
F1 animals								
Body weight								
- end of pre-mating period	511	501	492	461**	284	284	272	275
- final weight	601	602	585	562	315	314	303	313
Body weight gain								
- pre-mating period	447	437	428	398**	223	223	211	216
- final weight gain	538	538	521	499	254	253	242	253

Table 3.10.1.1-3

Dose (mg/kg food)	**0**		**40**		**200**		**1000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
F0 animals									
Mortality	0/24	0/24	0/24	0/24	0/24	0/24	1/24	0/24	
Clinical signs	No treatment-related findings								
Body weight									
- Pre-mating period					dc		dc	d	m
- Breeding period							dc	d	
- final weight							dc		
Body weight gain									
- Pre-mating period					dc		dc	d	m
- Breeding period							dc	d	
- final weight							dc		
Food consumption							dc		
Mating/fertility/gestation	No treatment-related findings								
Oestrus cycle	No treatment-related findings								
Sperm evaluation	No treatment-related findings								
Organ weight									
- ovary								ic[a,r]	
Pathology									
macroscopy	No treatment-related findings								
Microscopy									
Ovary:									
- vacuolation, interstitial gland cell		1/23		1/24		0/24		6/24	
Thyroid:									
- increased small sized follicles	1/24	1/23	2/24	2/24	2/24	0/24	2/24	7/24*	
F1 pups									
Litter size	No treatment-related findings								

Dose (mg/kg food)	0		40		200		1000		dr
	m	f	m	f	m	f	m	f	
Viability index	No treatment-related findings								
Sex ratio	No treatment-related findings								
Clinical signs	No treatment-related findings								
Body weight									
- lactation day 21					dc	dc	dc	dc	m,f
- day 26 of age					dc	dc	dc	dc	m,f
Physical development									
- completion of vaginal opening (days)		29.0		29.8		31.0*		31.1*	
Organ weight									
- Thymus					$dc^{a}d^{r}$	$dc^{a}d^{r}$	$dc^{a}d^{r}$	$dc^{a}d^{r}$	m,f
Pathology									
macroscopy	No treatment-related findings								
F1 animals									
Mortality	0/24	2/24	1/24	1/24	0/24	0/24	0/24	0/24	
Clinical signs	No treatment-related findings								
Body weight									
- Premating period							dc		
- Breeding period							dc		
- final weight							d		
Body weight gain									
- Premating period							dc		
- Breeding period							dc		
- final weight							d		
Food consumption	No treatment-related findings								
Mating/fertility/gestation	No treatment-related findings								
Oestrus cycle	No treatment-related findings								
Sperm evaluation	No treatment-related findings								
Organ weight									
- testes					ic^{a}		$ic^{a,r}$		m
- ovary						$ic^{a,r}$		$ic^{a,r}$	f
- brain								ic^{a}	
Pathology									
macroscopy	No treatment-related findings								
Microscopy									
Ovary:									
- vacuolation, interstitial gland cell		1/23		0/23		0/24		8/24*	
Thyroid:									
- increased small sized follicles	3/24	1/23	2/24	3/23	2/24	2/24	3/24	7/24*	
F2 pups									
Litter size	No treatment-related findings								
Viability index	No treatment-related findings								
Sex ratio	No treatment-related findings								
Clinical signs	No treatment-related findings								

Dose (mg/kg food)	0		40		200		1000		dr
	m	f	m	f	m	f	m	f	
Body weight - lactation day 21 - lactation days 0-21 - day 26 of age					dc	dc	dc dc	dc dc	m,f
Physical development - completion of vaginal opening (days)		29.6		29.5		30.3		31.3*	
Organ weight - thymus					$dc^{a}d^{r}$	$dc^{a}d^{r}$	$dc^{a,r}$	$dc^{a}d^{r}$	m,f
Pathology									
macroscopy	No treatment-related findings								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
* statistically significant

Table 3.10.1.1-4: Effect on completion of vaginal opening

Preliminary study (F1)				
Dose (mg/kg food)	**0**	**100**	**500**	**1000**
Completion of vaginal opening (days)	30.1	29.6	30.9	30.8
Weight at vaginal opening (g)	108	104	104	101
F1 (main study)				
Dose (mg/kg food)	**0**	**40**	**200**	**1000**
Completion of vaginal opening (days)	29.0	29.8	**31.0***	**31.1***
Weight at vaginal opening (g)	90.5	93.8	94.8	94.4
F2 (main study)				
Dose (mg/kg food)	**0**	**40**	**200**	**1000**
Completion of vaginal opening (days)	29.6	29.5	30.3	**31.3***
Weight at vaginal opening (g)	95.4	98.2	97.5	**104.6***

Acceptability
The study is considered acceptable.

Conclusions
In the 2-generation reproduction study, P and F1 parental animals showed no treatment-related mortality or clinical signs. Statistically significant lower body weights and body weight gains were noted in P males at 200 and 1000 mg/kg food (weight gain was retarded approximately 10% at 200 ppm, 15% at 1000 ppm in P males), throughout the pre-mating and breeding period. Final body weights and body weight gains of P males were decreased (91 and 88% of controls, respectively). Decreased body weights were also noted for P females at 1000 mg/kg food, however, only incidentally (week 4, 8, 9, gestation day 20, lactation day 4 and 14). Decreased body weight gains were noted for P females throughout the pre-mating period (10% at 1000 ppm in P females). Statistically lower body weights and body weight gains were noted in F1 males at 1000

mg/kg food, in both the pre-mating and breeding period. Final body weight and body weight gains of F1 males were only slightly decreased (94 and 93% of controls, respectively, not statistically significant).
Food consumption was decreased in P males at 200 and 1000 mg/kg food, mainly during the pre-mating period.
There were no changes observed between P and F1 parental animals of the treated and control groups in mating indices, fertility indices, gestation indices, implantation sites, oestrus cycle and sperm evaluation. A statistically significant decrease in the duration of gestation was noted in F1 animals at 200 and 1000 mg/kg food, however, this change was only slight (22.0 days for both dose levels compared to 22.4 days for controls) and within the historical control range of this strain (22.0-22.6 days). No further details on the HCD were available in the study report.
F1 males showed increased absolute testes weights at 200 and 1000 mg/kg food (105 and 108% of controls, respectively), and relative increased testes weights at 1000 mg/kg food (114% of controls).
Increased absolute ovary weights were noted in P and F1 females at 1000 mg/kg food (111 and 122% of controls) and in F1 females at 200 mg/kg food (112% of controls). A slightly increased absolute brain weight was noted in F1 females at 1000 mg/kg food (103% of controls). Increased relative ovary weights were also seen in P and F1 females at 1000 mg/kg food (115 and 122% of controls) and in F1 females at 200 mg/kg food (116% of controls). Other changes in absolute (e.g. liver and spleen) and relative organ weights (e.g. thyroids, kidneys, brain, epididymes) were considered to be due to the decrease in body weight gain.
At necropsy no treatment-related abnormalities were observed in P and F1 parental animals. Histopathological examination revealed an increased incidence of vacuolation of ovarian interstitial gland cells and of increased small-sized follicles in the thyroid in the P and F1 females at 1000 mg/kg food.
No treatment-related changes were detected in litter size, viability index or sex ratio or clinical signs of the F1 and F2 pups.
F1 male and female pups showed a decrease in body weight on lactation day 21 at 200 mg/kg food (91 and 92% of controls), and at 1000 mg/kg food (90% of controls for both sexes). At day 26 of age, male and female F1 pups showed a decrease in body weight at 200 mg/kg food (89 and 91% of controls) and at 1000 mg/kg food (87% of controls for both sexes).

F2 male and female pups showed a decrease in body weight on lactation day 21 at 200 mg/kg food (93% of controls for both sexes), and during lactation days 0-21 at 1000 mg/kg food (91 and 92% of controls on day 21). At day 26 of age, male and female F2 pups showed a decrease in body weight at 200 mg/kg food (92 and 93% of controls) and at 1000 mg/kg food (90% of controls for both sexes).

Changes in sexual development of F1 and F2 females were noted. In F1 females at 200 and 1000 mg/kg food and in F2 females at 1000 mg/kg food a delay in completion of vaginal opening was noted. In F1 females body weight at vaginal opening was not significantly affected. In F2 females there was a significant increase in body weight at vaginal opening in the high dose group (Table **3.10.1.1-4**). F1 male sexual development was not affected.

Statistically significant decreased absolute thymus weights were noted for F1 male and females pups at 200 mg/kg food (84 and 83% of controls) and at 1000 mg/kg food (80 and 77% of controls). Relative thymus weights of F1 male and female pups were also decreased at 200 mg/kg food (94 and 90% of controls) and at 1000 mg/kg food (93 and 88% of controls), but not statistically significant.

In F2 male and female pups, decreased absolute thymus weights were noted at 200 mg/kg food (85 and 89% of controls) and at 1000 mg/kg food (80 and 84% of controls). Statistically significances were also observed in F2 male at 200 and 1000 mg/kg food and F2 female at 1000 mg/kg food. Relative thymus weights of F2 male and female pups were decreased at 200 mg/kg food (91 and 96% of controls) and at 1000 mg/kg food (88 and 93% of controls), but gained only statistical significance in males at 1000 mg/kg food. Decreased

relative brain weights were noted in F1 and F2 pups at 200 and 1000 mg/kg food, but were considered to be due to the decrease in body weights.

At necropsy no treatment-related abnormalities were observed in F1 and F2 pups.

Organs of F1 and F2 pups were not investigated histopathologically.

In order to elucidate the changes in thymus weight of F1 and F2 pups, histopathology of the thymus was performed (IIA 5.6.1/03, separate report). Ten F2 male pups with significantly decreased thymus weight and ten control male F2 pups were selected for histopathological examination. No abnormalities were observed in any of the pups examined. Therefore, the decreases in absolute and relative thymus weight, might considered to be due to the lower pup weights.

No effects on fertility were noted in the present study. No treatment related changes were noted in oestrus cycle, sperm parameters, mating behaviour, conception and gestation. Therefore, the NOAEL for reproductive effects is set at 1000 mg/kg food (equivalent to 66.7 mg/kg bw/d).

Based on decreased body weight (gain) in P parental animals and increased testes and ovary weights in F1 parental animals at 200 and 1000 mg/kg food, the NOAEL for parental effects is set at 40 mg/kg food (equivalent to 2.8 mg/kg bw/day). At 1000 mg/kg food, histopathological changes in ovary (vacuolation of interstitial gland cells) and thyroid (increased small-sized follicles) were noted in P and F1 females. The NOAEL for developmental toxicity is set at 40 mg/kg food (equivalent to 2.8 mg/kg bw/day based on the decreased F1 and F2 pups weights and delay in the completion of vaginal opening in F1 pups).

3.10.1.2 Study 2 – developmental toxicity, rat

Characteristics

reference	:	IIA 5.6.10/02 Study No 00-0094	exposure	:	days 6-19 of gestation, gavage (1 ml/kg)
type of study	:	teratogenicity study	doses	:	0, 10, 50 or 250 mg/kg bw/day
year of execution	:	2001	vehicle	:	corn oil
test substance	:	S-1812 (pyridalyl), Lot PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	oral	guideline	:	OECD guideline 414 (2001)
species	:	Rat, Crj:CD (SD)	acceptability	:	acceptable
group size	:	24 females/dose	**NOAEL**mat	:	10 mg/kg bw/day
			NOAELdev	:	250 mg/kg bw/day
			teratogenic effects	:	none at the highest dose tested (250 mg/kg bw/day)

Study design

The study was performed in accordance with OECD guideline 414 (2001). Dose levels were based on a preliminary study. Rats (7/dose) were given 0, 50, 100, 200 or 300 mg/kg bw by gavage. Body weight, adjusted body weight, body weight gain were decreased at 100 mg/kg bw and above without a clear dose-response relationship.

Results

The results are summarised in table 3.10.1.2-1 and -2.

Table 3.10.1.2-1

Dose (mg/kg bw/day)	0	10	50	250
Number of females mated	24	24	23	24
Non-pregnant	0	0	0	1
Number of females with live foetuses	24	24	23	23
Clinical sign/Incidence of hair loss	0/24	2/24	4/23*	3/23
Maternal weight gain, days 6-18 (g)	71	71	63**	55**
Food consumption, days 9-12 (g/rat/day)	19.1	19.8	18.8	15.8**
Mean litter size (live foetuses)	15.1	15.3	14.5	14.1
Pre-implantation loss (%)	9.0	10.9	13.2	13.1
Resorptions & foetal death (%)	7.7	5.2	5.8	11.3
Number of foetuses	362	368	333	325
Sex ratio	0.494	0.505	0.483	0.462
Mean foetal weight (mg) Male	3497	3506	3681	3574
Female	3326	3341	3484	3443
Total litters with malformations	6/24	1*/24	0*/23	2/24
Total litters with malformations	6	1*	0*	2
No. foetuses with external malformation	0/362	2/368	0/333	1/325
No. foetuses with visceral malformation	3/174	0/179	0/162	0156
No. foetuses with skeletal malformations	3/188	1/189	0/171	2/169
Number of litters with variations	15/24	15/24	11/23	14/24
No. foetuses with thymic remnant	15/174	10/179	10/162	1/154**

Level of statistical significance: * $0.05 \geq p > 0.01$, ** $p \geq 0.01$ in comparison with control

Table 3.10.1.2-2

Dose (mg/kg bw/day)	0	10	50	250	dr
Maternal effects					
Mortality	None				
Clinical signs	No treatment-related findings				
Pregnant animals	24/24	24/24	24/24	23/24	
Body weight (gain)					
Day 6-18			dc		
Day 6-20				dc	
Food consumption					
Day 6-15				dc	
Uterus weight	No treatment-related findings				
Pathology					
macroscopy	No treatment-related findings				
Litter response					
Number of dams examined	24	24	23[1]	23	
Corpora lutea/dam	No treatment-related findings				
Dams with live foetuses	24	24	23	23	
Live foetuses/dam	No treatment-related findings				

Dose (mg/kg bw/day)	0	10	50	250	dr
Foetal weight	No treatment-related findings				
Placental weight	No treatment-related findings				
Post implantation loss	No treatment-related findings				
Sex ratio	No treatment-related findings				
Examination of the foetuses					
External observations	No treatment-related findings				
Skeletal findings	No treatment-related findings				
Visceral findings	No treatment-related findings				

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
1 One female was excluded from evaluation because this animal appeared to be injured by intubation.

Acceptability
The study was considered acceptable.

Conclusions
No effect on mortality or clinical signs was observed. Mean body weight was slightly decreased compared to controls from day 15 onwards at 250 mg/kg bw. Body weight gain at 250 mg/kg bw was reduced during the whole treatment period with a slight body weight loss during the first three days resulting in a reduced body weight gain at the end of the study (85% of control for unadjusted body weight and 41% of control for adjusted body weight). No effect on gravid uterine weight was observed. At 50 mg/kg bw body weight gain was reduced from day 6-18 (89% of control). Food consumption was reduced from day 6-15 at 250 mg/kg bw. No treatment-related findings were observed at macroscopy.
There were no significant differences in the number of corpora lutea or implantations, post-implantation loss, the number of live foetuses, sex ratio, foetal weight or placental weight. External foetus examination revealed no treatment-related findings. No significant changes in the incidence of soft tissue anomalies or skeletal malformations and variations were noted.
Based on the decrease in body weight gain in maternal females at 50 and 250 mg/kg bw, the NOAEL for maternal toxicity was set at 10 mg/kg bw/day. Based on the absence of treatment-related findings on foetuses the NOAEL for developmental toxicity was set at 250 mg/kg bw/day. Since no treatment–related irreversible structural effects were reported, the NOAEL for teratogenic effects was set at 250 mg/kg bw/day.

3.10.1.3 Study 3 – developmental toxicity, rabbit

Characteristics

Reference	:	IIA 5.6.10/01 Study No 00-0095	exposure	:	days 6-27 of gestation, gavage (0.5 ml/kg)
type of study	:	teratogenicity study	doses	:	0, 15, 50 or 150 mg/kg bw/day
year of execution	:	2001	vehicle	:	corn oil
test substance	:	S-1812 (pyridalyl), Lot PS-98041G, purity 93.7%	GLP statement	:	Yes
Route	:	oral	guideline	:	OECD guideline 414 (2001)
Species	:	Rabbit, Japanese White (Kbl:JW)	acceptability	:	acceptable
group size	:	25 females/dose (30 females at high dose)	**NOAEL**mat	:	50 mg/kg bw/day
			NOAELdev	:	50 mg/kg bw/day
			teratogenic effects	:	none at the highest dose tested (150 mg/kg bw/day)

Study design

The study was performed in accordance with OECD guideline 414 (2001). Dose levels were based on a preliminary study (Hojo, 2001a). Rabbits (6/dose) were given 0, 25, 50, 100 or 150 mg/kg bw by gavage. Body weight gain was decreased at 100 and 150 mg/kg bw (day 6-9 at 100 and day 6-21 at 150 mg/kg bw). At 150 mg/kg bw one rabbit aborted on day 24 of gestation and at 100 mg/kg bw one rabbit delivered prematurely on day 28.

Results

The results are summarised in table 3.10.1.3-1 and -2.

Table 3.10.1.3-1.

Dose (mg/kg bw/day)		0	15	50	150
Number of females inseminated		25	25	25	30
Died		0	0	0	1
Abortion		0	0	0	3
Premature delivery		0	0	0	1
Non-pregnant		0	0	1	1
Resorption sites only (no live young)		1	1	2	1
Number of females with live foetuses		24	24	22	23
Clinical signs		-	-	-	-
Mean weight gain (g) days 6 – 28		204	213	161	0**
Mean food consumption (g/rabbit/day)					
	days 6-9	182	185	178	170
	Days 12 – 15	169	169	162	129*
Mean litter size (live foetuses)		7.6	7.5	7.9	7.3
Pre-implantation loss (%)		22.5	19.7	19.5	20.0
Resorption & foetal death (%)		9.0	9.8	13.5	13.5
Number of foetuses		182	181	173	174
Sex ratio		0.516	0.497	0.509	0.500
Mean foetal weight (g)	Males	38.0	37.6	35.9	34.6
	Females	37.6	37.5	36.9	33.1*
Total litters with malformations		3/24	5/24	2/22	4/23
Foetuses with external malformations		0	0	1	2
Foetuses with visceral malformations		0	0	1	1
Foetuses with skeletal malformations		3	6	1	3
Total litters with variations		22/24	20/24	20/22	23/23

Level of statistical significance: * $0.05 \geq p > 0.01$, ** $p \geq 0.01$ in comparison with control

Table 3.10.1.3-2.

Dose (mg/kg bw/day)	0	15	50	150	dr
Maternal effects					
Mortality	0/25	0/25	0/25	1/30	
Clinical signs - premature birth	0	0	0	1	
Pregnant animals	24/25	24/25	22/25	23/30	
Body weight gain Day 6-28				dc	
Food consumption Day 12-15 and 18-21				dc	
Uterus weight				d	
Pathology					
macroscopy	No treatment-related findings				
Litter response					
Number of dams examined	24	24	22	24	
Corpora lutea/dam	No treatment-related findings				

Dose (mg/kg bw/day)	0	15	50	150	dr
Dams with live foetuses	24	24	22	23	
Live foetuses/dam	No treatment-related findings				
Foetal weight					
- males	38.0	37.6	35.9	34.6	
- females	37.6	37.5	36.9	33.1*	
Placental weight	No treatment-related findings				
Post implantation loss/dam	0.79	0.75	0.91	0.79	
Sex ratio	No treatment-related findings				
Examination of the foetuses					
External observations	No treatment-related findings				
Skeletal findings	No treatment-related findings				
Visceral findings	No treatment-related findings				

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
* significantly different from control at $p \leq 0.05$

Acceptability
The study was considered acceptable.

Conclusions
One female at 150 mg/kg bw was found dead on day 26 of gestation. Three animals at 150 mg/kg bw aborted on day 24, 26 and 27 of gestation. One female gave birth prematurely on day 28. Body weight was decreased at 150 mg/kg bw from day 15 onwards (not statistically significant). Adjusted body weight was slightly decreased. Body weight gain was significantly decreased at 150 mg/kg bw with incidental body weight losses during the whole treatment period. Gravid uterine weight was decreased at 150 mg/kg bw. Food consumption was reduced from day 12 onwards at 150 mg/kg bw with statistically significant reductions at day 12-15 and 18-21. The animals that were found dead, had aborted or prematurely delivered, all showed reduced food consumption. At macroscopy no treatment-related findings were observed in surviving animals. In animals found dead or killed before termination at 150 mg/kg bw two showed a hair bolus in the stomach, one of which had also a pale liver, and one animals had an accentuated lobular pattern on the liver.
There were no significant differences in the number of corpora lutea or implantations, post-implantation loss, the number of live foetuses, sex ratio or placental weight. Foetal weight was statistically significantly decreased in females and decreased in males at 150 mg/kg bw.
External foetus examination revealed no treatment-related findings. No significant changes in the incidence of soft tissue anomalies or skeletal malformations and variations were noted.
Based on mortality, decreased body weight gain in maternal animals at 150 mg/kg bw, the NOAEL for maternal toxicity was set at 50 mg/kg bw/day. Based on decreased foetal weight at 150 mg/kg bw the NOAEL for developmental toxicity was set at 50 mg/kg bw/day. Since no treatment-related irreversible structural effects were seen, the NOAEL for teratogenic effects was set at 150 mg/kg bw/day.

Data from the rat reproduction toxicity and oral semichronic toxicity studies indicated that pyridalyl may affect lipid metabolism and consequently hormone levels. Vacuolization was observed in endocrine organs such as the ovary and adrenal glands in rats, and decreases in testosterone and oestuarial were noted in rats.

Three mechanistic studies were performed to further investigate the effect of pyridalyl on steroid hormone biosynthesis.

3.10.2 Human data

No data.

3.10.3 Other data (e.g. studies on mechanism of action)

3.10.3.1 Study 1 – Investigation of hormonal activity

Characteristics

Reference	:	IIA 5.6.9/01 Study No. S0998	exposure	:	28 days, diet
Type of study	:	4-week hormone study	dose	:	0, 100, 500, 1000 and 2000 mg/kg food[1]
year of execution	:	2002	vehicle	:	None
test substance	:	S-1812 (pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	No
route	:	oral	guideline	:	Not applicable
species	:	rat, Crj:CD(SD)	acceptability	:	acceptable
group size	:	8 males/dose 16 females/dose	**NOAEL**	:	not derived

[1]equivalent to 0, 5.5, 25.5, 49.9 or 94.9 mg/kg bw/day in males and 0, 6.1, 29.5, 54.9 or 102.2 mg/kg bw/d for females

Study design

The study was designed to investigate the effects on the endocrine system, based on the effects observed in the 2-generation reproduction study (delay vaginal opening, increased ovary weight, vacuolation of ovarian interstitial gland cells). In addition, in the 90-day oral toxicity study in rats (IIA 5.3.2/01), histopathological changes in adrenals (vacuolation), and decreases in testosterone and oestradiol were observed. In the present study male and female rats were fed doses of 0, 100, 500, 1000 or 2000 mg/kg food for 4 weeks. Serum hormone levels (males: testosterone and corticosterone; females: oestradiol, progesterone and corticosterone), the oestrus cycle, the endocrine organ weights (males: seminal vesicles with coagulating gland, ventral and dorso-lateral prostate, testes, epididymes and adrenals; females: uterus, ovaries and adrenals), gross pathology and histopathology (ovary) were evaluated. In addition, clinical signs, body weights, food consumption, liver and kidney weights and necropsy were performed.

Chemical analysis of the diets in the study were reported separately (Saka, 2003).

Results

The results are summarised in table 3.10.3.1-1 and -2.

Table 3.10.3.1-1.

	Males					Females				
Diet concentration	**0**	**100**	**500**	**1000**	**2000**	**0**	**100**	**500**	**1000**	**2000**
Bodyweight gain (g), 0-4	95	91	72*	60**	56**	37	32	30	25**	20**
Food consumption, week 1 (g/rat/day)	21	18	16**	14**	14**	14	15	13	10**	9**
Intake (mg /kg bw/day)		5.5	25.5	49.9	94.9		6.1	29.5	54.9	102.2
Hormone analyses										
Testosterone (ng/mL)	1.1	1.3	1.1	0.7	1.0					
Oestradiol (pg/mL)						29.4	<26.8	34.8	<32.7	33.8
Progesterone (ng/mL)						6.8	3.6	3.4	4.5	4.2
Corticosterone (ng/mL)	<25	<17	<27	40	<15	51	42	73	111	99
Organ Weights										
Bodyweight (g)	450	447	431	417*	407**	274	275	272	258	256*
Prostate (dorso-lateral) (mg)	409	379	345	347	330**					
Testes (g)	3.26	3.10	3.32	3.09	3.25					
Seminal vesicles (g)	1.44	1.36	1.24	1.20**	1.17**					
Ovaries (mg)						94	75*	89	83	87
Histopathology										
Ovary: interstitial gland vacuolation						0/16			0/16	10/16 **

Level of statistical significance: * 0.05≥ p > 0.01, ** 0.01≥ p in comparison with control

Table 3.10.3.1-2

Dose (mg/kg food)	**0**		**100**		**500**		**1000**		**2000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	None										
Clinical signs	No treatment-related findings										
Body weight gain					dc		dc	dc	dc	dc	
Body weight											
Food consumption							dc	dc	dc	dc	
Hormone analysis											
- Testosterone (ng/ml)	1.1		1.3		1.1		0.7		1.0		
- Oestradiol (pg/ml)		29.4		<26.8		34.8		<32.7		33.8	
- Progesterone (ng/ml)		6.8		3.6		3.4		4.5		4.2	
- Corticosterone (ng/ml)	<25	51	<17	42	<27	73	40	111	<15	99	
Organ weights											
- prostate (dorso lateral)					d^{ar}		d^{ar}		$dc^a d^r$		
- seminal vesicle					d^{ar}		$dc^a d^r$		$dc^a d^r$		
- liver										ic^r	
Pathology	No treatment-related findings										
macroscopy											
microscopy											
Ovary:											
- vacuolation, interstitial gland cell		0/16		0/0		0/0		0/16		10/16	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative

Acceptability
The study is considered acceptable.

Conclusions
Rats were administered 100, 500, 1000 or 2000 mg/kg food of S-1812 via the diet for 28 days. No mortality and no treatment-related clinical signs were seen. Body weight gain was decreased in males at 500, 1000 and 2000 mg/kg food (76, 64 and 59% of control, respectively) and in females at 1000 and 2000 mg/kg food (68 and 54% of controls, respectively). Final body weights were decreased in males at 1000 and 2000 mg/kg food (93 and 90% of controls), and in females at 1000 and 2000 mg/kg food (94 and 92% of controls).
Food consumption was decreased in males at 1000 mg/kg food (67-87% of controls) and at 2000 mg/kg food (67-87% of controls) during the whole treatment period. In females, food consumption was also decreased in males at 1000 mg/kg food (71-88% of controls) and at 2000 mg/kg food (64-82% of controls), throughout the study period.
No treatment-related changes were observed in analysis of testosterone, oestradiol and progesterone. Higher values for corticosterone were noted for females at 500, 1000 and 2000 mg/kg food, due to high individual values in each group, but not attaining statistical significance. The observed change in corticosterone in females is of unknown toxicological significance, as no historical control data were available. No changes in corticosterone values were noted in males.
A decrease in the weight of the dorso-lateral prostate was noted in males at 500, 1000 and 2000 mg/kg food (85, 85 and 81% of controls), but gained only statistical significance at 2000 mg/kg food.
Relative prostate weights were decreased (89, 92 and 89% of controls) at 500, 1000 and 2000 mg/kg food, but gained no statistical significance. A decrease in absolute weight of the seminal vesicles was noted for males at 500, 1000 and 2000 mg/kg food (87, 83 and 81% of controls), statistical significant at 1000 and 2000 mg/kg food. Relative weights of seminal vesicles were noted in males at 500, 1000 and 2000 mg/kg food (90% of controls for all three dose groups), but gained no statistical significance.
An increased relative liver weight at 2000 mg/kg food in females (111% of controls), was considered to be due to a decrease in body weight.
Macroscopic examination showed no treatment related changes. Histopathology revealed an increased incidence of vacuolation of ovarian interstitial gland cells in females at 2000 mg/kg food.

In conclusion, S-1812, did not affect testosterone, oestradiol and progesterone levels in rats after 4 week dietary exposure. However, in females higher values for corticosterone were noted at 500, 1000 and 2000 mg/kg food, due to high individual values in each group, but not attaining statistical significance. The observed change in corticosterone in females is of unknown toxicological significance. In addition, effects on dorso-lateral prostate and seminal vesicle weights were noted in males at 500, 1000 and 2000 mg/kg food. Furthermore, histopathology revealed an increased incidence of vacuolation of ovarian interstitial gland cells in females at 2000 mg/kg food. Overall, it is concluded that an effect on the endocrine system cannot be completely ruled out, however, equivocal effects occured at dose levels equal or higher than dose levels showing general toxicity (e.g. decreased body weight).

3.10.3.2 Study 2 – Investigation of hormonal activity

Characteristics

Reference	:	IIA 5.6.9/02 Study No. X0091	exposure	:	5-48 hours incubation

Type of study	:	Sex steroid hormone biosynthesis	concentrations	:	0, 1, 3, 10, 20 µM
year of execution	:	2002	vehicle	:	DMSO
test substance	:	S-1812 (pyridalyl), Lot no. KOBE-95006, purity 98.4%	GLP statement	:	No
route	:	Not applicable	guideline	:	Not applicable
species	:	rat, Crj:CD(SD), Leydig and ovary cells	acceptability	:	Acceptable
group size	:	Not applicable	**Effect**	:	See conclusions

Study design

The study was designed to investigate the effects on the production of hormones and the activity of enzymes that catalyze sex steroid hormone biosynthesis.

The following tests were performed.

- S-1812 was added to Leydig cell or ovary cell culture medium at concentrations of 0, 1, 3, 10 or 30 µM. Progesterone, 17α-OH-Progesterone, androstenedione, testosterone and oestradiol in medium were analysed by radioimmunoassay.
- Incubations were performed to compare the activity of S-1812 with human chorionic gonadotropin (to stimulate oestrogen production in ovarian cells) or lutenising hormone (to stimulate production of testosterone from Leydig cells). In Leydig cells ketoconazole and human chorionic gonadotropin were used as positive controls.
- Leydig and ovarian cells were incubated with [^{14}C]androstenedione and analysed for unconverted androstenedione, as a measure of 17β-hydroxysteroid dehydrogenase activity.
- In addition, ovarian cells were incubated with [^{14}C]testosterone and analysed for [^{14}C]oestradiol, a metabolite of testosterone as a measure of aromatase activity or inhibition.

Results

Effects on hormone concentrations in Leydig cells

S-1812 caused a significant increase in concentrations of androstenedione at doses of 10 and 30 µM (122 and 164% of controls, respectively). No changes were noted in testosterone and 17α-OH-progesterone. Progesterone and oestradiol were not detected before and after S-1812 treatment.

Incubation with ketoconazole caused a decrease in production of androstenedione and testosterone, the incubation with human chorionic gonadotropin caused an increase in the production of 17α-OH-progesterone, androstenedione and testosterone. Incubation with S-1812 at 10 µM, resulted in an increase in androstenedione production (200-203% of controls at 5-48 hours). Incubation with S-1812 at 10 µM caused a non statistical significant increase in 17 α-OH-progesterone (247 and 188% of controls at 24 and 48 hours, respectively. Incubation with S-1812 at 10 µM did not affect testosterone production.

After incubation of [^{14}C]androstenedione, the addition of S-1812 at concentrations of 3 and 30 µM a caused a significant decrease in androstenedione metabolite production, indicating a inhibition of 17β-hydroxysteroid dehydrogenase activity. However, the decrease was only slight, 93% and 94% of controls at 3 and 30 µM, respectively, and no decrease was noted at 10 µM.

Effects on hormone concentrations in ovary cells

S-1812 at concentrations of 0, 1, 3, 10 or 30 µM, did not affect the concentrations of progesterone, 17α-OH-progesterone, androstenedione and oestradiol. Testosterone was not detected before and after S-1812 treatment.

After incubation of [^{14}C]androstenedione, the addition of S-1812 at concentrations of 3, 10 and 30 µM a caused a decrease in androstenedione metabolite production (86.2, 86.0 and 89% of controls, respectively), only statistical significant at 10 µM.

After incubation of the ovarian cells with [^{14}C]testosterone and S-1812 at concentrations of 0, 1, 3, 10 and 30 µM, no change in [^{14}C]oestradiol production was noted.

Acceptability
The study is considered acceptable.

Conclusions
In cultures of rat Leydig cells, S-1812 caused an increase in androstenedione and α-OH-progesterone levels. In addition, S-1812 caused a slight decrease in androstenedione metabolite production, indicating an inhibition of 17β-hydroxysteroid dehydrogenase activity. No change was noted in testosterone levels.
In cultures of rat ovary cells, S-1812 caused did not affect the concentrations of progesterone, 17α-OH-progesterone, androstenedione and oestradiol. After incubation of [^{14}C]androstenedione, S-1812 caused a decrease in androstenedione metabolite production in ovary cells. Incubation of the ovarian cells with [^{14}C]testosterone and S-1812, did not change [^{14}C]oestradiol production.
In conclusion, slight changes in the steroid hormone biosynthesis pathway were noted after exposure of Leydig and ovarian cells to S-1812, however, the changes did not result in alterations in testosterone or oestradiol levels.

3.10.3.3 Study 3 – Reported gene assay

Characteristics

Reference	:	Saito, 2002	exposure	:	40 hours incubation
Type of study	:	Reporter gene assay	concentrations	:	0, 10 nM, 100 nM or 1 µM
year of execution	:	2002	vehicle	:	DMSO
test substance	:	S-1812 (pyridalyl), Lot no. 1980202-1, purity 94.2%	GLP statement	:	No
route	:	Not applicable	guideline	:	Not applicable
species	:	HeLa cells from human cervical carcinoma	acceptability	:	Acceptable
group size	:	Not applicable	**Effect**	:	See conclusions

Study design
The study was designed to investigate the effects of S-1812 on transactivation by human oestrogen receptor alpha (hERα), androgen receptor (hAR) and thyroid hormone receptor alpha (hTRα), using mammalian cell-based (HeLa cells from human cervical carcinoma) luciferase reporter gene assays (reporter gene assays).
HeLa cells were transfected with receptor-expressing plasmid (luciferase) for either the oestrogen α-receptor, androgen receptor or throxine α-receptor.
Cells were exposed to S-1812 at 10 nM, 100 nM or 1 µM to detect an effect on the receptors. Cells were also exposed to S-1812 together with known agonist (oestradiol, 100 pM for ERα; dihydrotestosterone, 100 pM for AR; T_3, 50 nM for TRα). After incubation for 40 hours, cells were lysed and supernatant diluted. Luminous intensity (due to luciferase) was measured after adding enzyme substrate solution.

Results

Reporter gene assay with ERα
There were no effects of S-1812 on transcriptional activation by ERα.
Oestradiol showed an agonistic effect and 4-hydroxytamoxifen showed an antagonistic response.
It was therefore concluded that S-1812 showed no agonistic or antagonistic effect on ERα.

Reporter gene assay with AR
There were no effects of S-1812 on transcriptional activation by AR.

Dihydrotestosterone showed an agonistic effect and hydroxyflutamide showed an antagonistic response.
It was therefore concluded that S-1812 showed no agonistic or antagonistic effect on AR.

Reporter gene assay with TRα
There were no effects of S-1812 on transcriptional activation by TRα.
T_3 showed an agonistic effect. It was therefore concluded that S-1812 showed no agonistic or antagonistic effect on AR.

Acceptability
The study is considered acceptable.

Conclusions
S-1812 had no direct agonistic effects on the oestrogen receptor alpha, the androgen receptor and the thyroid hormone receptor alpha. S-1812 had also no effect on the expression due to the agonists, therefore also had no antagonistic response. Overall, it can be concluded that S-1812 did not show a direct effect on human oestrogen, androgen or thyroid receptors.

3.11 Specific target organ toxicity – single exposure

3.11.1 Animal data

See sections 3.1, 3.2 and 3.3 for the summary of the acute toxicity studies of the oral, dermal and inhalation route, respectively.

3.11.2 Human data

No data.

3.11.3 Other data

No data.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Study 1 – 28-day oral toxicity, rat

Characteristics

Reference	:	IIA 5.3.1/01 Study no. S0418	exposure	:	28 days, diet (+ control and highest dose 14 days)
Type of study	:	28-day oral toxicity study	dose	:	0, 70, 200, 700 and 2000 mg/kg food (nominal)[1]
year of execution	:	1995/96	vehicle	:	none
test substance	:	S-1812 (pyridalyl), Lot no. NSA-950525, purity 98.7%	GLP statement	:	no
route	:	oral	guideline	:	predominantly in accordance with OECD 407 (1995)

species	:	rat, Crj:CD(SD)	acceptability	:	acceptable as range-finding study
group size	:	6/sex/dose (+ additional 6/sex in control and highest dose for interim sacrifice)	**NOAEL**	:	not derived

[1]equivalent to 0, 7.05, 19.5, 64.6 and 182 mg/kg bw/d for males and 0, 7.21, 19.6, 66.4 and 188 mg/kg bw/d for females (corrected for actual intake of food)

Study design

The study was generally in compliance with OECD 407 (1995). However, functional observations were not performed and weight of epididymides was not determined. For histopathology only the following tissues were preserved from all control and high dose group animals: lung, heart, liver, kidney, spleen, testis, adrenal (all dose groups) and ovary (all dose groups). Dietary concentrations were not confirmed by analysis.

Additionally, haematology and clinical biochemistry parameters were determined in the control and high dose group after 2 weeks of treatment. Urinalysis was performed in the control and high dose group after 2 weeks and in all dose groups after 4 weeks of treatment. Tissue sections from liver, lungs and adrenals from 2 animals/sex in the control and high dose group were additionally examined by electron microscopy.

Dose levels were based on a 2-week oral toxicity study in rat (not submitted), in which animals were given S-1812 (pyridalyl) at 7000 mg/kg food. It was reported that treated animals showed a decrease in body weight gain (56% of control), and increased leukocyte count, changes in lipid parameters and histopathological changes of the lung.

Results

The results are summarised in table 3.12.1.1-1 and 3.12.1.1-2.

Table 3.12.1.1-1

	Males					Females				
Diet concentration (ppm)	0	70	200	700	2000	0	70	200	700	2000
Bodyweight, week 4 (g)	328	342	320	314	295**	201	211	196	185	195
Food consumption, week 4 (g/rat/day)	23	24	22	22	20*	16	17	16	15	15
mg test material/kg bw/day	0	7.1	19.5	64.6	182	0	7.2	19.6	66.4	188
Haematology										
Fibrinogen, mg/dl Week 2	238				215*	191				207
Week 4	253	230**	239*	237*	234*	192	198	195	207	226**
Blood Chemistry, week 2										
Glucose, mg/dL	114				137*	115				122
T. cholesterol, mg/dL	70				112**	64				105**
Triglycerides (mg/dL)	43				58	20				25
Phospholipids (mg/dL)	99				139*	101				145**
T. Bilirubin (mg/dL)	0.15				0.15	0.15				0.15
Direct bilirubin (mg/dL)	0.09				0.06*	0.06				0.06
Gamma-GT (U/L)	1				1	1				2*
Chloride (mEq/L)	104				103	106				103**
Blood Chemistry, week 4										
T. cholesterol, mg/dL	70	62	66	96*	93*	62	72	72	80	88
Triglycerides (mg/dL)	86	62	61	71	37**	19	20	22	20	37
Phospholipids (mg/dL)	108	95	101	130	126	106	126	122	129	133
T. Bilirubin (mg/dL)	0.17	0.17	0.16	0.16	0.15**	0.19	0.19	0.18	0.19	0.18
Gamma-GT (U/L)	1	1	2	2	2*	2	2	2	2	3*
Sodium (mEq/L)	137	137	137	138	138	137	137	139	139*	140**
Potassium (mEq/L)	3.7	3.6	3.6	3.7	3.7	3.5	3.4	3.0**	3.3	3.0*
Organ weights (week 2)										
Bodyweight (g)	222				210	146				146
Lung (g)	1.08				1.17*	0.85				0.93
Lung (% bw)	0.49				0.56**	0.58				0.64*
Liver (% bw)	3.45				3.71	3.36				3.84**
Organ weights (week 4)										
Bodyweight (g)	304	316	298	292	273**	192	195	187	175	184

	Males					Females				
Lung (g)	1.18	1.25	1.20	1.20	1.26	0.99	0.99	0.96	0.89*	1.04
Lung (% bw)	0.39	0.39	0.40	0.41	0.46**	0.52	0.51	0.52	0.51	0.57**
Ovaries (mg)						73	78	72	69	97**
Liver (% bw)	3.04	3.13	3.09	3.21*	3.28**	2.89	3.01	3.10*	3.13**	3.40**
Histopathology (incidence, 6 rats/group) Week 4										
Adrenal cortex: vacuolation	0	0	0	0	1	0	0	0	0	6
Ovary: interstit. degen						0	0	0	0	4
Ovary: corpus luteum. persistence						0	0	0	0	4
Ovary; interstit. vacuolation						0	0	0	1	6

Level of statistical significance: * 0.05≥ p > 0.01, ** 0.01≥ p in comparison with control

Table 3.12.1.1-2

Dose (mg/kg food)	**0**		**70**		**200**		**700**		**2000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	None										
Clinical signs	No treatment-related findings										
Body weight gain									dc		
Food consumption									dc		
Haematology											
After 2 weeks											
- APTT									ic		
- fibrinogen									dc		
After 4 weeks											
- fibrinogen			dc		dc		dc		dc	ic	
Clin. Chemistry											
After 2 weeks											
- total cholesterol									ic	ic	
- phospholipids									ic	ic	
- direct bilirubin									dc		
- gamma-GTP										ic	
- chloride										dc	
After 4 weeks											
- A/G									ic		
- total cholesterol							ic	ic	ic	ic	f
- triglycerides									dc	i	
- phospolipids									i		
- total bilirubin									dc		
- gamma-GTP									ic	ic	
- LDH									dc		
- sodium										ic	
- potassium										dc	
Urinalysis	No treatment-related findings										

Dose (mg/kg food)	0		70		200		700		2000		dr
	m	f	m	f	m	f	m	f	m	f	
Organ weights											
After 2 weeks											
- lung									$ic^{a,r}$	ic^{r}	
- liver										ic^{r}	
- ovaries										$i^{a,r}$	
After 4 weeks											
- lung									ic^{r}	ic^{r}	
- liver						ic^{r}	ic^{r}	ic^{r}	ic^{r}	$ic^{a,r}$	m/f
- ovaries										$ic^{a,r}$	
- brain									ic^{r}		
- testes									ic^{r}		
Pathology											
macroscopy											
After 2 weeks											
- liver, dark	0/6	0/6							5/6	1/6	
- lung, pale	1/6	1/6							4/6	1/6	
After 4 weeks											
- liver, dark	0/6	0/6	1/6	0/6	5/6	1/6	6/6	2/6	5/6	4/6	m/f
microscopy											
After 2 weeks											
adrenal:											
- cortex degeneration	0/6	0/6							2/6	3/6	
- cortex vacuolation	0/6	0/6							5/6	6/6	
After 4 weeks											
adrenal:											
- cortex degeneration	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	3/6	
- cortex vacuolation	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6	6/6	
ovary:											
- degen. interstitial gland cells		0/6		0/6		0/6		0/6		4/6	
- persistent corpus luteum		0/6		0/6		0/6		0/6		4/6	
- vacuol. interstitial gland cells		0/6		0/6		0/6		1/6		6/6	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative

Acceptability

As no individual values were available making it impossible to check whether effects were real effects and diet concentrations were not confirmed by analysis, the study is acceptable as range-finding study and a NOAEL is not derived.

Conclusions

Rats were administered 70, 200, 700 or 2000 mg/kg food of S-1812 via the diet for 28 days. No mortality and no treatment-related clinical signs were seen. Body weight gain was decreased in males at 2000 mg/kg food (84% of control). Food consumption was decreased in males at 2000 mg/kg food during the whole treatment period.

Activated partial thromboplastin time (APTT) was increased (114% of control) and fibrinogen was decreased (90% of control) after 2 weeks of treatment in males at 2000 mg/kg food. Fibrinogen was decreased in all treated males after 4 weeks of treatment without a dose-response relationship (91-94% of control). This decrease after 4 weeks may be due to incidental high control values of one or more individuals based on comparison of 2-week and 4-week mean control values.

Total cholesterol and phospholipids were increased after 2 weeks of treatment in both sexes at 2000 mg/kg food (160-164% and 140-144% of control, resp.). Total cholesterol was still increased after 4 weeks of treatment at 2000 mg/kg food (133-142% of control) and also at 700 mg/kg food (129-137% of control). Phospholipids were only increased in males at 2000 mg/kg food after 4 weeks of treatment (117% of control). Triglycerides were significantly decreased in males (43% of control) and increased in females (195% of control) at 2000 mg/kg food. Direct bilirubin was decreased (67% of control) after 2 weeks of treatment and total bilirubin was decreased (88% of control) after 4 weeks of treatment in males at 2000 mg/kg food. Gamma-glutamyltranspeptidase was increased in females after 2 weeks and 4 weeks of treatment (150-200% of control) and in males after 4 weeks of treatment (200% of control) at 2000 mg/kg food. The changes in cholesterol, phospholipids, triglycerides, bilirubin and gamma-GTP may reflect disturbance of lipid synthesis/metabolism and induction of microsomal enzymes. The decrease in lactate dehydrogenase at 2000 mg/kg in males after 4 weeks of treatment was not considered to be toxicologically relevant. Sodium was increased after 4 weeks of treatment (102% of control) and chloride was decreased after 2 weeks of treatment (97% of control) in females at 2000 mg/kg food. Potassium was significantly decreased in females at 2000 mg/kg food after 4 weeks of treatment.

Urinalysis showed no treatment-related findings.

After 2 weeks of treatment absolute lung weight was increased in males at 2000 mg/kg food, and increased relative weight of the lungs in both sexes and of the liver in females at 2000 mg/kg food. Absolute and relative ovary weight was increased after 2 weeks of treatment and statistically significantly increased after 4 weeks of treatment in females at 2000 mg/kg food. After 4 weeks absolute liver weight in females at 2000 mg/kg food was increased and relative liver weight in both sexes at 2000 mg/kg food and 700 mg/kg food and in females at 200 mg/kg food. After 4 weeks of treatment relative weight of lungs was increased in both sexes at 2000 mg/kg food and of the brain and testes in males at 2000 mg/kg food. The increased relative weight of lung, brain and testes in males may be due to decreased body weight gain.

Macroscopic examination showed a dark liver in most males and one female at 2000 mg/kg food and pale lungs in 4 males and one female at 2000 mg/kg food after 2 weeks of treatment. After 4 weeks a dark liver was noted in most animals at 2000 mg/kg food. No histopathological confirmation of the effects seen in the liver or lungs was found. After 4 weeks the adrenal cortex was noted to be slightly degenerated and contained mild vacuolation in females at 2000 mg/kg food, which was already present after 2 weeks. Electron microscopy showed swelling of the mitochondria and increased fatty vacuolation in the zona

reticularis. In males at 2000 mg/kg food only after 2 weeks similar effects as in females were found. After 4 weeks the ovaries showed degeneration of the interstitial gland cells and persistent corpora lutea in 4/6 females and vacuolation of the interstitial gland cells in all females at 2000 mg/kg food.

In conclusion, the main target organs are the liver, lungs, adrenals and ovaries. Changes in body weight, food consumption, clinical biochemistry (changes in cholesterol, phospholipids, triglycerides, bilirubin and gamma-GTP), increased liver, lung and ovary weights and histopathological changes in adrenals and ovary were noted at 2000 mg/kg food. Changes in cholesterol and liver weight were noted at 700 mg/kg food. No effects were observed at 70 and 200 mg/kg food (dark liver was not confirmed by histopathology).

3.12.1.2 Study 2 – 13-week oral study in rat

Characteristics

Reference	:	IIA 5.3.2/01 Study No. S0450	exposure	:	13 weeks, diet
Type of study	:	13-week oral toxicity study	dose	:	0, 70, 700, 2000 and 3500 mg/kg bw/day (nominal)[1]
year of execution	:	1996	vehicle	:	none
test substance	:	S-1812 (pyridalyl), Lot no. KOBE951006, purity 98.4%	GLP statement	:	no
route	:	oral	guideline	:	predominantly in accordance with OECD 408 (1998)
species	:	rat, Crj:CD(SD)	acceptability	:	acceptable
group size	:	10/sex/dose (+ 6/sex/dose for hormone analysis)	**NOAEL**	:	4.68 mg/kg bw/day for males 5.37 mg/kg bw/day for females

[1]equivalent to 0, 4.68, 47.4, 133 and 233 mg/kg bw/d for males and 0, 5.37, 55.5, 153 and 256 mg/kg bw/d for females (corrected for actual food intake)

Study design

The study was generally in compliance with OECD 408 (1998). Functional observations were not performed.

Dose levels were based on a 2-week diet study in which male rats were given 0 or 7000 mg/kg food of S-1812 (pyridalyl). It was reported that treated rats showed reduced body weight gain (56% of control), foamy cell accumulation in alveoli of the lung and vacuolation in the adrenal cortex.

Additional to the requested observations, urinalysis was performed on 6 animals/sex/group in week 12 or 13. Electron microscopical examination of lung, adrenal and liver tissue from 2 animals/sex from the control and highest dose group was performed. Liver tissue was immunohistochemically analysed with glutathione-S-transferase placental type. Analysis of plasma hormones was performed on the control and satellite group (6/sex for control and 3500 mg/kg food). Oestrous cycle of females was checked starting 8 days prior to sacrifice.

Results

The results are summarised in table 3.12.1.2-1 to -3.

Table 3.12.1.2-1

	Males					Females				
Diet concentration	**0**	**70**	**700**	**2000**	**3500**	**0**	**70**	**700**	**2000**	**3500**
Bodyweight gain (g)	341	336	313	298*	276**	151	160	133*	122**	122**
Food consumption, week 13 (g/rat/day)	23	22	22	21	20**	16	16	16	14	13**
mg test material/kg bw/day	0	4.7	47.4	133	233	0	5.4	55.5	153	256
Haematology										
HGB (g/dL)	14.6	14.9	15.0*	15.1*	15.3**	14.6	14.8	15.2	14.9	15.1
HCT (%)	41.7	42.5	43.2*	43.7**	43.6**	41.3	41.9	42.6	42.3	42.7
MCV (fL)	50.8	50.2	51.8	52.4*	52.5*	52.9	52.5	52.0	52.7	53.2
WBC (10^3/µL)	7.7	8.7	7.7	9.5	10.2*	5.4	6.1	7.3*	7.8**	10.4**
Lymphocytes (10^3/µL)	6.0	7.4	6.1	7.8*	8.6**	4.4	5.0	6.2*	6.5*	9.3**
PT (sec)	19.1	20.6	18.8	22.6*	25.0**	17.2	17.5	18.3*	17.4	16.3*
APTT (sec)	19.8	20.8	19.1	22.0**	25.3**	16.3	16.0	16.0	15.3**	15.5*
Blood Chemistry										
Total protein (g/dL)	6.4	6.3	6.3	6.3	6.2	7.0	6.7*	6.7*	6.7	6.5*
A/G ratio	0.92	0.94	0.99*	1.01**	1.08**	1.23	1.22	1.23	1.28	1.25
Glucose (mg/dL)	157	159	149	148	133**	132	125	121	121	122
T. cholesterol (mg/dL)	62	67	88**	102**	126**	81	79	82	114**	115**
HDL-Chol. (mg/dL)	38	28**	33	31**	37	44	43	48	54*	43
Triglycerides (mg/dL)	58	64	75	67	79	34	30	35	39	38
Phospholipids	94	98	125**	140**	172**	151	149	148	191**	176
Gamma-GT (U/L)	2	2	2	2**	3**	2	2	2*	2*	3**
Hormone analyses										
Testosterone (ng/mL)	1.3				0.6*					
Progesterone (ng/mL)						6.3				13.1
Oestradiol						33				9**
Organ Weights										
Necropsy bodyweight (g)	468	463	442	423*	406**	253	260	237	224**	226**
Testes (g)	3.4	3.2	3.3	2.9**	3.1*					
Pituitary (mg)	14	13	12**	12**	12**	17	17	15	14*	14**
Epididymides (g)	1.32	1.24	1.24	1.19*	1.17**					
Ovaries (mg)						74	88	84	85	110**
Lung (g%)	0.31	0.31	0.31	0.33**	0.33*	0.43	0.42	0.44	0.46	0.55**
Adrenals (mg)	55	51	54	51	54	63	65	62	62	73
Adrenals (mg%)	11.7	11.1	12.3	12.2	13.2*	25.1	24.9	26.1	27.7	32.2**
Liver (g%)	2.50	2.63	2.64	2.71*	2.86**	2.57	2.62	2.67	2.75*	3.03**

Level of statistical significance: * $0.05 \geq p > 0.01$, ** $p \leq 0.01$ in comparison with control

	Males					Females				
Diet concentration	**0**	**70**	**700**	**2000**	**3500**	**0**	**70**	**700**	**2000**	**35000**
Histopathology										
Adrenal:										
Vacuolation, Z. reticularis	0	0	0	0	6**	0	0	0	3	10**
Decreased vacuolation, Z. glomerulosa	0	0	0	0	0	0	0	0	0	8**
Liver:										
Hypertrophy, centrilobular	0	1	0	6**	8**	0	0	0	3*	7**
Vacuolation, periportal	8	7	3*	0**	0**	1	2	2	0	0
Mononuclear cell infiltration	10	10	10	10	10	4	4	8*	10**	10**
Necrosis, single cell	10	10	10	10	10	4	4	8*	10**	10**
GST-P +ve foci	14				28	13				20
Lung:										
Foamy/Eosinophilic cells in alveoli	0	0	0	0	3	0	0	0	0	7**
Ovaries:										
Interstitial cell vacuolation						2	2	4	10**	10**

Level of statistical significance: * $0.05 \geq p > 0.01$, ** $p \leq 0.01$ in comparison with control

[1] Diet concentration of 35000 should be 3500 ppm in females.

Table 3.12.1.2-2

Dose (mg/kg food)	**0**		**70**		**700**		**2000**		**3500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Liver											
Single cell necrosis, **total**	**10**	**4**	**10**	**4**	**10**	**8***	**10**	**10**	**10**	**10****	
- grade 1 slight	8	4	7	4	4	6	3	2	4	4	
- grade 2 mild	2	0	3	0	6	2	7	8	6	6	
- grade 3 moderate		0		0		0		0		0	
- grade 4 severe		0		0		0		0		0	
Ovary											
vacuol. interstitial gland cells, **total**		**2**		**2**		**4**		**10****		**10****	
- grade 1 slight		2		2		4		2		0	
- grade 2 mild		0		0		0		8		10	
- grade 3 moderate		0		0		0		0		0	
- grade 4 severe		0		0		0		0		0	

* P<0.05
** P<0.01

Table 3.12.1.2-3

Dose (mg/kg food)	**0**		**70**		**700**		**2000**		**3500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	None										
Clinical signs	No treatment-related findings										
Body weight (13 w)							dc	dc	dc	dc	m
Body weight gain						dc	dc	dc	dc	dc	m

Dose (mg/kg food)	0		70		700		2000		3500		dr
	m	f	m	f	m	f	m	f	m	f	
Food consumption							dc	dc	dc	dc	
Ophthalmoscopy	No treatment-related findings										
Haematology											
- haemoglobin					ic		ic		ic		m
- haematocrit					ic		ic		ic		
- MCV							ic		ic		m
- MCH									ic		
- WBC						ic		ic	ic	ic	f
- lymphocytes						ic	ic	ic	ic	ic	m/f
- monocytes								ic		ic	
- basophils										ic	
- prothrombin time							ic		ic	dc	m
- APTT							ic	dc	ic	dc	m
- platelets								ic		ic	
Clin. Chemistry											
- total protein										ic	
- A/G					ic		ic		ic		m
- glucose									dc		
- total cholesterol					ic		ic	ic	ic	ic	m/f
- phospolipids					ic		ic		ic		m
- direct bilirubin									dc		
- gamma-GTP						ic	ic	ic	ic	ic	m/f
- CPK									ic		
- ALP				ic		ic		ic		ic	
- ChE										dc	
- potassium										dc	
- chloride										dc	
Urinalysis	No treatment-related findings										
Hormone analysis											
- testosterone									dc		
- oestradiol										dc	
Organ weights											
- testes							dc[a]		dc[a]		
- pituitary					dc[a]		dc[a]	dc[a]	dc[a]	dc[a]	
- epididymides							dc[a]		dc[a]		
- kidneys									ic[r]	ic[a,r]	
- ovaries								ic[r]		ic[a,r]	
- lungs							ic[r]		ic[r]	ic[a,r]	
- heart							ic[r]		ic[r]	ic[r]	
- liver							ic[r]	ic[r]	ic[r]	ic[r]	m/f
- adrenals									ic[r]	ic[r]	
- brain							ic[r]	ic[r]	ic[r]	ic[r]	
- thyroid							ic[r]		ic[r]		
- thymus				ic[r]		ic[r]		ic[r]		ic[r]	
Pathology											
<u>macroscopy</u>											
- adrenal, enlarged	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/10	
- adrenal, pale	0/10	0/10	0/10	0/10	0/10	0/10	0/10	6/10	0/10	6/10	
- liver, dark	0/10	0/10	1/10	0/10	2/10	1/10	6/10	0/10	10/10	4/10	m
- ovary, enlarged		0/10		0/10		1/10		0/10		6/10	
<u>microscopy</u>											
adrenal:											
- vacuolation, zona reticularis	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/10	6/10	10/10	f
- vacuolation, zona fasciculata	8/10	0/10	6/10	1/10	10/10	0/10	10/10	1/10	9/10	7/10	
- dc vacuolation, zona glomerulosa	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	8/10	
liver:											
- centrilob. hypertrophy	0/10	0/10	1/10	0/10	0/10	0/10	6/10	3/10	8/10	7/10	m/f

Dose (mg/kg food)	0		70		700		2000		3500		dr
	m	f	m	f	m	f	m	f	m	f	
hepatocytes											
- mononuclear cell infiltr.	10/10	4/10	10/10	4/10	10/10	8/10	10/10	10/10	10/10	10/10	f
- single cell necrosis, hepatocytes	10/10	4/10	10/10	4/10	10/10	8/10	10/10	10/10	10/10	10/10	f
lung, foamy/eosin. cell, alveoli	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/10	7/10	
ovary:											
- vacuol. interstitial gland cells		2/10		2/10		4/10		10/10		10/10	f

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative

Acceptability

The study is considered acceptable. Although diet concentrations were not analysed, effects seen coincide with the effects observed in the other 90-day study.

Conclusions

Rats were administered 70, 700, 2000 or 3500 mg/kg food of S-1812 via the diet for 13 weeks. No mortality and no treatment-related clinical signs were seen. Body weight was decreased in males and females at 1000 and 2000 mg/kg food during the whole study. At study termination body weights were decreased when compared to controls at 2000 mg/kg food (90 and 89% of control) and 3500 mg/kg food (87 and 89% of control) in males and females, respectively. Body weight gain was decreased in males and females at 1000 and 2000 mg/kg food (81-87% of control for males and 81% of control for both female groups). In females at 700 mg/kg food body weight gain was also decreased (88% of control). Food consumption was decreased in both sexes at 1000 and 2000 mg/kg food during the whole treatment period. Ophthalmoscopy revealed no treatment-related findings.

Haemoglobin and haematocrit were minimally increased at 700, 2000 and 3500 mg/kg food in males (103-105 and 104-105% of control, resp.). Mean cell volume was minimally increased at 2000 and 3500 mg/kg food (both 103% of control) and mean cell haemoglobin was minimally increased at 3500 mg/kg food in males (104% of control). The changes in red blood cell parameters may point to a slight dehydration. No effect on reticulocytes was noted. White blood cells were increased at 3500 mg/kg food in males (131% of control) and females (195% of control). In females white blood cells were also increased at 700 and 2000 mg/kg food (136-145% of control). Lymphocytes were increased at 2000 and 3500 mg/kg food in males (131-144% of control) and at 700, 2000 and 3500 mg/kg food in females (142-210% of control). Monocytes were increased at 2000 and 3500 mg/kg food in females (175-194% of control). Basophils were increased (200% of control) at 3500 mg/kg food in females. The changes in white blood cell parameters may be related

to abnormal fatty metabolism. Platelets were increased at 2000 and 3500 mg/kg food in females (111-116% of control), which may point to dehydration. Prothrombin time was increased in males at 2000 and 3500 mg/kg food (118-131% of control) and decreased in females at 3500 mg/kg food (95% of control). Activated partial thromboplastin time (APTT) was increased in males at 2000 and 3500 mg/kg food (111-128% of control) and decreased in females at the same dose levels (94-95% of control). Elongation of both coagulation times may reflect disturbed liver function as a number of coagulation proteins are synthesised in the liver. Other statistically significant changes at lowest and/or intermediate dose levels (not presented in the Table) were not considered to be toxicologically relevant because changes were not dose-related.

Total protein was increased in females and A/G ratio was increased in males at 700, 2000 and 3500 mg/kg food with a dose-related response (108-117% of control). Glucose was decreased at 3500 mg/kg food in males (85% of control). Total cholesterol was increased in both sexes at 2000 and 3500 mg/kg food and at 700 mg/kg food in males (142-203% of control for males and 141-142% of control for females). Phospholipids was increased at 700, 2000 and 3500 mg/kg food in males with a dose-related response (133-183% of control). Gamma glutamyltranspeptidase (gamma-GTP) was increased in both sexes at 2000 and 3500 mg/kg food and at 700 mg/kg food in males (150% of control at 3500 mg/kg food for both sexes). Changes in glucose, total cholesterol, phospholipids and gamma-GTP may reflect disturbance of liver function; A/G ratio and gamma-GTP increase may indicate enzyme induction and increased cholesterol and phospholipids in specific may point to affected lipid synthesis/metabolism. Direct bilirubin was decreased (83% of control) and creatine phosphokinase (CPK) was increased (123% of control) in males at 3500 mg/kg food. Alkaline phosphatase was increased in all treated females without a dose-response relationship. However, ALP values were within historical control range at 70, 700 and 3500 mg/kg food and at 2000 mg/kg food the increase was caused by one animal. Therefore, the changes in ALP are not considered to be toxicologically relevant. Cholinesterase, potassium and chloride were decreased at 3500 mg/kg food in females (ChE: 65% of control; K: 86% of control; Cl: 96% of control). Other statistically significant changes at lowest and/or intermediate dose levels (not presented in the Table) were not considered to be toxicologically relevant because changes were not dose-related or values were within historical control range.

Urinalysis showed no treatment-related findings.

Testosterone was decreased in males (46% of control) and oestradiol was decreased in females (27% of control) at 3500 mg/kg food. An abnormal oestrous cycle was noted in one female at 3500 mg/kg food (dioestrous period lasted 7 days).

Absolute ovary weight was increased at 3500 mg/kg food (149% of control) and relative ovary weight was increased at 2000 and 3500 mg/kg food (129-166% of control). Absolute lung weight was increased at 3500 mg/kg food in females (113% of control), and relative lung weight was increased in both sexes at 3500 mg/kg food and in males at 2000 mg/kg food (both 106% of control in males and 128% of control in

females). Relative liver weight was increased in males (108-114% of control) and females (107-118% of control) at 2000 and 3500 mg/kg food. Relative adrenal weight was increased at 3500 mg/kg food in both sexes (113 and 128% of control, resp.). Relative heart weight was increased at 2000 mg/kg food in males and at 3500 mg/kg food in both sexes (115-118% of control; may be caused by reduced lung function). In the absence of histopathological findings the changes in absolute or relative organ weight of testes, pituitary (not dose related), epididymides, kidneys, brain and thyroid are considered to be not toxicologically significant and related to the reduced body weight gain. The increased relative thymus weights in all treated females were within the historical control range.

Macroscopical examination showed a dark liver with an increased incidence in males at 2000 and 3500 mg/kg food and in females at 3500 mg/kg food. Histopathology showed an increased incidence of centrilobular hypertrophy of hepatocytes in both sexes at 2000 and 3500 mg/kg food, which was due to proliferation of the smooth endoplasmatic reticulum (SER) as determined by electron microscopy. An increased incidence of mononuclear cell infiltration and single cell necrosis of hepatocytes was noted at 700, 2000 and 3500 mg/kg food in females compared to controls; these effects were noted in all males including controls, and in males and females the severity is only slight to mild, with no clear dose response. Adrenals were noted to be enlarged in 3/10 females at 3500 mg/kg food and pale in 6/10 females at 2000 and 3500 mg/kg food. Vacuolation of the zona reticularis was seen in 6/10 males and in all females at 3500 mg/kg food and in 3/10 females at 2000 mg/kg food with increased severeness in females at 3500 mg/kg food (none in the controls). In females at 3500 mg/kg food an increased incidence of vacuolation of the zona fasciculata was noted, while decreased vacuolation of the zona glomerulosa was seen. Adrenal vacuolation was confirmed to be fatty in nature by electron microscopy. An enlarged ovary was observed in females at 3500 mg/kg food and vacuolation of interstitial gland cells was increased in incidence at 700 mg/kg food and higher with increased severity from slight to mild at 2000 and 3500 mg/kg food (no further indication for organ dysfunction). In the alveoli of the lung an increased incidence of accumulation of foamy/eosinophilic cells was seen in both sexes at 3500 mg/kg food; electron microscopically no abnormalities were observed.

Effects seen on the liver, i.e. increased total cholesterol, phospholipids and gamma-GTP, and increased relative weights and centrilobular hypertrophy caused by SER proliferation point to disturbance of lipid synthesis/metabolism and induction of enzymes. Hormone synthesis/excretion seems to be disrupted in adrenals as well as ovaries with concomitant vacuolation of the zona reticularis in the adrenal and the interstitial gland cells in the ovaries. The NOAEL is set at 70 mg/kg food (equivalent to 4.68 mg/kg bw/d for males and 5.37 mg/kg bw/d for females) based on decreased body weight gain, changes on white blood cell parameters and effects on the liver and ovaries.

3.12.1.3 Study 3 – 13-week oral study in rat

Characteristics

Reference	:	IIA 5.3.2/02	exposure	:	13 weeks, diet

		Study No 98-0075			
Type of study	:	13-week oral toxicity study	dose	:	0, 100, 1000 and 2000 mg/kg food (nominal)[1]
year of execution	:	1998	vehicle	:	none
test substance	:	S-1812 (pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	yes
route	:	oral	guideline	:	in accordance with OECD 408 (1998)
species	:	rat, Sprague-Dawley (Crj:CD(SD))	acceptability	:	acceptable
group size	:	10/sex/dose	**NOAEL**	:	5.56 mg/kg bw/day for males 6.45 mg/kg bw/day for females

[1]equal to 0, 5.56, 56.0 and 111.3 mg/kg bw/d for males and 0, 6.45, 64.0 and 128.6 mg/kg bw/d for females (actual test substance intake)

Study design

The study was in compliance with OECD 408 (1998). Dose levels were based on the earlier 90-day study in rats (IIA 5.3.2/01). Additionally, urinalysis was performed on all surviving animals in week 13.

Results

The results are summarised in table 3.12.1.3-1 to -3.

Table 3.12.1.3-1

	Males				Females			
Diet concentration	**0**	**100**	**1000**	**2000**	**0**	**100**	**1000**	**2000**
Bodyweight gain (g)	373	373	330**	319**	195	193	170*	173
Food consumption, weeks 1 - 13 (g/rat/day)#	22.1	21.9	20.3	19.8	17.3	16.9	16.0	16.2
mg test material/kg bw/day		5.6	56	111		6.5	64	129
Blood Chemistry								
Total protein (g/dL)	6.5	6.3	6.3	6.3	6.4	6.5	6.6	6.4
A/G ratio	0.82	0.84	0.86	0.89*	1.03	1.03	1.03	1.02
T. cholesterol (mg/dL)	52	49	62	80**	62	64	78	96**
Triglycerides (mg/dL)	104	127	100	114	82	106	86	83
Gamma-GT (U/L)	1	1	1	1	2	1	2	2*
CPK (U/L)	191	169	142	130*	123	135	125	132
Organ Weights								
Necropsy bodyweight (g)	501	504	458**	449**	313	312	290	292
Liver (g)	12.4	12.5	12.0	12.2	7.29	7.56	7.20	7.88
Liver (% bodyweight)	2.48	2.48	2.62	2.71**	2.33	2.41	2.48*	2.69**
Histopathology								
Liver								
Centrilobular hepatocyte hypertrophy	0/10	0/10	0/10	8/10**	0/10	0/10	0/10	5/9*
Single-cell hepatocyte necrosis	10/10	10/10	10/10	10/10	4/10	5/10	7/10	9/9**
Lung								
alveolar foam cells accumulation	2/10	2/10	5/10	5/10	2/10	3/10	5/10	5/10
Ovary								
Interstitial cell vacuolation					0/10	0/10	2/10	8/9**
Adrenal Cortex								
Zona reticularis cell vacuolation	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/9

Level of statistical significance: * $0.05 \geq p > 0.01$, ** $p \leq 0.01$ in comparison with control
No statistical analysis applied.

Table 3.12.1.3-2

Dose (mg/kg food)	0		100		1000		2000		dr
	m	f	m	f	m	f	m	f	
Ovary									
vacuol. interstitial gland cells, total		0		0		2		8**	
- slight		0		0		2		8	
- moderate		0		0		0		0	
- severe		0		0		0		0	

* P<0.05
** P<0.01

Table 3.12.1.3-3

Dose (mg/kg food)	0		100		1000		2000		dr
	m	f	m	f	m	f	m	f	
Mortality	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	
Clinical signs	No treatment-related findings								
Body weight (13 w)					dc	d	dc	d	
Body weight gain					dc	dc	dc	d	m
Food consumption					dc	d	dc	d	
Functional observations	No treatment-related findings								
Ophthalmology	No treatment-related findings								
Haematology	No treatment-related findings								
Clin. Chemistry									
- total cholesterol							ic	ic	
- CPK							dc		
- A/G ratio							ic		
- gamma-GTP								ic	
Urinalysis	No treatment-related findings								
Organ weights									
- pituitary					dc[a]		d[a]		
- spleen							dc[a]		
- brain					ic[r]		ic[r]		
- heart							ic[r]	ic[r]	
- liver						ic[r]	ic[r]	ic[r]	
- kidneys						ic[r]	ic[r]	ic[r]	
- lungs							ic[r]		
- epididymides					ic[r]				
Pathology									
macroscopy									
- liver, dark	0/10	0/10	0/10	0/10	0/10	0/10	5/10	5/9	
- liver, enlarged	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/9	
microscopy									
lung:									
- alveolar, foamy cell accumulation	2/10	2/10	2/10	3/10	5/10	5/10	5/10	5/9	
liver:									
- hepatocyte, single cell necrosis									
- grade 1, slight	9/10	4/10	8/10	5/10	9/10	5/10	9/10	5/9	f

Dose (mg/kg food)	0		100		1000		2000		dr
	m	f	m	f	m	f	m	f	
- grade 2, moderate	1/10	0/10	2/10	0/10	1/10	2/10	1/10	4/9	
- hepatocyte, centrilob. hypertrophy	0/10	0/10	0/10	0/10	0/10	0/10	8/10	5/9	
adrenal:									
- zona reticularis, vacuolation	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/9	
ovary:									f
- interstitial gland cells, vacuolation		0/10		0/10		2/10		8/9	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative

Acceptability

The study is considered acceptable.

Conclusions

Rats were administered 100, 1000 or 2000 mg/kg food of S-1812 via the diet for 13 weeks. The death of the female at 2000 mg/kg food in week 9 was found to be related to hepatic necrosis. No treatment-related clinical signs were seen. Body weight was decreased in males and females at 1000 and 2000 mg/kg food during the whole study (at study termination 92 and 89% in males and 93% in females). Total body weight gain was decreased in males and females at 1000 and 2000 mg/kg food (86-89% of control) due to lower body weight gains during the first 5-7 weeks of treatment. Food consumption was decreased in males at 1000 and 2000 mg/kg food (90-92% of control) during the whole treatment period and in females at 1000 and 2000 mg/kg food (92-94% of control) during the first 6 weeks of treatment. The decrease for female food consumption was not statistically significant. Functional observations and ophthalmology revealed no treatment-related findings.

Clinical biochemistry revealed changes indicative of liver toxicity. Total cholesterol was significantly increased in both sexes at 2000 mg/kg food (154-155% of control). Gamma glutamyltranspeptidase (gamma-GTP) was increased in females at 2000 mg/kg food (140% of control). Creatine phosphokinase (CPK) was decreased (68% of control) and albumin/globulin ratio was increased (109% of control) in males at 2000 mg/kg food; the toxicological significance of both effects are not clear.

Urinalysis showed no treatment-related findings.

Decreases in absolute weight of the pituitary and spleen and increases in the relative weight of brain, heart, kidneys, lungs and epididymides at 1000 and 2000 mg/kg food are all considered to be related to decreased body weight gain. The increased relative liver weight in males (109% of control) and females (115% of

control) at 2000 mg/kg food and females at 1000 mg/kg food (106% of control) may be related to the histopathological effects found.

Macroscopic examination showed a dark and enlarged liver in half of males and females at 2000 mg/kg food, which was also seen in the female that had died in week 9. Histopathology showed centrilobular hypertrophy of hepatocytes in both sexes at 2000 mg/kg food. In females at 1000 and 2000 mg/kg food increased single cell necrosis of hepatocytes (slight to moderate) was noted with an increased incidence compared to controls and also in the female that died. In both sexes an increased incidence of foamy cell accumulation of the alveolar space was found at 1000 and 2000 mg/kg food compared to the controls. Vacuolation of the adrenal zona reticularis was seen in 3/10 females at 2000 mg/kg food. In ovary, vacuolation of interstitial gland cells was observed in 2/10 and 8/10 females at 1000 and 2000 mg/kg food, respectively.

The NOAEL is set at 100 mg/kg food (equal to 5.56 mg/kg bw/d for males and 6.45 mg/kg bw/d for females) based on decreased body weight gain, decreased food consumption, increased liver weights and histopathological changes seen in liver and ovary.

3.12.1.4 Study 4 – 13 week oral study in mouse

Characteristics

Reference	:	IIA 5.3.2/03 Study No. SUT-0004	exposure	:	13 weeks, diet
Type of study	:	13-week oral toxicity study	dose	:	0, 70, 700, 3500 and 7000 mg/kg food (nominal)[1]
year of execution	:	1998	vehicle	:	none
test substance	:	S-1812 (pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	yes
route	:	oral	guideline	:	in accordance with OECD 408 (1998)
species	:	mouse, ICR (Crj:CD-1)	acceptability	:	acceptable
group size	:	12/sex/dose	**NOAEL**	:	8.17 mg/kg bw/day for males 86.8 mg/kg bw/day for females

[1]equal to 0, 8.17, 81.7, 379 and 721 mg/kg bw/d for males and 0, 9.50, 86.8, 415 and 879 mg/kg bw/d for females (actual test substance intake)

Study design

The study was generally in compliance with OECD 408 (1998). Functional observations and ophthalmoscopy were not performed, and potassium, sodium and chloride in blood were not determined. Dose levels were based on a 2-week toxicity study (Nishimoto, 1997; not available to the reviewer). Haematology and clinical biochemistry parameters were determined after 13 weeks of treatment. Histopathology was performed on all tissues from all animals at 0 and 7000 mg/kg food and on liver, kidneys, lungs, adrenals, testes, ovaries, uterus, vagina, and gross lesions from all animals at 70, 700 and 3500 mg/kg food.

Results

The results are summarised in table 3.12.1.4-1 and -2.

Table 3.12.1.4-1

	Males					Females				
Diet concentration	**0**	**70**	**700**	**3500**	**7000**	**0**	**70**	**700**	**3500**	**7000**
Bodyweight gain (g)	16.4	18.1	17.2	12.7	12.2	10.8	8.8	11.2	7.3	7.8
Food consumption, weeks 1 - 13 (g/mouse/day)	4.9	4.9	4.9	4.3	4.0	4.0	4.2	3.9	3.5	3.8
mg test material/kg bw/day	0	8.2	81.7	379	721	0	9.5	86.8	415	879
Haematology										
Hb (g/dL)	13.8	13.6	13.5	12.9**	12.7**	13.7	14.1	13.4	13.0	13.0
Ht (%)	40.3	40.6	40.2	37.8*	37.1**	39.8	41.1	39.1	37.2*	37.4*
RBC (10^6/mm^3)	8.15	8.08	8.06	7.64*	7.65*	8.01	8.29	7.79	7.60	7.65
Blood Chemistry										
ALP (U/L)	37	36	35	53**	66**	55	59	54	59	67
SGOT (U/L)	59	46	48	56	64*	56	53	72	65	52
SGPT (U/L)	39	31	32	39	67**	27	25	29	25	27
Total protein (g/dL)	4.5	4.5	4.7	4.8*	4.7	4.6	4.6	4.5	4.8	4.9**
Albumin	1.87	1.95	2.02*	2.11**	2.13**	2.20	2.22	2.19	2.32	2.44**
A/G ratio	0.71	0.75	0.75	0.79*	0.83**	0.94	0.92	0.94	0.94	1.00
Glucose (mg/dL)	169	185	186	175	187	184	177	182	160	159
T. cholesterol (mg/dL)	112	113	143*	152**	139	88	76	88	149**	154**
Triglycerides (mg/dL)	145	149	173	122	76**	144	89*	121	104	92*
Organ weights										
Terminal bodyweight (g)	47.2	48.8	47.9	43.4	43.0	35.7	33.7	36.1	32.2*	32.7
Liver (g)	2.2	2.3	2.3	2.5*	2.9**	1.74	1.58	1.78	1.79	2.09**
Kidney (% bwt)	1.46	1.48	1.43	1.38	1.30*	1.08	1.17	1.11	1.12	1.19
Ovarian weight (mg)						22.2	23.1	20.3	17.5	15.8*
Histopathology										
Liver: hepatocyte hypertrophy, centrilobular	0/11	0/12	0/12	9/12**	12/12**	0/12	0/12	0/12	0/12	10/12**
Liver; hepatocyte vacuolation, centrilobular	0/11	0/12	0/12	0/12	12/12**	0/12	0/12	0/12	0/12	0/12
Kidney: tubular basophilic change	1/11	4/12	2/12	1/12	6/12*	2/12	1/12	0/12	3/12	8/12*
Adrenal: brown pigment, cortico-medullary junction	1/11	1/12	2/12	3/12	7/12*	0/12	0/12	0/12	0/12	0/12
Ovary: atrophy						0/12	0/12	0/12	4/12*	7/12**

Level of statistical significance: * $0.05 \geq p > 0.01$, ** $0.01 \geq p$ in comparison with control

No statistical analysis applied.

Table 3.12.1.4-1

Dose (mg/kg food)	0		70		700		3500		7000		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	No treatment-related findings										
Clinical signs	No treatment-related findings										
Body weight							d	dc	d	d	
Body weight gain							d	dc	d	dc	m
Food consumption							d	d	dc		m
Haematology											
- haemoglobin							dc	d	dc	d	m
- haematocrit							dc	dc	dc	dc	m
- RBC							dc	d	dc	d	
Clin. Chemistry											
- alkaline phosphatase							ic		ic		m
- ALT									ic		
- AST									ic		
- total protein							ic			ic	
- albumin					ic		ic		ic	ic	m
- A/G ratio							ic		ic		m
- total cholesterol					ic		ic	ic	i	ic	f
- triglycerides									dc	dc	
- creatinine								ic		ic	
- calcium										ic	
Organ weights											
- liver							ic^{a,r}	ic^{r}	ic^{a,r}	ic^{a,r}	m/f
- kidneys							dc^{a}		dc^{a,r}		m
- ovaries								d^{a,r}		dc^{a}, d^{r}	f
Pathology											
<u>macroscopy</u>											
- liver, accentuated lobular pattern	0/12	0/12	0/12	0/12	0/12	0/12	1/12	0/12	10/12	0/12	
<u>microscopy</u>											
liver:											
- centrilob. vacuolation hepatocyte	0/11	0/12	0/12	0/12	0/12	0/12	0/12	0/12	12/12	0/12	
- centrilob. hypertrophy hepatocyte	0/11	0/12	0/12	0/12	0/12	0/12	9/12	0/12	12/12	10/12	m
- kidney, basophilic change, tubular cell	1/11	2/12	4/12	1/12	2/12	0/12	1/12	3/12	6/12	8/12	
- adrenal, brown pigment deposition, cortico-medullary junction	1/11	0/12	1/12	0/12	2/12	0/12	3/12	0/12	7/12	0/12	
- ovary, atrophy		0/12		0/12		0/12		4/12		7/12	f

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative

Acceptability

The study is considered acceptable.

Conclusions

Mice were administered 70, 700, 3500 or 7000 mg/kg food of S-1812 via the diet for 13 weeks. No treatment-related mortality (one control male died) or clinical signs were seen. Body weight was slightly decreased at 3500 and 7000 mg/kg food in males and females during the whole study (91-92% and 90-92% of controls for males and females, respectively at study termination). Total body weight gain was decreased at 3500 and 7000 mg/kg food in males (73-76% of control; not statistically significant) and females (68-72% of control). Food consumption was decreased in males at 3500 and 7000 mg/kg food (82-88% of control) during the whole treatment period. Food consumption in females at 3500 mg/kg food was decreased (88% of control), but at 7000 mg/kg food the decrease (95% of control) was equal to the increase at 70 mg/kg food and therefore, this decrease is not considered to be toxicologically relevant.

Haematocrit, haemoglobin and erythrocyte count were decreased at 3500 and 7000 mg/kg food in males (92-94%, 92-93% and 94% of control, resp.) and females (93-94%, 95% and 95-96% of control). The decrease in haemoglobin and erythrocyte count in females was not statistically significant. No effect on reticulocytes was noted.

Alkaline phosphatase was significantly increased at 3500 and 7000 mg/kg food in males in a dose-related manner (143-178% of control). Alanine aminotransferase and aspartate aminotransferase were increased in males at 7000 mg/kg food (172 and 108% of control, resp.). Total protein was increased at 3500 mg/kg food in males (106% of control) and 7000 mg/kg food in females (107% of control). Albumin was increased at 700, 3500 and 7000 mg/kg food in males (108-114% of control; dose-related) and at 7000 mg/kg food in females (111% of control). Concomitantly, the A/G ratio was increased at 3500 and 7000 mg/kg food in males (111-117% of control; dose-related). Total cholesterol was increased at 3500 and 7000 mg/kg food in males (124-136% of control) and females (169-175% of control; dose-related) and in males at 700 mg/kg food (128% of control). Triglycerides were decreased at 7000 mg/kg food in males (52% of control) and females (64% of control). Creatinine was increased at 3500 and 7000 mg/kg food in females (both 122% of control). Calcium was increased at 7000 mg/kg food in females (104% of control). Other statistically significant changes at the lowest and/or intermediate dose level (not presented in the Table) were not considered to be toxicologically relevant because changes were not dose-related. The changes in liver enzymes, total cholesterol and triglycerides are considered to be related to liver injury and/or changes in lipid metabolism.

Absolute liver weight was increased at 3500 and 7000 mg/kg food in males (115-132% of control; dose-related) and at 7000 mg/kg food in females (120% of control). Relative liver weight was increased at 3500 and 7000 mg/kg food in males (125-145% of control; dose-related) and in females (115-132% of control; dose-related). Absolute kidney weight was decreased at 3500 and 7000 mg/kg food in males (81-87% of control; dose-related) and relative kidney weight was decreased at 7000 mg/kg food in males (89% of control). Ovary weight was decreased in females at 3500 and 7000 mg/kg food (abs: 71-79% of control; rel:

77-89% of control; dose-related); only the decrease in absolute weight at 7000 mg/kg food was statistically significant.

Macroscopic examination revealed an accentuated lobular liver pattern in most males at 7000 mg/kg food. Histopathology showed centrilobular vacuolation in all males at 7000 mg/kg food and centrilobular hypertrophy of hepatocytes in all males and 10/12 females at 7000 mg/kg food and 9/12 males at 3500 mg/kg food. Staining of the vacuoles with Oil red O stain suggested they contained lipid. In the kidneys of both sexes at 7000 mg/kg food an increased incidence of basophilic change in tubular cells was noted. In the adrenals a brown pigment deposition in the cortico-medullary junction was seen with increased incidence at 7000 mg/kg food in males. The decreased ovary weight correlated with the atrophy noted in 4/12 and 7/12 females at 3500 and 7000 mg/kg food, respectively. The changes at ovary (atrophy and a decrease in organ weight) could be related to the effect at body weights.

The NOAEL is set at 70 mg/kg food (equal to 8.17 mg/kg bw/d for males and 9.50 mg/kg bw/d for females) based on increased albumin and total cholesterol in males at 700 mg/kg food and histopathological effects found in the liver at 3500 mg/kg food and higher.

3.12.1.5 Study 5 – 90-day oral study in dog

Characteristics

reference	:	IIA 5.3.3/01 Study No. 29814	exposure	:	Gelatine capsule, once daily, 90 days
type of study	:	90-day oral toxicity study	doses	:	0, 10, 100 or 300 mg/kg bw/day
year of execution	:	1998 – 1999	vehicle	:	None
test substance	:	S-1812 (pyridalyl), lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 409 (1998)
species	:	dog, Beagle	acceptability	:	Acceptable
group size	:	4/sex/dose	**NOAEL**	:	10 mg/kg bw/day

Study design

The study was generally in accordance with OECD 409 (1998). Initially, high dose animals received 1000 mg/kg bw/day. One male and one female of the high dose group died on day 2 and 3 of administration, respectively. Therefore, the high dose level was decreased to 300 mg/kg bw/day from day 15 of the study in males and day 8 in females. Two additional animals were assigned to the high dose group.

In addition to the standard examinations in a subchronic oral toxicity study in dogs according to OECD 409, bone marrow smears were prepared of all animals at study termination. Smears were examined microscopically for differential cell count.

Results

The results are summarised in table 3.12.1.5-1 and -2.

Table 3.12.1.5-1

	Males				Females			
Dose (mg/kg bw/day)	**0**	**10**	**100**	**1000/ 300**	**0**	**10**	**100**	**1000/ 300**
Mortality	0	0	0	1/5	0	0	0	2/5
Tachypnoea/wheezing	0	0	0	1/5				3/5
Bodyweight gain (kg)	2.34	2.09	1.74	1.25	1.39	1.33	0.92	0.71
Haematology								
Erythrocytes, (10^4/µL)week 2/3	643	583	574	555	626	649	608	659
Haemoglobin (g/dL) week 2/3	14.3	13.2	12.8	12.2*	13.4	13.9	13.2	14.4
Haematocrit (%) week 2/3	42.5	39.6	38.5	36.4*	40.2	42.1	39.4	43.1*
Blood Chemistry								
Glucose (mg/dL) week 2/3	86	91	94*	98**	91	87	93	94
Glucose (mg/dL) week 4	98	97	97	100	89	91	97*	94
InP (mg/dL) week 13	5.8	5.2	5.1	4.8**	4.7	4.4	4.4	4.9
Ca^{2+} (mg/dL) week 13	11.4	11.2	11.4	11.1	11.2	11.1	10.8*	10.6**
Potassium (mEq/L) week 13	4.8	4.8	4.5	4.7	5.0	4.5**	4.3**	4.7
Organ Weights (survivors)								
Necropsy bodyweight (g)	10.7	10.6	10.0	9.8	8.9	9.0	8.5	8.1
Lung (g)	71.1	77.7	89.2	94.4*	67.3	73.7	91.2**	83.7
Liver (g)	276	261	270	295	216	220	232	265*
Kidney (g)	46.8	43.3	43.7	41.5	32.9	36.7	37.0	37.7
Histopathology#								
Lung: artery – wall thickened	0/4	0/4	0/4	1/5	0/4	0/4	1/4	3/5
Lung: arteriole-wall thickened	0/4	0/4	0/4	1/5	0/4	0/4	1/4	2/5
Lung: cellular infiltration, perivascular, lymphocyte	0/4	0/4	0/4	1/5	0/4	0/4	1/4	1/5
Adrenal: vacuolation, zona fasciculata	0/4	0/4	0/4	4/5	0/4	0/4	3/4	4/5
Liver: hepatocellular hypertrophy	0/4	0/4	0/4	0/5	0/4	0/4	0/4	2/5
Liver: hepatocyte inclusion	0/4	0/4	0/4	0/5	0/4	0/4	2/4	3/5
Liver; hepatocyte vacuolation	0/4	0/4	0/4	0/5	0/4	0/4	1/4	2/5
Kidney: proximal tubule – brown pigment	0/4	0/4	0/4	0/4	0/4	0/4	1/4	1/5

Level of statistical significance: * 0.05≥ p > 0.01, ** 0.01≥ p in comparison with control
Incidence expressed as dogs affected/ dogs examined, no statistical analysis applied.

Table 3.12.1.5-2

Dose (mg/kgbw/day)	**0**		**10**		**100**		**1000/300[1]**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality							1	1	
Clinical signs									
- abnormal respiration						+	+	+	

Dose (mg/kgbw/day)	0		10		100		1000/300[1]		dr
	m	f	m	f	m	f	m	f	
Body weight (day 89)					d	d	d	d	
Body weight gain					d	d	d	d	
Food consumption	No treatment-related findings								
Ophthalmoscopy	No treatment-related findings								
Haematology									
- erythrocytes							d		
- haemoglobin							d		
- haematocrit							d		
Clinical chemistry					See text conclusions				
Urinalysis	No treatment-related findings								
Organ weights									
- lung					ic^{ar}	$ic^{r}i^{a}$	ic^{ar}	$ic^{r}i^{a}$	
- liver							i^{ar}	$ic^{a}i^{r}$	
- kidneys								ic^{r}	
Pathology									
macroscopy	No treatment-related findings								
microscopy									
lung:									
- artery wall thickened	0/4	0/4	0/4	0/4	0/4	1/4	1/4	3/5	
- arteriole wall thickened	0/4	0/4	0/4	0/4	0/4	1/4	1/4	2/5	
- cellular infiltration perivascular, lymphocyte	0/4	0/4	0/4	0/4	0/4	1/4	1/4	1/5	
adrenal:									
- vacuolation, zona fasciculate	0/4	0/4	0/4	0/4	0/4	3/4	4/4	4/5	
liver:									
- hepatocellular hypertrophy	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/5	
- hepatocytes inclusion	1/4	0/4	0/4	0/4	0/4	2/4	0/4	3/5	
- hepatocytes vacuolation	0/4	0/4	0/4	0/4	0/4	1/4	0/4	2/5	
kidney:									
- proximal tubule – brown pigment	0/4	0/4	0/4	0/4	0/4	1/4	0/4	1/5	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
1 One male and one female at 1000 mg/kg bw/day died on day 2 and 3 of administration, respectively. Therefore, the high dose level was decreased to 300 mg/kg bw/day from day 15 of the study in males and day 8 in females. Two additional animals were assigned to the high dose group.

Acceptability

The study is considered acceptable.

Stability of the test substance in capsule was separately investigated in a stability test of S-1812 in capsules (Dohi, 1999). Recovery of the test substance was 94.0% on day 0 of storage and 93.6% on day 14 of storage.

Conclusions

Dogs were exposed to 0, 10, 100 and 300/1000 mg/kg bw/d pyridalyl in a gelatin capsule for 13 weeks. One male and one female receiving 1000 mg/kg bw/day died on days 2 and 3, respectively. Therefore, the high

dose level was decreased to 300 mg/kg bw/day. One female at 300 mg/kg bw/day died on day 38 of the study. Abnormal respiration as tachypnea, wheezing, abdominal respiration and/or dyspnea were observed in high dose males and in females at 100 mg/kg bw/day and above.

Decreased body weight gain was observed in females at 300 mg/kg bw/day throughout the study period and in males at 300 mg/kg bw/day from day 49. At 100 mg/kg bw/day decreased body weight gain was observed in males and females from day 56 and 77, respectively. At termination of the study, body weights of males and females at 100 mg/kg bw/day (94% and 96% of controls) and at 300 mg/kg bw/day (93% and 92% of controls) were reduced. No changes in food consumption and ophthalmoscopy were observed.

A decrease in erythrocytes (86 and 90% of controls), haemoglobin (85% and 89% of controls) and haematocrit (86% and 90% of controls) was noted among males at 300 mg/kg bw/day in week 3 and 4 of the study, respectively. Similar changes were noted in one female at 300 mg/kg bw/day in week 4, 8 and 13. No treatment-related changes in reticulocytes and in bone marrow smears were noted.

Slight changes indicative of liver toxicity were noted in males and females at 300 mg/kg bw/day. As the number of animals per group is rather small and the parameters have broad physiological ranges, no clear statistical changes were observed. One female animal at 300 mg/kg bw/day showed an increase in alkaline phosphatase in week 4, 8 and 13 (>280% of pre-test). The same female showed an increase in cholesterol (190% of pre-test). A slight decrease in calcium was noted in females at 100 and 300 mg/kg bw/day at the end of the study period (96% and 95% of control, respectively). A decrease in inorganic phosphate was noted at 300 mg/kg bw/day in males (83% of controls). Some statistically significant changes were noted when compared with control animals, e.g. glucose, and gamma GT in males at 300 mg/kg bw/day. However, when the same data were compared with pre-test values of the same group, no treatment-related changes were apparent.

When animals showed apparent moribundity, additional biochemical examinations were performed, one male at 1000 mg/kg bw/day at day 2, one female at 100 mg/kg bw/day on day 10 and one female at 300 mg/kg bw/day on day 37. The male at 1000 mg/kg bw/day showed increases in ASAT, alkaline phosphatase, blood urea nitrogen, inorganic phosphate and potassium. The female at 100 mg/kg bw/day showed an increase in alkaline phosphatase and a decrease in sodium. The female at 300 mg/kg bw/day showed increased ALAT, blood urea nitrogen, gamma GT and cholesterol and a decrease in sodium. Other changes in clinical biochemistry were considered incidental as the changes were observed at one time point and were not dose-related.

No changes in urinalysis were observed.

Absolute lung weights were increased at 100 and 300 mg/kg bw/d in males (125 and 133% of controls) and females (135 and 129% of controls). Relative lung weights were increased at 100 and 300 mg/kg bw/d in both males (134 and 144% of controls) and females (142 and 139% of controls). Absolute and relative liver weights were increased at 300 mg/kg bw/d in males (107 and 118% of controls) and females (122 and 138%

of controls). Relative kidney weights were increased at 300 mg/kg bw/day in females only (126% of controls).

Histopathology showed changes in liver, adrenals, lungs and kidneys. In liver, vacuolation of hepatocytes was noted in females at 100 and 300 mg/kg bw/day, and hypertrophy of the centrilobular hepatocytes was noted in females at 300 mg/kg bw/day. Hepatocyte inclusion was noted in females at 100 and 300 mg/kg bw/day. In adrenals, vacuolation of the cortical cells in the zona fasciculate was observed in the dead and surviving females at 100 and 300 mg/kg bw/day, and surviving males at 300 mg/kg bw/day. As there were no biochemical correlates, the toxicological significance of this finding is unclear.

In lungs, thickening of the arterial and arteriolar wall and cellular infiltration of lymphocytes were noted in females at 100 and 300 mg/kg bw/day and in males at 300 mg/kg bw/day. Additional histopathological changes were seen in animals that died during the study, a.o. haemorrhage of the perivascular region, oedema of the perivascular region and alveoli. Changes in lungs are related to the abnormal respiration seen in females at 100 and 300 mg/kg bw/day and males at 300 mg/kg bw/day.

In kidneys, deposition of brown pigment in the proximal tubules was observed in the surviving females at 100 and 300 mg/kg bw/day.

Based on changes in body weight, and histopathological changes in adrenals, liver and lungs at 100 and 300 mg/kg bw/d, the NOAEL is set at 10 mg/kg bw/day.

3.12.1.6 Study 6 – 1-year oral study in dog

Characteristics

reference	:	IIA 5.3.4/01 Study No. 29917	exposure	:	Gelatine capsule, once daily, 1-year
type of study	:	1-year oral toxicity study	doses	:	0, 1.5, 5, 20 or 80 mg/kg bw/day[1]
year of execution	:	1999 – 2000	vehicle	:	None
test substance	:	S-1812 (pyridalyl), lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 409 (1998)
species	:	dog, Beagle	acceptability	:	Acceptable
group size	:	4/sex/dose	**NOAEL**	:	20 mg/kg bw/day

Study design

The study was generally in accordance with OECD 409 (1998).

Results

The results are summarised in table 13.12.1.6-1 and -2.

Table 13.12.1.6-1

	Males					Females				
Dose (mg/kg bw/day)	**0**	**1.5**	**5**	**20**	**80**	**0**	**1.5**	**5**	**20**	**80**
Haematology										
MCH (pg) Week 13	22.0	22.3	21.0	21.8	20.4*	22.0	22.2	22.0	22.0	21.0*
Platelets (10^4/µL), week 26	25.0	28.9	21.9	24.1	24.6	19.4	18.7	20.7	22.2	28.3*
Blood Chemistry										
ALP (IU/L) Week 39	83	83	98	104	168*	94	117	83	113	180
Organ Weights										
Necropsy bodyweight (kg)	11.0	12.1	11.6	11.4	11.0	11.2	10.3	9.6	10.0	11.1
Liver (g)	234.7	241.1	227.7	256.5	266.0	219.5	228.7	210.9	220.8	284.5
Liver (g/kg bodyweight)	21.5	19.8	19.8	22.5	24.5	19.5	22.1	22.2	22.3	25.7*

Level of statistical significance: * $0.05 \geq p > 0.01$ in comparison with control

Table 13.12.1.6-2

Dose (mg/kg bw/day)	**0**		**1.5**		**5**		**20**		**80**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No morality										
Clinical signs	No treatment-related findings										
Body weight	No treatment-related findings										
Body weight gain	No treatment-related findings										
Food consumption	No treatment-related findings										
Ophthalmoscopy	No treatment-related findings										
Haematology - MCH									dc	dc	
Clinical chemistry - alkaline phosphatase									d	d	
Urinalysis	No treatment-related findings										
Organ weights - liver - lungs - epididymes									i^{ar}	i^{a} ,ic^{r} i^{ar} d^{ar}	
Pathology											
macroscopy	No treatment-related findings										
microscopy	No treatment-related findings										

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight

Acceptability

The study is considered acceptable.

Stability of the test substance in capsule was separately investigated in a stability test of S-1812 in capsule (Dohi, 1999). Recovery of the test substance was 94.0% on day 0 of storage and 93.6% on day 14 of storage. Historical control values were given for haematology and clinical biochemistry. However, values were presented in absence of detailed data on e.g. species (strain, supplier), performing laboratory and study period. Therefore, the reported historical control data are of limited value.

Conclusions

Dogs were administered 0, 1.5, 5, 20 or 80 mg/kg bw/day of S-1812 in gelatin capsules for 1-year. No mortality and clinical signs were observed. No changes in body weight gain, food consumption and ophthalmoscopy were noted.

A decrease in MCH was noted in males at 80 mg/kg bw/day in week 13 (93% of controls) and in females at 80 mg/kg bw/day in week 13, 26 and 39 (95-96% of controls). Changes in MCH were only slight, but considering the anaemia as seen in the 90-day study (IIA 5.3.3/01), a relation to treatment with the test substance cannot be excluded.

Other changes (e.g. increase in platelets in females at 80 mg/kg bw/day in week 26, in decreased neutrophils at 20 mg/kg bw/day in week 52), were not considered toxicologically relevant since changes were not consistent throughout the study period, were not dose related or were considered to be due to abnormal control values in a specific week. No treatment-related changes in bone marrow smears were noted.

Changes indicative of liver toxicity were noted at clinical biochemistry. An increase in alkaline phosphatase was noted in males at 80 mg/kg bw/day in week 13, 26, 39 and 52 (157-218% of controls) and in females at 80 mg/kg bw/day in week 39 and 52 (135-191% of controls). Further changes in clinical biochemistry were not considered toxicologically relevant as changes were not consistent throughout the study period, were not dose related or were considered to be due to abnormal control values in a specific week.

Non statistically significant changes in organ weights were noted in liver (except for relative liver weight in females), lungs and epididymes at 80 mg/kg bw/day. Absolute liver weights were increased in males and females at 80 mg/kg bw/day (113 and 130% of controls, respectively) and relative weights were increased in both sexes at 80 mg/kg bw/day (114 and 132% of controls). Absolute and relative lung weights were increased at 80 mg/kg bw/day in males (117 and 120% of controls, respectively). Absolute and relative epididymes weights were decreased in males at 80 mg/kg bw/day (83 and 82% of controls, respectively). Changes in organ weights were not accompanied by macroscopic or histopathological changes.

Based on changes in MCH, liver (organ weight, clinical biochemistry) and lungs (organ weight) at 80 mg/kg bw/d, the NOAEL is set at 20 mg/kg bw/day .

3.12.1.7 Study 7 - 28-day dermal toxicity study rat

Characteristics

Reference	:	IIA 5.3.7/02 Study No. 20047	exposure	:	28 days, 6 hours/day, 10% body surface, dorsal area, occlusive
type of study	:	subacute dermal toxicity	dose	:	0 (distilled water), 30, 100 and 1000 mg/kg bw/day
year of execution	:	2001	vehicle	:	None
test substance	:	S-1812 (pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	Yes
Route	:	Dermal	guideline	:	In accordance with OECD 410 (1981)
Species	:	Rats, Crj:CD(SD)	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL**	:	100 mg/kg bw/day

Study design

The study was performed in accordance with OECD 410 (1981). Dose levels were based on a range-finding study in rats given a dermal dose of 0, 100, 300 or 1000 mg/kg bw S-1812 (Ogata, 2001). No mortality, clinical signs or skin reactions were noted. No treatment-related findings were observed on body weight, food consumption, haematological and biochemical parameters and necropsy. Cholesterol was increased at 300 and 1000 mg/kg bw in males (127-128% of control) and females (110-113% of control) without a dose-relationship. The absolute and relative adrenal weight were increased at 1000 mg/kg bw in males.

Results

The results are summarised in table 13.12.1.7-1 and -2.

Table 13.12.1.7-1

	Males				Females			
Dose (mg/kg bw/day)	**0**	**30**	**100**	**1000**	**0**	**30**	**100**	**1000**
Food consumption (g/rat/day) day 8	27.8	27.0	26.5	26.4	20.9	19.7	19.4	17.9**
Day 28	28.5	27.9	27.4	27.5	20.4	20.6	19.9	18.1*
Blood chemistry								
T. cholesterol (mg/dL)	58	55	61	71*	66	75	66	77
Creatinine (mg/dL)	0.5	0.4**	0.5	0.4*	0.6	0.6	0.6	0.5
Glucose (mg/dL)	93	86	78**	84	92	88	81*	84
Organ weights								
Terminal bodyweight (g)	374	366	360	357	219	221	215	217
Brain weight (g)	2.09	2.09	2.05	2.09	1.91	1.93	1.97	2.00*
Brain weight (mg/100g bwt)	0.56	0.57	0.57	0.59	0.88	0.88	0.92	0.92
Lung weight (g)	1.32	1.29	1.26	1.24	0.94	0.99	0.95	1.01
Lung weight (mg/ 100 g bwt)	0.35	0.35	0.35	0.35	0.43	0.45	0.45	0.47*

Table 13.12.1.7-2

Dose (mg/kg food)	0		30		100		1000		dr
	m	f	m	f	m	f	m	f	
Mortality	No treatment-related findings								
Clinical signs	No treatment-related findings								
Skin irritation	No treatment-related findings								
Body weight gain	No treatment-related findings								
Food consumption								dc	
Ophthalmology	No treatment-related findings								
Haematology									
- segm. neutrophils							dc		
- lymphocytes							ic		
Clin. Chemistry									
- total cholesterol							ic		
- creatinine			dc				dc		
- glucose					dc				
Organ weights									
- brain								ic^r	
- lungs								ic^r	
Pathology									
macroscopy	No treatment-related findings								
microscopy	No treatment-related findings								f

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative

Acceptability

The study is considered acceptable.

Conclusions

Dermal exposure to test substance concentrations of 0, 30, 100 and 1000 mg/kg bw/day did not result in local skin effects. No treatment-related effects on mortality, clinical observations, ophthalmoscopy and body weight were noted. Food consumption was decreased at 1000 mg/kg bw in females after 1 and 4 weeks (14% and 11%). The overall food consumption over the entire study period was marginally decreased in all dose groups (-1.8%, -6.1% and -7.1% in low, mid and high dose).

Segmented neutrophils were decreased and lymphocytes were increased at 100 and 1000 mg/kg bw in males. No changes were noted in leukocyte count. The values were well within the historical control range and the effects are considered to be not toxicologically relevant.

Total cholesterol was increased at 1000 mg/kg bw in males (122% of control; without other liver effects). Creatinine was decreased at 30 and 1000 mg/kg bw in males (both 80% of control). However, the values were well within the historical control range and in the absence of a dose-response relationship or

histopathological effects the change in creatinine is considered to be not toxicologically relevant. The statistically significant decrease in glucose at 100 mg/kg bw in males was not dose-related and therefore, considered to be not toxicologically relevant.

Relative brain and lung weight were marginally increased at 1000 mg/kg bw in females (105 and 109% of control, resp.). Since the changes were slight and the values were within the historical control range the increases were not considered to be toxicologically relevant.

Macroscopic pathology and histopathology showed no treatment-related effects.

Based on increased cholesterol in the highest dose group and in view of related liver effects seen in the oral studies, the NOAEL for systemic effects is set at 100 mg/kg bw/day. No local effects were observed.

3.12.1.8 Study 8 - 7-day dermal toxicity study rat

Characteristics

Reference	:	IIA 5.3.7/01	exposure	:	7 days, 6 hours/day, 10% body surface, dorsal area, occlusive
type of study	:	7-day, subacute dermal toxicity, range finding study	dose	:	0 (distilled water), 100, 300 and 1000 mg/kg bw/day
year of execution	:	2001	vehicle	:	None
test substance	:	S-1812 (pyridalyl), Lot no. PS-98041G, purity 93.7%	GLP statement	:	No
Route	:	Dermal	guideline	:	None (dose range finding study)
Species	:	Rats, Crj:CD(SD)	acceptability	:	Acceptable as drf
group size	:	5/sex/dose			

Study design

The study was only partly performed in accordance with OECD 410 (1981) since it was intended as a dose range finding study. Main deviations: the study period was only 7 days, and the group size was smaller than required for OECD 410.

Results

The results are summarised in table 3.12.1.8-1 and -2.

Table 3.12.1.8-1

	Males				Females			
Dose (mg/kg bw/day)	**0**	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
Terminal bodyweight (g)	293	294	284	290	197	198	199	201
Adrenal weight (Right) (mg)	28.7	27.5	29.9	32.6	36.0	35.2	34.6	33.8
Adrenal weight (Left) (mg)	30.1	28.7	32.2	35.5	38.5	38.2	36.6	36.6
Adrenal weight (Right) (mg/ kg bwt)	98	93	106	112	183	179	174	167
Adrenal weight (Left) (mg/kg/day)	103	98	114	122*	196	195	184	180

No significance achieved at: * 0.05≥ p in comparison with control

Table 3.12.1.8-2

Dose (mg/kg food)	**0**		**30**		**100**		**1000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No treatment-related findings								
Clinical signs	No treatment-related findings								
Skin irritation	No treatment-related findings								
Body weight gain	No treatment-related findings								
Food consumption	No treatment-related findings								
Ophthalmology	Not performed								
Haematology	No treatment-related findings								
Clin. Chemistry - cholesterol	49±9	52±11	57±10	52±7	i 63±6	i 57±18	i 62±10	i 59±11	
Organ weights - adrenals							ic[r]		
Pathology									
macroscopy	No treatment-related findings								
microscopy	Not performed								

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative

Acceptability

The study is considered acceptable as a dose range finding study.

Conclusions

In a 7-day dermal dose range finding toxicity study, exposure to test substance concentrations of 0, 30, 100 and 1000 mg/kg bw/day did not result in mortality, clinical signs or skin reactions. No treatment-related findings were observed on body weight, food consumption, haematological and biochemical parameters and necropsy. Cholesterol was increased at 300 and 1000 mg/kg bw in males (127-128% of control) and females (110-113% of control) without a dose-relationship. The absolute and relative adrenal weight were increased at 1000 mg/kg bw in males.

3.12.2 Human data

No data.

3.12.3 Other data

No data.

3.13 Aspiration hazard

3.13.1 Animal data

No data.

3.13.2 Human data

No data.

3.13.3 Other data

No data.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability

Characteristics

reference	:	IIA 7.7/01, Study No. 850273	test system	:	activated sludge, manometric respirometry
year of execution	:	2003	incubation time	:	28 d
GLP statement	:	yes	nominal concentration	:	100 mg//L

guideline	:	EEC C.4-D, OECD 301F	temperature	:	22°C
test substance	:	Pyridalyl, batch no. PS-98041G	result	:	not readily biodegradable
purity	:	93.7% pure	acceptability	:	acceptable

Methods
The ready biodegradability of pyridalyl was studied in a 28-day biodegradation test by following the Biological Oxygen Demand (BOD) using manometric methods according to OECD 301F.

Test solutions (250 mL, duplicate) containing pyridalyl (100 mg/L; suspended by means of ultrasound dispersion) and activated sludge inoculum (30 mg solids/L) were incubated in 500 mL airtight flasks in the dark for 28 days at 22°C under continuous magnetic stirring. Duplicate flasks for inoculum blank controls (inoculum, no test substance) and the reference substance (sodium benzoate, 100 mg/L), and single flasks for the abiotic control (pyridalyl, 100 mg/L, poisoned with mercury dichloride at 10 mg/L) and the inhibition control (pyridalyl and sodium benzoate, both 100 mg/L) were included. The CO_2 generated as a result of oxygen consumption was absorbed into soda lime, the pressure drop recorded by a manometer and the consumed oxygen replaced by electrolytically generated oxygen from a copper sulphate solution. The oxygen consumption was recorded each workday.

Results
BOD in the inoculum controls (8 and 9 mg/L after 28 days) satisfied the validity criterion of OECD 301F (≤60 mg/L). The pass level for the reference substance (60% degradation) was reached within 4 days. After 28 days, the BOD in the flasks with pyridalyl was 7 and 9 mg/L, indicating that pyridalyl was not readily biodegradable in this test. This was not due to inhibitory effects of pyridalyl since the time course of the BOD in the toxicity control and the procedural control was similar during the test, with comparable levels after 28 days (157 mg/L in toxicity control, 157-159 mg/L in the procedural control).

Conclusions

Pyridalyl was not readily biodegradable in a biodegradation test following the Biological Oxygen Demand according to OECD 301F.

Guidelines & Limitations
The study was performed in accordance with OECD 301F and is acceptable.

4.1.2 Other degradability studies

4.1.2.1 Soil degredation

STUDY 1

Characteristics

reference	:	IIA 7.1.1/01, Study 0333/211-D2149	study type	:	aerobic degradation
year of execution	:	2003-2004	incubation time	:	up to 120 d

GLP statement	:	yes	nominal concentration	:	0.60 mg/kg
guideline	:	OECD 307 (2002)	temperature	:	20°C
test substance	:	a) [pyridyl-2,6-14C]-pyridalyl, lot no. RIS2003-001. b) [dichlorophenyl-U-14C]-pyridalyl, lot no. RIS2003-003.	DT50	:	see results
purity	:	a) chemical purity not reported, radiochemical purity 99.3%. b) chemical purity not reported, radiochemical purity 100.0%.	metabolites	:	see results
soils	:	loam/sandy loam & sandy loam & sandy clay loam & silt loam	acceptability	:	acceptable

Study design

The test soils (soil properties see Table B.4.1.2.1-01) were collected from sites untreated with pesticides for 5 years. Following field collection and sieving (2 mm) they were stored aerobically at the test facility for about two months at 4°C. Aliquots of 50 g dry weight portions of each of the 4 soils were dispensed into flasks, adjusted to moisture levels of 21% (PT 102), 10% (PT 103), 23% (SK 912091) and 33% (SK 15556090) (equivalent to pF 2.0 to pF 2.5) and incubated in the dark a 20±2°C for two weeks to stimulate microbial activity. At the end of this period, [pyridyl-2,6-14C]-pyridalyl (designated PYR-pyridalyl) was applied in acetonitrile to the surface of the 50 g dry weight portions of all 4 soils at a rate of 0.6 mg/kg, whilst [dichlorophenyl-U-14C]-pyridalyl (designated DCP-pyridalyl) was applied in acetonitrile to the surface of the 50 g dry weight portions of the PT 102 soil only, at the same rate of 0.6 mg/kg. The solvent was allowed to evaporate following which treated soil was mixed and incubated under a continuous humid air supply in the dark at 20±2°C for up to 120 days. Polar and non-polar volatiles in effluent air were trapped in ethane diol (1 trap) and 2% liquid paraffin in xylene (1 trap), and CO_2 was trapped in 2M NaOH (2 traps). The soil moisture content was maintained at the above levels throughout incubation.

Table 4.1.2.1-01 Properties of study soils

Parameter	PT 102	PT 103	SK 920191	SK 15556090
% Sand/silt/clay[A]	52/35/13	73/15/12	47/23/30	20/61/19
Texture[A]	loam/sandy loam	sandy loam	sandy clay loam	silt loam
pH (water)	7.2	5.3	8.0	7.0
pH (1M KCl)	6.5	3.9	7.4	6.1
pH (0.01M $CaCl_2$)	6.4	3.9	7.3	6.2
% organic carbon	1.7	1.2	2.1	4.2
CEC (meq/100 g)	21.8	8.7	14.6	20.2
WHC at pF 0 (%)[C]	66.4	49.9	58.7	93.5
WHC at pF 2.0 (%)[C]	21.5	9.9	22.8	32.8
WHC at pF 2.5 (%)[C]	21.1	9.8	21.8	32.9
Microbial biomass (start)[B]	462 µg C/g (⇔ 2.7% of OC content)	552 µg C/g (⇔ 4.6% of OC content)	120 µg C/g (⇔ 0.6% of OC content)	286 µg C/g (⇔ 0.7% of OC content)
Microbial biomass (90 d)[B]	285 µg C/g	388 µg C/g	174 µg C/g	238 µg C/g
Microbial biomass (120 d)[B]	330 µg C/g	249 µg C/g	205 µg C/g	290 µg C/g

(A) USDA classification system.
(B) By fumigation/extraction method, in samples treated with acetonitrile.
(C) Whether w/w or v/v was not reported.

Duplicate soil samples were analysed immediately after treatment and on days 14, 30, 62, 90 and 120 post-treatment. Trapping solutions were sampled and replaced on the same days. Soil samples were extracted twice with 90 mL of acetonitrile containing 0.25 mL of 1% HCl, and twice with acetonitrile. Radioactivity in extracts and liquid traps was determined by LSC. The extracts were combined, concentrated and analysed by reversed phase HPLC with confirmation by normal phase TLC for day 120 samples. Compound identification was by co-chromatography with unlabelled reference standards. Radioactivity in Post-Extraction-Solids (PES) was determined by combustion/LSC. Day 120 PES was extracted by reflux in acetonitrile/water/HCl (50/50/1). Selected reflux extracts were extracted with ethyl acetate and ethyl acetate extracts were concentrated and analysed by reversed phase HPLC. PES from reflux extraction underwent extraction for fractionation into fulvic acid, humic acid and humin. Radioactivity in selected NaOH traps was confirmed to be CO_2 by $BaCl_2$ precipitation.

Results

Microbial activity of the test soil was confirmed at the start, during and at the end of aerobic incubation by determining the microbial biomass using the fumigation/extraction method (see table 4.1.2.1-01).

Polar and non-polar volatiles were not detected in any trap. Other results for the distribution and identification of radioactivity are given in Table 4.1.2.1-02 to Table 4.1.2.1-06. These values represent replicate means; individual values were in excellent agreement. Ranges given here are for both labels. The amount of extractables decreased from 99-100% AR on day 0 to 61-78% AR on day 120. CO_2 was evolved from the soil to 4.6-9.8% AR on day 90 and a maximum of 5.0-16% AR on day 120. PES increased to a maximum of 13-22% AR on day 120. Reflux extraction of selected day 120 PES samples with acetonitrile/water/HCl released 8.0-17% AR, and the remaining solids were shown to consist of humic acids (0.6-4.3% AR), humin (1.3-7.1% AR) and fulvic acids (1.5-4.0% AR).

Pyridalyl degraded to 40-60% AR after 120 days. Except for HTFP, the same metabolites were found for both labels. Metabolites S-1812-DP (max. 12.2% AR), S-1812-DP-Me (max. 12.4% AR) and HTFP (max. 14.7% AR) accounted for >10% AR at any time. No metabolites were detected at >5% AR during at least two successive samplings. The levels of the only other identified metabolite S-1812-Ph-CH_2COOH increased monotonously during the study in two soils, but the terminal levels were low (1.5-2.1% AR). In the remaining two soils the levels of S-1812-Ph-CH_2COOH were always <LOQ. Unidentified fractions in primary extracts were always <1% AR.

Table 4.1.2.1-02 Distribution and identification(A) of radioactivity after aerobic incubation at 20°C of soil PT 102 treated with [pyridyl-2,6-14C] pyridalyl at 0.6 mg/kg (% AR, duplicate means)

	extractable								
day	total	a.s.	S-1812-DP	S-1812-DP-Me	HTFP	S-1812-Ph-CH_2COOH	PES	CO_2	mass balance
0	98.8	98.1	0.2	nd	nd	nd	0.4	-	99.2
14	94.0	86.5	5.0	0.9	1.0	0.2	4.4	1.0	99.3
30	90.0	80.4	6.7	1.8	0.7	0.3	7.3	1.9	99.2
62	84.4	69.0	9.7	3.8	0.7	0.8	10.9	2.7	97.9
90	79.8	61.2	10.3	4.7	1.1	1.5	14.2	4.6	98.5
120	76.3	52.4	12.2	6.1	2.3	2.1	15.7(B)	5.0	96.9

nd = not detected

(A) Other degradates were at the most 0.6% AR, and unresolved background 0.7% AR.

(B) Reflux with acetonitrile/water/HCl (50/50/1) of day 120 PES of one replicate (17.0% AR) released 10.7% AR, containing 0.7% AR pyridalyl, 1.4% AR S-1812-DP, 0.1% AR S-1812-DP-Me, 1.9% AR HTFP, 1.6% AR S-1812-Ph-CH_2COOH, 4.4% AR other degradates (including 1.4% AR aqueous fraction) and 1.4% AR unresolved background.

Table 4.1.2.1-03 Distribution and identification(A) of radioactivity after aerobic incubation at 20°C of soil PT 103 treated with [pyridyl-2,6-14C] pyridalyl at 0.6 mg/kg (% AR, duplicate means)

	extractable								
day	total	a.s.	S-1812-DP	S-1812-DP-Me	HTFP	S-1812-Ph-CH_2COOH	PES	CO_2	mass balance
0	99.1	98.2	0.2	nd	nd	nd	0.7	-	99.8
14	87.3	75.2	4.4	0.1	6.9	0.3	7.5	2.9	97.7
30	81.4	62.2	6.3	0.2	11.5	0.9	11.9	4.7	98.0
62	66.6	40.7	8.1	0.7	14.7	1.4	21.4	9.5	97.4
90	66.4	41.9	9.3	0.9	11.4	1.2	19.8	9.8	95.9
120	60.9	40.0	9.9	1.1	6.3	1.6	22.3(B)	15.5	98.7

nd = not detected

(A) Other degradates were at the most 1.5% AR (individual compounds <1% AR), and unresolved background 0.6% AR.

(B) Reflux with acetonitrile/water/HCl (50/50/1) of day 120 PES of one replicate (23.7% AR) released 16.8% AR, containing 0.6% AR pyridalyl, 1.2% AR S-1812-DP, 3.6% AR HTFP, 1.0% AR S-1812-Ph-H_2COOH, 7.8% AR other degradates (including 3.4% AR aqueous fraction) and 2.8% AR unresolved background.

Table 4.1.2.1-04 Distribution and identification(A) of radioactivity after aerobic incubation at 20°C of soil SK 920191 treated with [pyridyl-2,6-14C] pyridalyl at 0.6 mg/kg (% AR, duplicate means)

	extractable								
day	total	a.s.	S-1812-DP	S-1812-DP-Me	HTFP	S-1812-Ph-CH_2COOH	PES	CO_2	mass balance
0	98.5	97.7	0.2	nd	nd	nd	0.7	-	99.1
14	92.7	84.7	4.6	1.4	1.2	nd	5.7	0.5	98.9
30	87.9	75.0	5.3	4.4	2.9	nd	8.2	2.0	98.1
62	79.4	61.4	4.7	8.9	3.8	<0.1	14.2	4.6	98.1
90	73.3	53.7	4.5	11.0	3.4	nd	17.7	6.2	97.6
120	68.5	46.8	4.6	12.4	4.1	nd	19.3	9.8	97.5

nd = not detected
(A) Other degradates were at the most 0.4% AR, and unresolved background 0.7% AR.

Table 4.1.2.1-05 Distribution and identification(A) of radioactivity after aerobic incubation at 20°C of soil SK 15556090 treated with [pyridyl-2,6-14C] pyridalyl at 0.6 mg/kg (% AR, duplicate means)

	extractable								
day	total	a.s.	S-1812-DP	S-1812-DP-Me	HTFP	S-1812-Ph-CH_2COOH	PES	CO_2	mass balance
0	98.7	98.0	0.3	nd	nd	nd	1.0	-	99.7
14	92.0	85.3	4.2	0.7	1.0	nd	4.1	1.4	97.5
30	89.2	80.0	5.7	1.5	1.3	nd	6.2	3.1	98.5
62	81.6	68.3	7.6	3.1	1.0	nd	9.8	7.0	98.4
90	77.8	63.7	7.8	3.5	0.9	nd	11.3	9.4	98.4
120	73.8	60.1	7.8	3.9	0.5	nd	13.3	10.1	97.1

nd = not detected
(A) Other degradates were at the most 1.7% AR (individual compounds <1% AR), and unresolved background 0.4% AR.

Table 4.1.2.1-06 Distribution and identification(A) of radioactivity after aerobic incubation at 20°C of soil PT 102 treated with [dichlorophenyl-U-14C] pyridalyl at 0.6 mg/kg (% AR, duplicate means)

	extractable								
day	total	a.s.	S-1812-DP	S-1812-DP-Me	HTFP	S-1812-Ph-CH_2COOH	PES	CO_2	mass balance
0	99.8	99.5	nd	nd	na	nd	0.4	-	100.2
14	94.1	87.7	4.8	0.8	na	nd	5.0	0.8	99.9
30	92.1	83.3	6.5	1.7	na	0.3	7.0	1.0	100.0
62	86.7	74.8	8.3	2.2	na	0.9	11.5	1.8	99.9
90	81.5	65.0	10.4	3.6	na	1.5	15.3	3.2	99.9
120	78.0	59.2	10.9	5.6	na	1.5	16.7(B)	4.6	99.3

nd = not detected; na = not applicable (not detectable with DCP label position)
(A) Other degradates and unresolved background were at the most 0.7% AR.
(B) Reflux with acetonitrile/water/HCl (50/50/1) of day 120 PES of one replicate (17.4% AR) released 8.0% AR, containing 0.9% AR pyridalyl, 1.6% AR S-1812-DP, 0.2% AR S-1812-DP-Me, 0.7% AR S-1812-Ph-H_2COOH, 3.1% AR other degradates (including 1.8% AR aqueous fraction) and 1.4% AR unresolved background.

Degradation rates

DT50 values were calculated by the RMS following the recommendations and procedures of the "Guidance document on estimating persistence and degradation kinetics from Environmental Fate studies on pesticides in EU registration" (SANCO/10058/2005 version 2.0). Calculations were based on the individual replicate measurements and the time zero values for pyridalyl were adjusted for % AR in metabolites (considered to be justified by the high measured radiochemical purity prior to dosing) and PES. All calculations were performed with ModelMaker v 4.0 software (SFO = single first-order, FOMC = first-order multi-compartment, DFOP = Double-First-Order in Parallel mode, HS = Hockey-Stick).

Pyridalyl

The conceptual model assumes that parent pyridalyl is converted into a sink (degradation products, PES) with a flow F1. The flow F1 is mathematically described by the equations given in the Guidance Documents for the various models.

Endpoints for persistence

For persistence endpoints, SFO and FOMC were run first (M0 free, all data, no weighting). For all five data sets FOMC gave a better fit than SFO. There were no scientific grounds to justify data elimination, and therefore fitting the two models was undertaken after fixing M0 to the initial measured value (adjusted for % AR in metabolites and PES). In case FOMC gave the better fit, DFOP was run unmodified and with M0 fixed. The optimisation results are shown in Table 4.1.2.1-07. When comparing the results obtained with the same model with M0 free or M0 fixed, it should be noted that for these two situations the visual fits were mostly comparable, that the coefficient of correlation for the model (r^2) with M0 free was equal to or higher than that with M0 fixed, but that the χ^2 (err) values for the fit with M0 fixed were nearly always slightly lower than for the fit with M0 free. The latter observation does not imply that the fitting with M0 fixed is better than that with M0 free, but is attributable to the fact that fixing M0 leads to one parameter less to be estimated, and hence one additional degree of freedom. The best fit model was considered to be the model with M0 free, which scored best on the points visual fit, χ^2 (err) and r^2. The persistence endpoints for the best fit model are summarised in Table 4.1.2.1-08.

In all cases the persistence endpoints were obtained from biphasic models, and the estimated DT90 persistence value was in no case reached within the experimental period (in two of four soils, the estimated DT50 persistence was also not reached within the experimental period).

Endpoints for modelling

The first run of SFO (M0 free, all data, no weighting) gave an acceptable fit, both visually and statistically for all data sets. The modelling endpoints for the data sets of all soils were therefore obtained from SFO (M0 free, all data, no weighting). There were no scientific grounds to justify data elimination, and therefore fitting the SFO model after fixing of M0 to the initial measured value (adjusted for % AR in metabolites and PES) was undertaken.. The optimisation results are shown in Table 4.1.2.1-07, and the modelling endpoints (not normalised for moisture content) are presented in Table 4.1.2.1-08.

Table 4.1.2.1-07 Optimisation results of the Modelmaker runs for estimation of half-lives of pyridalyl in four soils treated with [pyridyl-2,6-14C] pyridalyl or [dichlorophenyl-U-14C] pyridalyl at 0.6 mg/kg and incubated in the dark at 20°C and pF 2.0 to pF 2.5.

							visual	**endpoint(G)**

soil	label	model	M0[C]	χ^2 (err)	residuals	r^2	fit[D]	P	M
PT 102	PYR	SFO[A]	-3.3	2.07	fair	0.964	4		M
		SFO[B]	0	2.67	poor	0.949	4		
		FOMC[A]	-1.2	1.56	good	0.972	5		
		FOMC[B]	0	1.51	good	0.971	5		
		HS	fitting not required						
		DFOP[A]	-1.1	0.72	good	0.978	5	P	
		DFOP[B]	0	no fit					
PT 102	DCP	SFO[A]	-3.7	2.25	fair	0.953	4		M
		SFO[B]	0	2.73	fair	0.930	4		
		FOMC[A]	-1.4	1.89	fair	0.964	4		
		FOMC[B]	0	1.78	fair	0.962	4		
		HS	fitting not required						
		DFOP[A]	-0.7	1.02	good	0.975	5	P	
		DFOP[B]	0	no fit					
PT 103	PYR	SFO[A]	-8.5	9.92	fair	0.870	4		M
		SFO[B]	0	11.53	fair	0.826	3		
		FOMC[A]	+0.4	4.62	fair	0.973	3	P	
		FOMC[B]	0	4.20	fair	0.973	3		
		HS[E]	0.0	8.54	fair	0.940	3		
		HS[F]	-1.3	2.78	good	0.988	4		
		DFOP[E]	no fit could be obtained (k_2 negative)						
SK 920191	PYR	SFO[A]	-4.2	3.14	fair	0.974	4		M
		SFO[B]	0	3.80	poor	0.958	3		
		FOMC[A]	-0.3	0.56	good	0.998	5		
		FOMC[B]	0	0.53	good	0.998	5		
		HS	fitting not required						
		DFOP[A]	-0.1	0.31	good	0.998	5	P	
		DFOP[B]	0	0.28	good	0.998	5		
SK 15556090	PYR	SFO[A]	-5.7	3.77	fair	0.918	3		M
		SFO[B]	0	4.70	poor	0.862	2		
		FOMC[A]	-0.2	1.02	good	0.983	5	P	
		FOMC[B]	0	0.93	good	0.983	5		
		HS	fitting not required						
		DFOP[A]	-0.4	1.31	good	0.981	5		
		DFOP[B]	0	1.16	good	0.981	5		

(A) M0 free.
(B) M0 fixed.
(C) % AR difference (A-B) between fitted value (A) and mean measured initial value (B).
(D) Scale of 1 tot 5, where 1 = poor, 2 = fairly poor, 3 = fair, 4 = fairly good, 5 = good.
(E) No constraints.
(F) M_0 free, T_b constrained (30 days < Tb < 80 days).
(G) Endpoint selected for Persistence (P) or Modelling (M).

Table 4.1.2.1-08 Optimised fitting parameters and half-lives for pyridalyl in four soils treated with [pyridyl-2,6-14C] pyridalyl or [dichlorophenyl-U-14C] pyridalyl at 0.6 mg/kg and incubated in the dark at 20°C and pF 2.0 to pF 2.5.

soil	label	parameter for	kinetics	Optimised fitting parameters	DT50 (20°C)	DT90 (20°C)
PT 102	PYR	persistence	DFOP	g = 0.0548996; k_1 = 0.399574; k_2 = 0.00469605; M_0 = 98.0488	133	476
		modeling	SFO	k_p = 0.00515252; M_0 = 95.8281	135	-
	DCP	persistence	DFOP	g = 0.062957; k_1 = 4365.47;	163	586

				k_2 = 0.00380518; M_0 = 99.4817		
		modeling	SFO	k_p = 0.00425404; M_0 = 96.4711	163	-
PT 103	PYR	persistence	FOMC	α = 0.453104; β = 14.7602; M_0 = 100.081	53	2363
		modeling	SFO	k_p = 0.0092356; M_0 = 89.7302	75.1	249.3
SK 920191	PYR	persistence	DFOP	g = 0.189247; k_1 = 0.0471875; k_2 = 0.00450458; M_0 = 99.0313	108	465
		modeling	SFO	k_p = 0.00643457; M_0 = 94.8953	108	-
SK 15556090	PYR	persistence	FOMC	α = 0.25773; β = 19.8149; M_0 = 99.4039	272	150302
		modeling	SFO	k_p = 0.00429794; M_0 = 93.9523	161	-

Metabolites

Levels of metabolites were generally low, and in case they exceeded 5% AR for most data points, the formation pattern showed a monotonous increase, except in the case of HTFP in soil PT 102 treated with DCP-label. Moreover, for all three major metabolites studies into the rate of degradation in the soils PT 102, PT 103 and SK 920191 have been conducted by treating each soil with the metabolite concerned (see studies 2, 3 and 4 of this present section). Therefore no modelling to derive endpoints for metabolites was performed on the above data sets.

Conclusions

In 4 soils treated with PYR- and/or DCP-labelled pyridalyl and incubated under aerobic conditions at 20°C in the dark, CO_2 was evolved to a maximum of 5.0-16% AR on day 120, and PES increased to a maximum of 13-22% AR on day 120. Pyridalyl degraded to 40-60% AR after 120 days. Metabolites S-1812-DP (max. 12.2% AR), S-1812-DP-Me (max. 12.4% AR) and HTFP (max. 14.7% AR) accounted for >10% AR at any time. No metabolites were detected at >5% AR during at least two successive samplings. The levels of the only other identified metabolite S-1812-Ph-CH_2COOH increased monotonously during the study in two soils, but the terminal levels were low (1.5-2.1% AR), and in the remaining two soils the levels of S-1812-Ph-CH_2COOH were always <LOQ. DT50 (persistence, 20°C) values of pyridalyl were in the range 53-272 days, DT90 (persistence, 20°C) values were in the range 465-150302 days, and DT50 (modelling, 20°C, non-normalised) values were in the range 75.1-163 days.

Guidelines & Limitations

1. The microbial activity (determined by fumigation/extraction) of soil SK 912091 and SK 15556090 at the start of incubation represented 0.6% and 0.7%, respectively, of the organic carbon content of these soils. This is slightly below the level of 1% of organic carbon recommended by OECD 307, but during incubation the microbial activity in soil SK 912091 increased to 1.0% of organic carbon, and that of SK 15556090 remained at levels comparable to that at the start. The results for these two soils are considered to be acceptable.
2. Acceptability: the study is acceptable.

The initial step in the formation of the major soil metabolites S-1812-DP and S-1812-DP-Me from parent pyridalyl is loss of the dichloropropenyl moiety.

A study into the route of degradation of [dichloropropenyl-14C]-pyridalyl in aerobic soil was submitted, to identify whether relevant metabolites are formed from this portion of the molecule.

STUDY 2

Characteristics

reference	:	(IIA 7.1.1/02, study No. 12152	study type	:	aerobic degradation
year of execution	:	1999-2002	incubation time	:	up to 361 d
GLP statement	:	yes	nominal concentration	:	0.93 µg/kg
guideline	:	US-EPA 162-1	temperature	:	25°C
test substance	:	a) [propenyl-2-^{14}C]-pyridalyl, lot no. RIS98018. b) [pyridyl-2,6-^{14}C]-pyridalyl, lot no. RIS97020. c) [dichlorophenyl-U-^{14}C]-pyridalyl, lot no. RIS98015.	DT50	:	see results
purity	:	a) chemical purity not reported, radiochemical purity 98.3%. b) chemical purity not reported, radiochemical purity 99.5%. c) chemical purity not reported, radiochemical purity 99.3%.	metabolites	:	see results
soil	:	sandy loam	acceptability	:	acceptable

Study design

The test substance includes three different labeling positions, the propenyl label [propenyl-2-^{14}C]-pyridalyl, the pyridalyl label [pyridyl-2,6-^{14}C]-pyridalyl and the phenyl label [dichlorophenyl-U-^{14}C]-pyridalyl. Identical experiments were conducted using each label position.

The test soil (soil properties see Table 4.1.2.1-09) was collected from an agricultural site representative of cotton or vegetable crops growing regions. Pesticide history was not reported. Following field collection the soil was sieved (2 mm) and soil moisture was determined following sieving. Final soil moisture was adjusted to 1/3 bar moisture level. The storage conditions and storage time prior to the test at the test facility were not reported.

Aliquots of 50 g dry weight portions were dispensed into glass beakers, adjusted to moisture levels of 1/3 bar and incubated in the dark a 25±1°C. The required dose of pyridalyl was delivered in approximately 250 µL of ethanol (0.5% of sample dry weight), to the surface of the 50 g dry weight portions, thus providing a concentration of 0.93 µg/kg soil, equivalent to a dose rate of 0.65 kg a.s./ha . Polar and non-polar volatiles in effluent air were trapped in Tenax® cartridges and CO_2 was trapped in 2M NaOH (2 traps). The soil moisture content was maintained at the above levels throughout incubation.

Table 4.1.2.1-09 Properties of study soil

Parameter	Hanford Soil
% Sand/silt/clay(A)	66/28/6
Texture(A)	sandy loam
pH (water)	6.0
% organic matter	0.7
CEC (meq/100 g)	6.9
WHC at 1/3 bar (%)(C)	12.4
Viable Soil Microbial Population Characterization	
Actinomycetes (Time 67.4 hours)	2,260,000 CFU/g dry wt. soil
Fungi (Time: 66.7 hours)	8,550 CFU/g dry wt. soil
Bacteria (Time: 67.7 hours)	794,000 CFU/g dry wt. soil

(A) USDA classification system.

(B) By fumigation/extraction method, in samples treated with acetonitrile.
(C) Whether w/w or v/v was not reported., 1/3 bar corresponds with pF = 2.5

Duplicate soil samples were analyzed immediately after treatment and on days 11, 32, 60, 88, 120, 179, 272 and 361 post-treatment. Trapping solutions were sampled and replaced on the same days. Soil samples were extracted twice with 180 mL of acetonitrile containing 0.5 mL of 1% HCl, and additional once with acetonitrile. Radioactivity in extracts and liquid traps was determined by LSC. The extracts were combined, concentrated and analysed by reversed phase HPLC with confirmation by normal phase TLC for day 120 samples. Compound identification was by co-chromatography with unlabelled reference standards. Radioactivity in Post-Extraction-Solids (PES) was determined by combustion/LSC. PES from reflux extraction underwent extraction for fractionation into fulvic acid, humic acid and humin. Radioactivity in selected NaOH traps was confirmed to be CO_2 by $BaCl_2$ precipitation.

Results

The results of [^{14}C]S-1812 metabolism in aerobic soil are discussed by grouping the degradation products into extractable radioactive products, soil-bound radioactivity, radioactivity mineralized to $^{14}CO_2$ and [^{14}C]volatiles other than $^{14}CO_2$. Reported values are the arithmetic means of duplicate samples, expressed as percentage (%) of applied [propenyl-2-^{14}C]-pyridalyl, [pyridyl-2,6-^{14}C]-pyridalyl and [dichlorophenyl-U-^{14}C]-pyridalyl.

The parent [^{14}C]S-1812 declined to concentrations between 22.4 and 42.9% AR after 361 days.

Only one [14C]S-1812 degradate, HTFP, was found in the soil extract at a concentration >10% of applied radioactivity. HTFP reached a peak of 14.5% AR at 272 DAT, and it declined to 10.9% AR by 361 DAT. Degradate S-1812-DP was observed in the soil extract at peak concentration of 2.49% AR at 11 DAT, S-1812-DP-Me reached 6.31% AR at 361 DAT. Two other degradates, S-1812-DHQ and HPHM were detected only in a single sampling event at trace amounts of 0.11 and 0.18% AR, respectively. An unknown degradate, Unknown-1, detected in both the dichlorophenyl and pyridalyl labeled studies, was found in amounts >5% AR on more than two consecutive time points. Its peak concentration of 7.58% AR at DAT 88 for the pyridyl label and 7.04 % AR at DAT 179 for the dichlorophenyl label was followed by decline. Therefore it is not expected to reach amounts >10% AR. Up to six unknowns were found in small amounts at various points of the study. The six unknowns combined peaked at 4.48% AR.

Evolved $^{14}CO_2$ acounted for 43.4, 14.5 and 7.81% of applied [propenyl-2-^{14}C]-pyridalyl, [dichlorophenyl-U-^{14}C]-pyridalyl and [pyridyl-2,6-^{14}C]-pyridalyl, respectively. Volatiles other than $^{14}CO_2$ were negligible (peak 0.024% AR 361 DAT)

Table 4.1.2.1-10 Distribution and identification of radioactivity after aerobic incubation at 25°C of Hanford soil treated with [propenyl-2-^{14}C]-pyridalyl at 0.93 mg/kg (% AR, duplicate means)

	extractable					
day	**total**	**a.s.**	**Others (4 unknowns)**	**PES**	**CO_2**	**mass balance**
0	103.4	101.6	1.78	2.13	-	105.5
11	93.8	92.8	0.95	8.97	0.79	103.6
32	85.0	83.6	1.35	14.2	3.84	103.0
60	76.6	75.1	1.51	18.3	8.17	103.1
88	69.0	69.0	n.d.	19.7	12.2	100.9
120	61.2	61.2	n.d.	22.5	16.4	100.1

179	55.8	55.1	0.69	24.9	23.2	103.9
272	43.7	42.0	1.66	23.6	32.5	99.8
361	22.8	22.4	0.36	30.0	43.4	96.2

nd = not detected

Table 4.1.2.1-11 Distribution and identification of radioactivity after aerobic incubation at 25°C of Hanford soil treated with [dichlorophenyl-^{14}C]-pyridalyl at 0.93 mg/kg (% AR, duplicate means)

	extractable									
day	total	a.s.	S-1812-DP	S-1812-DP-Me	S-1812-DHQ	Unk-1	Others	PES	CO_2	mass balance
0	102.8	101.5	0.09	n.d.	0.11	1.08	n.d.	2.40	-	105.2
11	97.4	93.9	2.20	0.29	n.d.	1.00	n.d.	7.13	0.12	104.7
32	92.9	86.5	1.73	0.79	n.d.	0.79	3.10	9.05	0.73	102.7
60	87.8	79.4	n.d.	1.53	n.d.	6.74	0.17	12.9	1.77	102.5
88	85.2	76.6	n.d.	2.45	n.d.	4.79	1.39	14.1	2.85	102.2
120	81.4	72.4	n.d.	2.61	n.d.	6.07	0.36	17.4	4.09	102.9
179	77.4	65.7	n.d.	4.22	n.d.	7.04	0.46	20.8	5.89	104.1
272	74.2	62.1	n.d.	4.46	n.d.	6.18	1.43	20.6	10.5	105.3
361	58.0	42.9	0.73	5.56	n.d.	6.81	1.98	27.2	14.5	99.7

nd = not detected

Table 4.1.2.1-12 Distribution and identification of radioactivity after aerobic incubation at 25°C of Hanford soil treated with [pyridyl-2,6-^{14}C] pyridalyl at 0.93 mg/kg (% AR, duplicate means)

	extractable										
day	total	a.s.	S-1812-DP	S-1812-DP-Me	HTFP	HPHM	Unk-1	Others	PES	CO_2	mass balance
0	103.7	101.9	0.26	n.d.	n.d.	n.d.	0.88	0.63	2.34	-	106.0
11	99.8	94.8	2.49	n.d.	0.59	0.18	1.26	0.48	6.44	0.07	106.3
32	94.1	85.0	1.88	1.49	1.95	n.d.	1.09	2.64	8.94	0.58	103.6
60	90.8	76.1	1.97	3.95	2.49	n.d.	2.10	4.19	11.6	1.31	103.7
88	90.0	74.2	0.00	2.56	3.31	n.d.	7.58	2.39	12.1	2.00	104.1
120	84.7	62.3	0.62	4.81	8.30	n.d.	5.90	2.76	14.9	2.82	102.4
179	78.1	51.0	0.22	5.59	12.3	n.d.	5.37	3.66	20.4	3.91	102.4
272	71.4	40.5	0.00	5.82	14.5	n.d.	6.14	4.48	21.9	6.39	99.7
361	57.3	30.2	0.98	6.31	10.9	n.d.	4.96	3.95	23.5	7.82	88.6

nd = not detected

Degradation rates

DT50 values were calculated for the three different labeling positions by the RMS following the recommendations and procedures of the "Guidance document on estimating persistence and degradation kinetics from Environmental Fate studies on pesticides in EU registration" (SANCO/10058/2005 version 2.0). Calculations were based on the individual replicate measurements and the time zero values for pyridalyl were adjusted for % AR in metabolites (considered to be justified by the high measured radiochemical purity prior to dosing) and PES. All calculations were performed with ModelMaker v 4.0 software (SFO = single first-order).

Pyridalyl

The conceptual model assumes that parent pyridalyl is converted into a sink (degradation products, PES) with a flow F1. The flow F1 is mathematically described by the equations given in the Guidance Documents for the various models.

The optimisation results are shown in Table 4.1.2.1-13. The first run of SFO (M0 free, all data, no weighting) gave an acceptable fit, both visually and statistically (the χ^2 (err) values) for all data sets

Table 4.1.2.1-13 Optimization results of the Modelmaker runs for estimation of half-lives of pyridalyl in Hanford soil treated with [pyridyl-2,6-14C] pyridalyl, propenyl-2-^{14}C]-pyridalyl or [dichlorophenyl-U-14C] pyridalyl at 0.93 mg/kg and incubated in the dark at 25°C and pF = 2.5.

soil	label	model	M0[(B)]	χ^2 (err)	residuals	visual fit[(C)]	endpoint			
							DT50 (25°C)	DT90 (25°C)	DT50 (20°C)	DT90 (20°C)
Hanford	PRO	SFO[(A)]	-4.8	4.16	fair	4	194.4	645.8	290.0	963.4
	DCP	SFO[(A)]	-6.5	4.27	fair	4	339.6	1128.1	506.6	1682.9
	PYR	SFO[(A)]	-4.0	2.95	fair	5	200.6	666.5	299.3	994.3
						Mean:	**244.9**	**813.5**	**365.3**	**1213.5**

(A) Mo free.
(B) % AR difference (A-B) between fitted value (A) and mean measured initial value (B).
(C) Scale of 1 tot 5, where 1 = poor, 2 = fairly poor, 3 = fair, 4 = fairly good, 5 = good.

Metabolites

Levels of metabolites were generally low, in case of the [propenyl-2-^{14}C] label no metabolites were detected. For the [dichlorophenyl-^{14}C] labelling position only S-1812-DP-Me reached levels of >5% (5.56% AR) at the study end after monotonous increase. For the [pyridyl-2,6-14C] labelling position only HTFP reached levels >10 AR (maximum 14.5% at 272 DAT, decline to 10.9% at 361 DAT) and S-1812-DP-Me reached levels of >5% at two consecutive time points (maximum 6.31% AR) at the study end after monotonous increase.

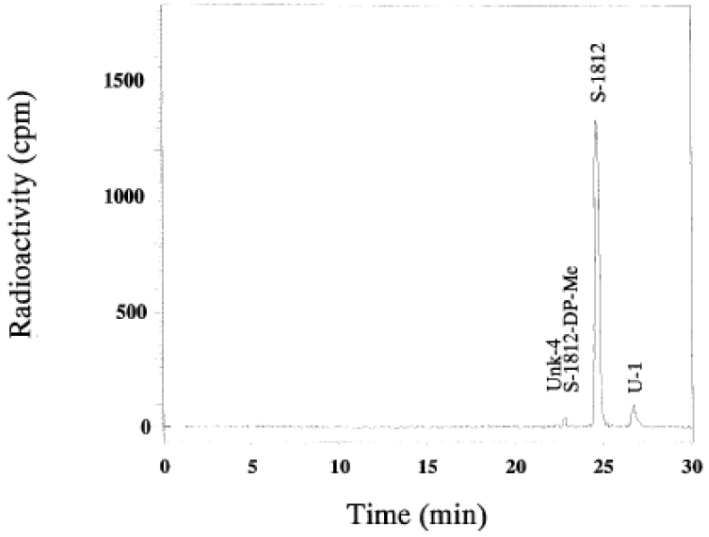
For the major metabolite HTFP and also for S-1812-DP-Me studies into the rate of degradation in the soils PT 102, PT 103 and SK 920191 have been conducted by treating each soil with the metabolite concerned (see studies 3, 4 and 5 of this present section). Therefore no modeling to derive endpoints for metabolites was performed on the above data sets.

Further information on Unk-1 was submitted by the applicant (T. Jarvis & V Montesano, 2012). Further information on 1) relative polarity of Unk-1 and pyridalyl and 2)kinetic formation fraction and DT50 values fro the metabolite should be provided.

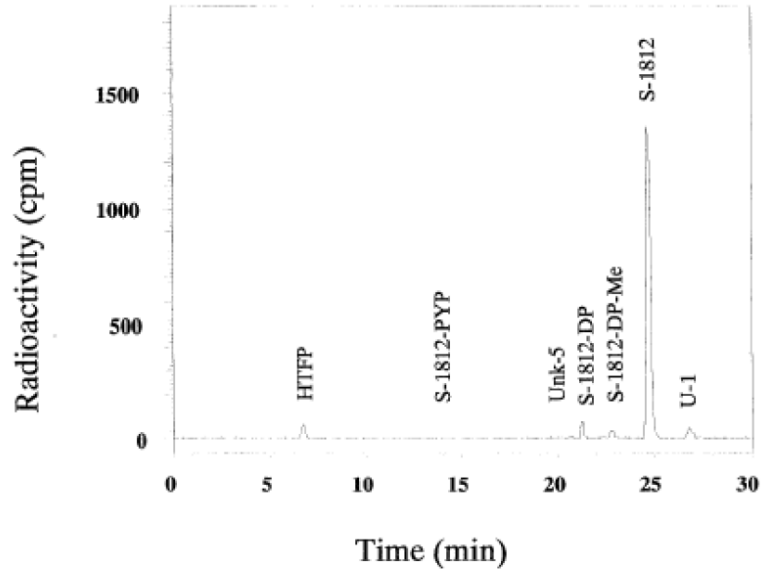
RELATIVE POLARITY OF UNK-1 AND PYRIDAYL

Unk-1 was present in both the pyridyl and dichlorophenyl labelled incubations of pyridalyl but was not observed in the propenyl-labelled incubations. Therefore the compound contains both the dichlorophenyl and pyridyl moieties but not the propenyl moiety. The retention time of Unk-1 was longer than that of pyridalyl on reversed-phase HPLC, therefore indicating that the compound has a lower polarity than pyridalyl. Figures 1 and 2 show chromatograms from the original study report and confirm the longer retention time.
As Unk-1 has a lower polarity than pyridalyl and since it only reaches a maximum of ca 8%, then it can be confidently concluded that this compound does not pose any leaching risk.

HPLC analysis of soil extract for the [Dichlorophenyl-^{14}C]S-1812 label 60 DAT



HPLC analysis of soil extract for the [Pyridyl 2,6l-^{14}C]S-1812 label 60 DAT



KINETIC CALCULATION FOR UNK-1

The modelling was performed following FOCUS (2006) recommendations using KinGui Version 2 (Bayer CropScience 2011) running on an IBM compatible PC running Windows 2007.

As requested by EFSA, data from the two radiolabelling positions were treated as replicates. Input values are shown in Table 4.1.2.1-13a. There is no evidence to clearly indicate that Unk-1 is formed via a metabolite of pyridalyl and hence, for simplicity, formation has been simulated as directly from the parent molecule.

SFO kinetics had been previously used for the parent data set in the DAR and the current examination also showed good fits to SFO kinetics, therefore this kinetic was used as the basis for metabolite fitting. All relevant parameters for parent (k, Mo) and metabolite (ff, k) were fitted simultaneously and results are shown in Table 4.1.2.1-13b. As a check, the parent alone was optimised and then used as fixed parameters for optimising the metabolite but this made no significant difference to the results.

Table 4.1.2.1-13a Input data for pyridalyl and Unk-1 used in Kingui 2.0

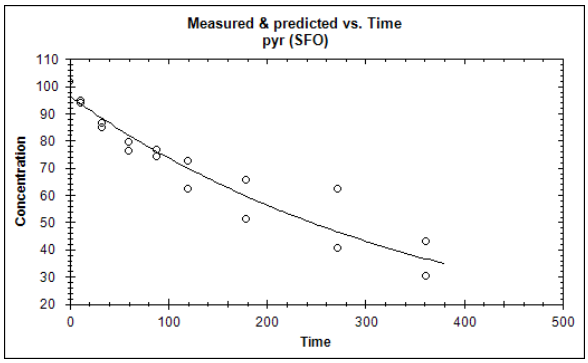
Time (day)	**pyridalyl**	**Unk-1**
0	101.5	1.08
0	101.9	0.88
11	93.9	1.00
11	94.8	1.26
32	86.5	0.79
32	85.0	1.09
60	79.4	6.74
60	76.1	2.10
88	76.6	4.79
88	74.2	7.58
120	72.4	6.07
120	62.3	5.90
179	65.7	7.04
179	51.0	5.37
272	62.1	6.18
272	40.5	6.14
361	42.9	6.81
361	30.2	4.96

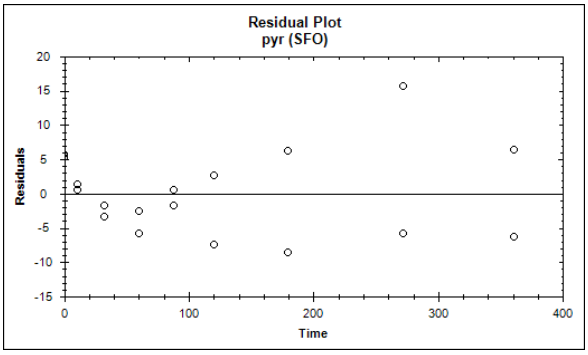
Table 4.1.2.1-13b: Summary of the results of the kinetic determinations

Parameter	Pyridalyl	Unk-1
Model	*SFO*	*SFO*
$\chi 2$ error (%)	3.65	14.23
k (day-1) *	0.00268 (1.52×10^{-12})	0.00808 (1.8×10^{-4})
Formation fraction	-	0.36
DT50 (day)	258	85.8
DT90 (day)	858	285

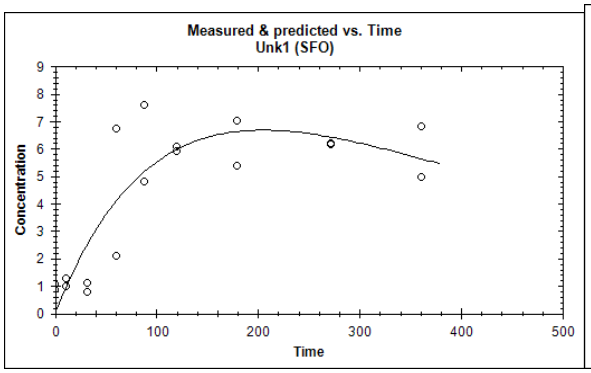
*P value from the t-test is given in brackets.

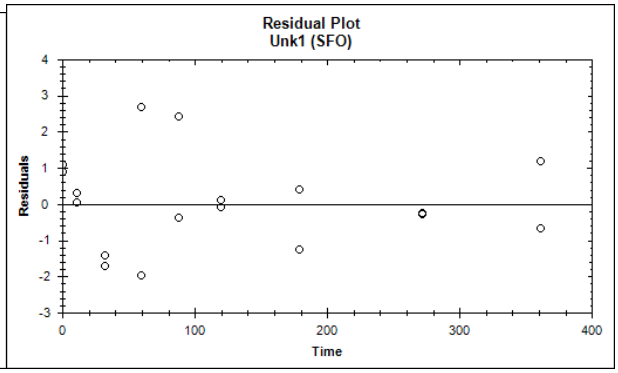
SFO Fits and residuals for pyridalyl





SFO Fits and residuals for Unk-1





The results showed a reasonable visual fit to the Unk -1 data with no bias of the residuals and statistical data ($\chi 2$ error and t-test) both showed acceptable/significant outcomes. Therefore the SFO DT50 value of 85.8 days was considered to be robust.
This was subsequently normalized to 20ºC and pF2 to give a corrected DT50 value of 138 days.

Conclusions

In one sandy loam soil treated with PYR-, PRO- or DCP-labelled pyridalyl and incubated under aerobic conditions at 25°C in the dark, CO_2 was evolved to a maximum of 8 – 43% AR on day 361, and PES increased to a maximum of 24 – 30% AR on day 361. Pyridalyl degraded to 22 – 43% AR after 361 days. For the PRO label no metabolites were found in significant amounts. Metabolite HTFP (max. 14.5% AR) accounted for >10% AR at any time. Metabolite S-1812-DP-Me was detected at >5% AR during at least two successive samplings. The metabolite S-1812-DP was detected irregularly and its maximum was 2,49% AR for the PYR label at11 DAT. Two other metabolites, S-1812-DHQ in case of DCP labeling and HPHM in case of the PYR label, were both detected on one time point only. Recalculated normalized DT_{50} values of pyridalyl were in the range 290 – 507 days, DT90 963 – 1683. The additional information submitted on metabolite Unk-1 showed that the fitted formation fraction is 0.36. The DT_{50} is in the same range or a bit lower than for the parent substance (138 days).

Guidelines & Limitations

3. Pesticide history was not reported.
4. Acceptability: Although pesticide history was not reported, the study is considered acceptable.

Additionally the notifier submitted a study on the aerobic soil metabolism of pyridalyl in Japanese upland soil (Aerobic Soil Metabolism of S-1812 in upland soil; Sunago, T, et al.; EF-2007-022; (2007))

This study is not considered applicable for this assessment report since the soil tested is a volcanic ash soil (andosol).

4.1.2.2 Water-sediment studies

STUDY 1

Characteristics

reference	:	IIA 7.8.3/01, Study No. 0333/212D-2149	incubation time	:	100 days
year of execution	:	2003-2004	nominal concentration	:	~70 µg/L
GLP statement	:	yes	temperature	:	20°C
guideline	:	OECD 308 (2002)	DT50	:	see results
test substance	:	a) [pyridyl-2,6-14C]-pyridalyl, lot no. RIS2003-001. b) [dichlorophenyl-U-14C]-pyridalyl, lot no. RIS2003-003.	metabolites	:	see results
purity	:	a) chemical purity not reported, radiochemical purity 99.9%. b) chemical purity not reported, radiochemical purity 99.2%.	acceptability	:	acceptable
test system	:	silt loam & sandy loam sediment			

Study design

The behaviour of [pyridyl-2,6-14C]-pyridalyl and [dichlorophenyl-U-14C]-pyridalyl was studied in two water/sediment systems.

Sediment and associated water were sampled from two locations A and B and sieved through a 2 mm (sediment) and 0.2 mm (water) sieve. The water/sediment properties are listed in Table 4.1.2.2-01. Sediment was dispensed into glass cylinders (4.5 cm inner diameter) to a depth of 3 cm, water was added to a depth of 9 cm above the sediment, and systems were pre-incubated at 20±2°C in the dark for 20 days prior to treatment. Aliquots (~1.4 mL) of [pyridyl-2,6-14C]-pyridalyl (designated PYR-pyridalyl) and [dichlorophenyl-U-14C]-pyridalyl (designated DCP-pyridalyl) in acetonitrile were then added to the water layer to give a concentration of about 70 µg/L water. The water/sediment systems were incubated at 20±2°C in the dark under slight agitation under a continuous humidified air supply. Volatiles were trapped in ethanediol (1 trap), 2% paraffin in xylene (1 trap) and 2M NaOH (2 traps).

Redox potential (sediment and water), pH (sediment and water) and dissolved oxygen (water) were measured at various time points in the equilibration phase and at samplings during the incubation phase. Systems had reached an equilibrium at the time of treatment. During the test period, the sediment redox potential of both systems was negative and that of the water positive (difference of about 300 mV), except for a post-treatment drop (day 0-30) in the redox potential of the water of A. The oxygen saturation values in the water were >80%, with the exception of a short post-treatment drop. The pH of the sediment of A rose from 6.5 on day 0 to about 8.3 from day 30 onwards, whilst that of the water of A rose from 7.5 on day 0 to about 9 from day 30 onwards. The pH of the sediment of B rose from 6.5 on day 0 to about 7.0 from day 15 onwards, whilst that of the water of A rose from 7.5 on day 0 to about 8.3-8.5 from day 15 onwards.

Table 4.1.2.2-01 Physico-chemical characterisation of water and sediment

parameter	**A**: silt loam (Calwich Abbey Lake)		**B**: sandy loam (Emperor's Son Lake)	
	water	sediment	water	sediment
textural class (USDA)	na	silt loam	na	sandy loam
% sand/silt/clay (USDA)	na	31/55/14	na	75/9/16
CEC [meq/100 g]	na	30.6	na	14.5
% organic carbon	na	5.2	na	1.6
DOC (mg/L)	8.4	na	18.0	na
suspended solids (mg/L)	40	na	110	na
pH (water)	8.00	7.4	6.6	6.7
pH (KCl)	na	7.1	na	5.7
pH ($CaCl_2$)	na	7.0	na	5.7
microbial biomass [µg C/g] (start) (A)	na	361	na	191
microbial biomass [µg C/g] (end) (A)	na	357	na	138

na = not applicable

(A) Determined by fumigation/extraction method.

Duplicate flasks and traps were analysed at 0, 14, 30, 49, 77 and 100 days after treatment (both labels) and after 1, 3 and 7 days (PYR-label). The water was separated from the sediment by aspiration.

The water samples were acidified with 1% HCl and extracted with ethyl acetate. Sediment samples were extracted twice with 90 mL of acetonitrile containing 0.25 mL of 1% HCl, and twice with acetonitrile. Radioactivity in extracts, residual water phases and liquid traps was determined by LSC. The combined water or sediment extracts were concentrated and analysed by reversed phase HPLC with confirmation by normal phase TLC for selected samples. Compound identification was by co-chromatography with unlabelled reference standards. Radioactivity in PES was determined by combustion/LSC. PES from the replicate with the highest level of unextracted radioactivity (one sample per label and system) was extracted by reflux in acetonitrile/water/HCl (50/50/1) followed by LSC of the extract. One day 100 reflux extract from PYR- and DCP label was extracted with ethyl acetate, followed by LSC of both phases and concentration and chromatography of the ethyl acetate extracts. Radioactivity in selected NaOH traps was confirmed to be CO_2 by $BaCl_2$ precipitation.

Results

The distribution of radioactivity is shown in Tables 4.1.2.2-02 to -05, which also show the levels of identified compounds found at ≥5% AR in water or sediment. These tables do not show replicate mean values per sampling time, but values for individual replicates, in order to highlight the differences between replicate values which existed on certain sampling times. The rather low recovery (<90%) in certain replicates on samplings up to day 7 for the systems treated with PYR-label were attributed by the author of the report to the test article sticking to probes used for determination of redox, pH and oxygen concentrations (after day 7 these determinations were carried out in control systems treated with acetonitrile). These probes however were washed with organic solvent and the rinses were added to the water extracts. The low amount of radioactivity in the water immediately after application (means 40-50% AR) is possibly not only due to rapid sorption to sediment (Koc of pyridalyl ≥402000 L/kg) but also to solubility problems (applied concentration about 70 µg/L, water solubility of pyridalyl is only 0.15 µg/L at 20°C). The presence of undissolved particulate pyridalyl in certain samples may also have caused the rather large differences in pyridalyl levels between certain replicates at samplings late during the study. The authors stated that the application rate of 10 µg per unit was chosen to provide sufficient analytical sensitivity to detect degradation products representing 1% AR.

Values stated in the text below refer to replicate means unless noted differently. The radioactivity level in water decreased from 40-50% AR on day 0 to 4-8% AR on day 100, except for system A, PYR-label, where a level of 5% AR was recorded on day 77, and a somewhat anomalous result of 24% AR on day 100. The amount of radioactivity partitioning into the sediment increased from 51-59% AR on day 0 to 90-95% AR on day 14-77, then decreasing to 62-85% AR (PYR-label) and 90-91% AR (DCP-label) on day 100. The non-extractable fraction in sediment increased to a maximum of 10-15% AR, reached at the end of incubation, except for system B, PYR-label, where the maximum of 10% AR was reached on day 77 (8% AR on day 100). Reflux extraction of PES with the highest level of radioactivity (12-13% AR and 16-20% AR, respectively, for PYR- and DCP-label) released 4% AR and 6-9% AR. CO_2 increased to 1-9% AR on day 100. Radioactivity in other traps and in unit rinses did not exceed 1.6% AR.

The level of parent pyridalyl in water fell from 39-49% AR on day 0 to 1-7% AR by day 100 except in system A, PYR-label, where the lowest value of 2% AR was reached on day 77 (13% AR on day 100). The levels of parent pyridalyl reached a maximum in sediment of 78-85% AR on day 14-49, and were 38-66% AR on day 100. The level of parent pyridalyl in the whole system fell from 96-99% AR on day 0 to 51-72% AR on day 100. The main metabolite was S-1812-DP, which reached maximum levels in water and sediment of 0.4-1.7% AR and 11-18% AR respectively. Besides pyridalyl and S-1812-DP, the following metabolites were found at low levels: S-1812-Ph-CH_2COOH (max. 1.9% AR in water and 4.2% AR in sediment), HTFP (max. 4.4% AR in water and 1.6% AR in sediment) and S-1812-DP-Me (max. 2.0% AR in water and 3.5% AR in sediment). Other degradates, unresolved background and unanalysed radioactivity did not exceed 2.5% AR. The reflux extracts contained pyridalyl (1-3%AR), S-1812-Ph-CH_2COOH (4% AR) and S-1812-DP (3% AR).

Table 4.1.2.2-02 Distribution and identification of radioactivity (% AR, replicate values) in water-sediment system A treated with [pyridyl-2,6-14C] pyridalyl at 70 µg/L and incubated at 20°C in the dark

	water			sediment extract					
day	total	a.s.	S-1812-DP	total	a.s.	S-1812-DP	PES	CO_2	mass balance
0	40.3	39.3	0.3	58.4	58.2	nd	0.5	-	99.2
0	39.2	39.0	0.2	56.7	55.9	nd	0.5	-	96.4
1	43.7	41.9	0.5	53.3	53.0	nd	0.6	nd	97.6
1	33.2	32.0	0.2	48.4	47.8	nd	0.6	nd	82.2
3	33.7	32.7	0.3	52.2	52.3	0.1	0.7	nd	86.9
3	38.3	36.8	0.5	52.8	52.5	0.1	0.7	nd	91.8
7	27.6	26.4	0.2	67.8	67.8	0.3	1.3	nd	97.5
7	23.3	21.8	0.4	69.3	70.2	0.4	2.0	nd	95.9
14	17.6	16.4	0.4	77.5	75.7	2.9	3.7	0.1	100.6
14	16.3	13.8	0.2	79.7	80.0	1.0	3.0	nd	100.4
30	26.4	24.8	0.5	67.6	67.7	1.9	2.3	0.1	98.4
30	10.7	5.6	0.6	83.7	72.9	7.2	4.6	0.2	99.6
49	10.7	7.4	nd	83.4	79.6	2.2	3.4	1.5	99.5
49	25.6	16.0	nd	62.2	56.4	2.7	6.8	3.1	97.7
77	7.1	3.3	0.2	85.9	74.4	7.9	5.2	1.4	99.9
77	3.7	1.0	nd	82.6	58.8	18.2	10.0	1.7	98.6
100	30.5	19.2	3.1	58.5	35.4	13.6	5.6	2.1	96.9
100	17.9	6.1	nd	47.0	41.2	1.9	11.8	16.4	93.8

nd = not detected

Table 4.1.2.2-03 Distribution and identification of radioactivity (% AR, replicate values) in water-sediment system B treated with [pyridyl-2,6-14C] pyridalyl at 70 µg/L and incubated at 20°C in the dark

	water			sediment extract					
day	total	a.s.	S-1812-DP	total	a.s.	S-1812-DP	PES	CO_2	mass balance
0	44.4	44.0	0.2	53.5	53.2	nd	0.9	-	98.8
0	38.1	37.9	0.2	61.5	61.2	nd	0.8	-	100.4
1	47.3	45.6	0.4	43.6	43.4	nd	0.4	nd	91.3
1	37.7	36.2	0.3	58.2	57.7	nd	0.5	nd	96.4
3	52.0	50.3	0.5	54.5	54.0	0.4	0.8	nd	107.3
3	26.5	25.9	0.3	71.3	70.2	0.5	0.8	nd	98.6
7	24.1	22.2	1.2	55.4	54.5	0.8	1.1	nd	80.6
7	13.7	11.6	0.9	86.2	85.3	0.5	1.0	nd	100.9
14	11.2	8.2	1.2	87.5	84.6	3.2	2.5	nd	101.5
14	10.2	6.8	1.3	87.7	83.0	4.3	2.9	nd	101.2
30	32.9	29.8	0.7	61.8	55.1	5.8	5.1	0.1	99.9
30	12.4	7.6	1.5	81.8	72.2	8.7	4.8	0.2	99.5
49	14.2	11.4	0.5	74.2	62.7	9.6	9.4	0.8	99.0
49	9.3	6.6	0.3	77.7	66.5	9.0	9.5	1.1	98.5
77	10.2	8.2	0.3	75.4	62.7	9.9	12.7	1.7	100.3
77	8.7	4.6	0.2	80.5	63.0	12.9	8.2	2.7	100.1
100	6.5	6.0	0.2	82.3	72.2	8.8	7.4	1.8	98.8
100	9.9	4.9	nd	72.8	60.5	8.3	8.1	4.8	96.1

nd = not detected

Table 4.1.2.2-04 Distribution and identification of radioactivity (% AR, replicate values) in water-sediment system A treated with [dichlorophenyl-U-14C] pyridalyl at 70 µg/L and incubated at 20°C in the dark

	water			sediment extract					
day	total	a.s.	S-1812-DP	total	a.s.	S-1812-DP	PES	CO_2	mass balance
0	46.8	46.7	nd	53.8	53.7	nd	0.5	-	101.1
0	52.1	51.3	0.1	47.0	46.9	nd	0.4	-	99.5
14	22.2	19.8	0.5	72.8	71.8	1.2	2.7	0.1	98.2
14	14.3	13.3	nd	80.8	81.0	0.6	3.3	nd	99.4
30	14.3	13.4	0.1	78.4	77.3	1.7	3.0	0.1	96.6
30	8.4	7.8	0.3	81.8	77.9	3.6	7.0	nd	98.0
49	5.2	4.7	nd	85.8	82.6	2.3	7.5	0.4	99.1
49	3.4	3.4	nd	92.4	87.7	3.4	3.6	0.2	100.0
77	0.8	nd	nd	93.4	86.3	4.7	5.2	0.4	99.8
77	10.2	6.7	0.1	83.4	76.2	1.7	5.2	0.3	99.1
100	8.9	6.6	0.7	87.0	79.7	5.5	4.2	0.5	100.7
100	2.0	nd	nd	71.0	40.0	17.3	20.3	2.4	95.8

nd = not detected

Table 4.1.2.2-05 Distribution and identification of radioactivity (% AR, replicate values) in water-sediment system B treated with [dichlorophenyl-U-14C] pyridalyl at 70 µg/L and incubated at 20°C in the dark

	water			sediment extract					
day	total	a.s.	S-1812-DP	total	a.s.	S-1812-DP	PES	CO_2	mass balance
0	42.7	41.4	nd	55.4	54.9	nd	0.8	-	98.9

0	37.7	37.6	nd	61.1	61.1	nd	0.6	-	99.4
14	10.7	8.0	1.3	84.7	80.9	3.6	3.8	0.2	99.7
14	11.8	11.3	0.2	85.4	81.1	3.4	2.6	0.2	100.0
30	13.4	11.0	1.1	77.3	65.6	9.2	7.7	1.0	99.7
30	10.1	8.8	0.3	83.6	74.2	7.6	5.5	0.7	100.1
49	11.7	8.6	1.5	68.6	49.4	15.0	13.9	3.2	97.8
49	11.2	8.7	1.0	75.3	59.6	13.2	10.9	2.2	99.8
77	2.9	2.5	nd	79.8	60.3	15.2	12.6	3.1	98.7
77	6.9	6.1	0.3	87.1	77.6	7.2	5.5	1.1	100.8
100	5.2	1.3	2.6	72.0	42.9	21.6	15.7	5.1	97.5
100	2.3	1.2	0.7	79.5	62.3	14.5	13.3	3.7	98.4

nd = not detected

DT50 estimations

Level P-I DT_{50} values of pyridalyl were calculated following the recommendations and procedures of the "Guidance document on estimating persistence and degradation kinetics from Environmental Fate studies on pesticides in EU registration" (SANCO/10058/2005). SFO = single first-order, FOMC = first-order multi-compartment, DFOP = double first-order parallel model, HS = hockey stick. The data used are summarised in the table below. All calculations were performed with ModelMaker v 4.0 software.

S-1812-DP was a major sediment metabolite. No decline of S-1812-DP was observed in the sediment and systems treated with DCP-label. In the sediment and systems treated with PYR-label, the decline phase consisted of one data point after the maximum, with only 25-29% decline except for the sediment of system 1 (41% decline), but this data point is insufficiently reliable due to the large difference between S-1812-DP replicate values. Since no decline was observed (DCP-label) or the decline phase was too short (PYR-label), no level M-I DT_{50} values were calculated for S-1812-DP.

Table 4.1.2.2-06 Levels of pyridalyl (% AR, replicate values) in water, sediment and complete water-sediment systems treated with [pyridyl-2,6-14C] pyridalyl or [dichlorophenyl-U-14C] pyridalyl at 70 µg/L and incubated at 20°C in the dark

	system A, PYR			system B, PYR			system A, DCP			system B, DCP		
day	water	sed.	syst.	water	sed.	syst.	water	sed.	syst.	water	sed.	syst.
0	39.3	58.2	97.4	44.0	53.2	97.3	46.7	53.7	100.4	41.4	54.9	96.3
0	39.0	55.9	94.9	37.9	61.2	99.1	51.3	46.9	98.2	37.6	61.1	98.7
1	41.9	53.0	94.9	45.6	43.4	89.0						
1	32.0	47.8	79.8	36.2	57.7	93.9						
3	32.7	52.3	85.0	50.3	54.0	104.2						
3	36.8	52.5	89.3	25.9	70.2	96.1						
7	26.4	67.8	94.2	22.2	54.5	76.7						
7	21.8	70.2	92.0	11.6	85.3	96.9						
14	16.4	75.7	92.1	8.2	84.6	92.8	19.8	71.8	91.6	8.0	80.9	88.9
14	13.8	80.0	93.8	6.8	83.0	89.8	13.3	81.0	94.3	11.3	81.1	92.4
30	24.8	67.7	92.4	29.8	55.1	84.9	13.4	77.3	90.7	11.0	65.6	76.6
30	5.6	72.9	78.4	7.6	72.2	79.7	7.8	77.9	85.8	8.8	74.2	83.0
49	7.4	79.6	87.0	11.4	62.7	74.1	4.7	82.6	87.3	8.6	49.4	58.0
49	16.0	56.4	72.3	6.6	66.5	73.1	3.4	87.7	91.0	8.7	59.6	68.3
77	3.3	74.4	77.8	8.2	62.7	71.0	nd	86.3	86.3	2.5	60.3	62.8
77	1.0	58.8	59.8	4.6	63.0	67.5	6.7	76.2	82.9	6.1	77.6	83.7
100	19.2	35.4	54.6	6.0	72.2	78.3	6.6	79.7	86.3	1.3	42.9	44.2
100	6.1	41.2	47.3	4.9	60.5	65.5	nd	40.0	40.0	1.2	62.3	63.5

nd = not detected; for the calculations set to 0.5xLOD = 0.05% AR (reported LOD for LSC was 0.1% AR, LOD for HPLC measurement not reported).

Pyridalyl

The conceptual model for disappearance of parent pyridalyl from the entire water/sediment system assumes that pyridalyl is converted into a sink (degradation products, PES) with a flow F1. In case of disappearance from water and sediment, the flow F1 not only includes degradation but also transfer from water to sediment (for disappearance from water) or from sediment to water (for disappearance from sediment). The flow F1 is mathematically described by the equations given in the Guidance Documents for the various models. DT50 values for dissipation of pyridalyl in sediment were calculated using the data from the point of maximum occurrence onwards. No DT50 (modelling) values were calculated for disappearance of parent pyridalyl from water and sediment, since these processes involve not only degradation but also mass transfer.

Day 0 values of pyridalyl in the whole water-sediment system were not adjusted for the levels of metabolites since these levels were very low (0.2-1.8% AR) and consisted mainly of unresolved background and not analysed radioactivity. SFO, FOMC, and where required HS and DFOP models were evaluated by the RMS. Modified fitting with M0 fixed and/or elimination of outliers was performed where required according to the recommendations of the Guidance document. The results for the best fit model are presented in Table 4.1.2.2-07. Where the best fit was obtained by another model than SFO, the optimised fitting parameter are presented in the footnotes. The error value was >15% for the best fit kinetics in the water phase of the B systems. This was mainly due to data scatter for the later time points, whereas the initial decline phase accounting for the majority of the dissipating was reasonably well fitted. Therefore the DT50 (water), which is the main parameter from this fitting procedure, was considered to be an acceptable estimate in these cases.

Table 4.1.2.2-07 Half-lives for pyridalyl in various compartments of water/sediment systems treated with [pyridyl-2,6-14C] or [dichlorophenyk-U-14C] pyridalyl at 70 µg/L and incubated at 20°C in the dark

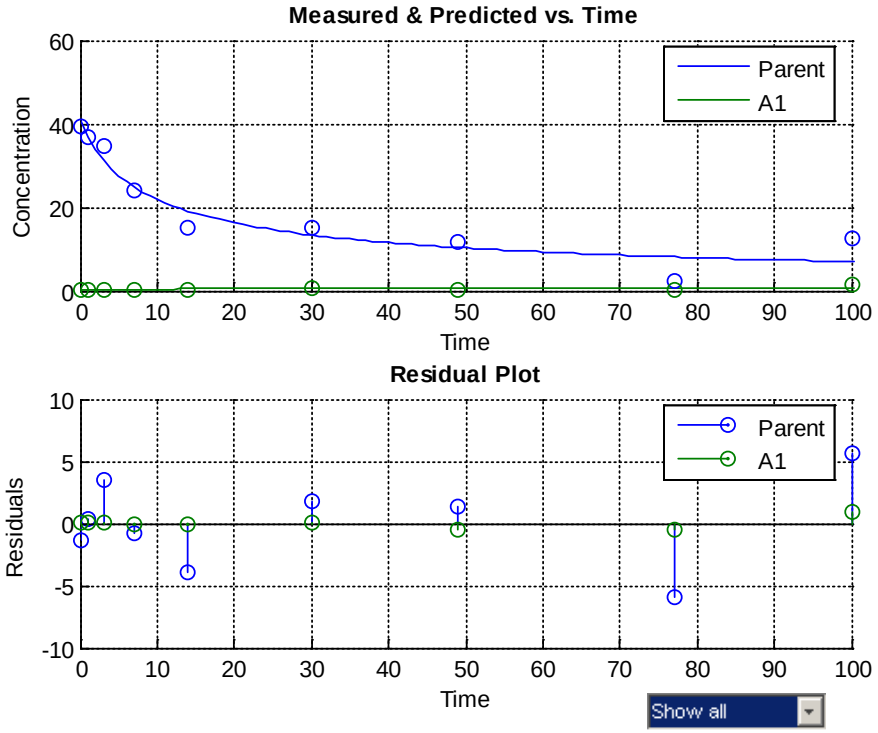
system	label	compart-ment	process	endpoint for	best fit kinetics	Chi^2 (error)	r^2	DT50 (d)	DT90 (d)
A	PYR	water	degradation & mass transfer	persistence	FOMC	13.4		12.1	258
		sediment	degradation & mass transfer	persistence	SFO	8.76	0.59	121	402
		system	degradation	persistence	SFO[(E)]	4.06	0.81	139	462
			degradation	*modelling*	SFO	4.06	0.81	139	-
	DCP	water	degradation & mass transfer	persistence	FOMC[(G)]	5.80	0.97	7.8	57
		sediment	degradation & mass transfer	persistence	-[(A)]				
		system	degradation	persistence	SFO[(D)]	1.76	0.75	366	1216
			degradation	*modelling*	SFO[(D)]	1.76	0.75	366	-
B	PYR	water	degradation & mass transfer	persistence	HS[(C)]	18.06	0.78	6.5	149
		sediment	degradation & mass transfer	persistence	SFO[(H)]	7.31	0.38	244	812
		system	degradation	persistence	SFO[(H)]	3.85	0.81	182	604
			degradation	*modelling*	SFO[(H)]	3.85	0.81	182	-
	DCP	water	degradation & mass transfer	persistence	SFO	29.33	0.86	11	38
		sediment	degradation & mass transfer	persistence	-[(F)]				
		system	degradation	persistence	SFO	6.33	0.71	129	428
			degradation	*modelling*	SFO	6.33	0.71	129	-

(A) SFO and FOMC based on day 49-100 data gave a poor visual fit with r^2 0.37. It is suspected that the day 100 data is unreliable in view of the large difference between replicates (40% AR and 80% AR), but leaving out the day 100 data would give only 2 time points for fitting which is insufficient for reliable estimates. Therefore no reliable degradation parameters could be estimated.
(B) Endpoints are based on the entire data set (M0 fixed did not improve the fit).
(C) Endpoints are based on the entire data set (excluding the day 7 replicate A value and/or M0 fixed did not improve the fit). Optimised HS parameters were: k_1 = 0.105848, k_2 = 0.00774879, M_0 = 44.2502, t_b = 11.7225.
(D) SFO and FOMC based on all data gave a poor visual fit with r^2 0.46. It is suspected that the day 100 data is unreliable in view of the large difference between replicates (40% AR and 86% AR). The above fit was obtained leaving out the day 100 data.
(E) Endpoints are based on the data set excluding the day 1 replicate B value (mass balance only 82%); exclusion of this sample resulted in the best fit.
(F) No acceptable visual fit could be obtained due to data scatter.
(G) Optimised FOMC parameters were: α = 1.21615, β = 10.1148, M_0 = 48.9765.
(H) Endpoints are based on the data set excluding the day 7 replicate A value (mass balance only 81%); exclusion of this sample resulted in the best fit.

Kinetics graphs

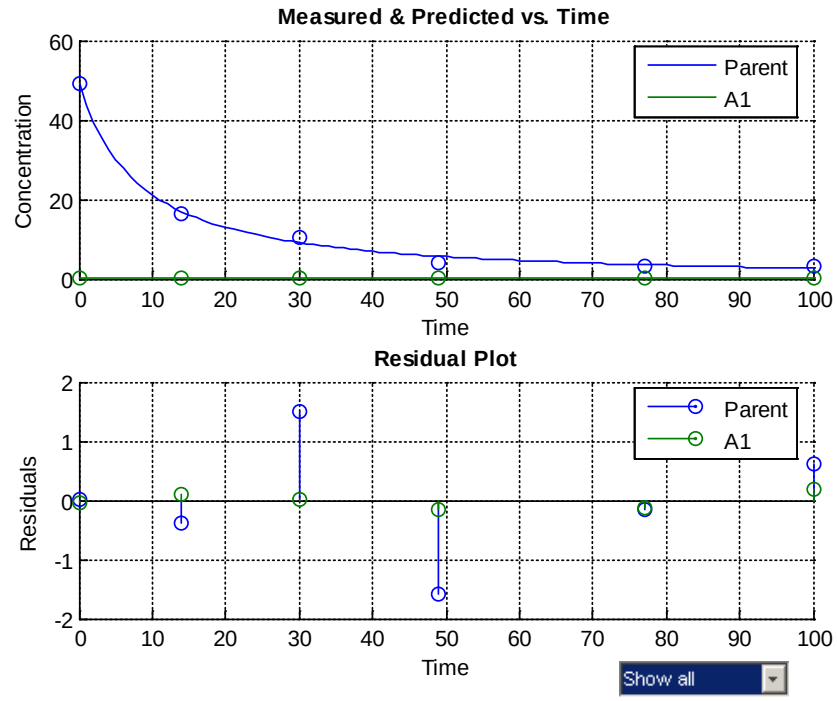
System A PYR label, dissipation in water

Best fit, FOMC-SFO



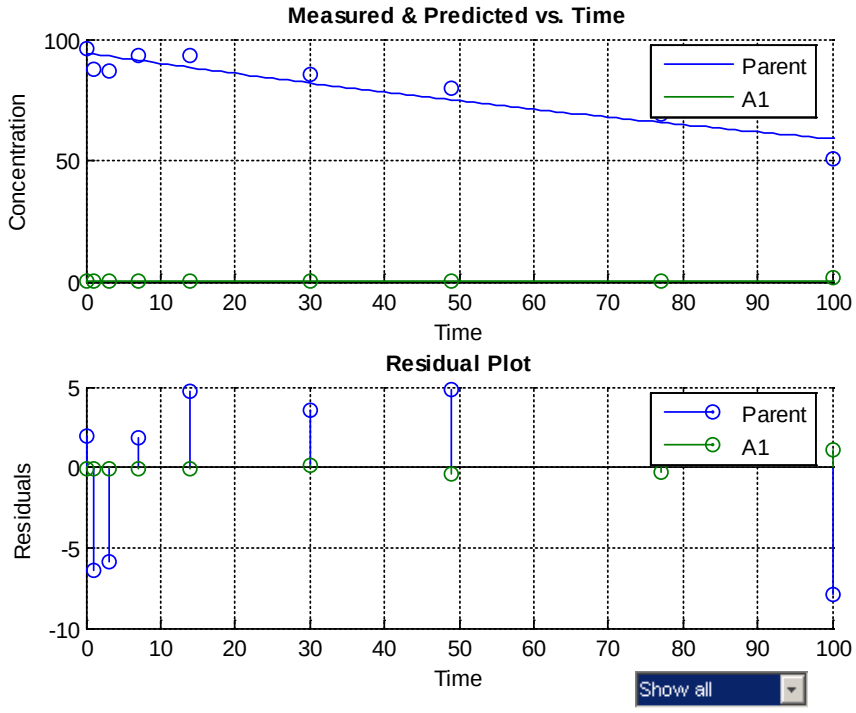
System A DCP label, dissipation in water

Best fit, FOMC-SFO



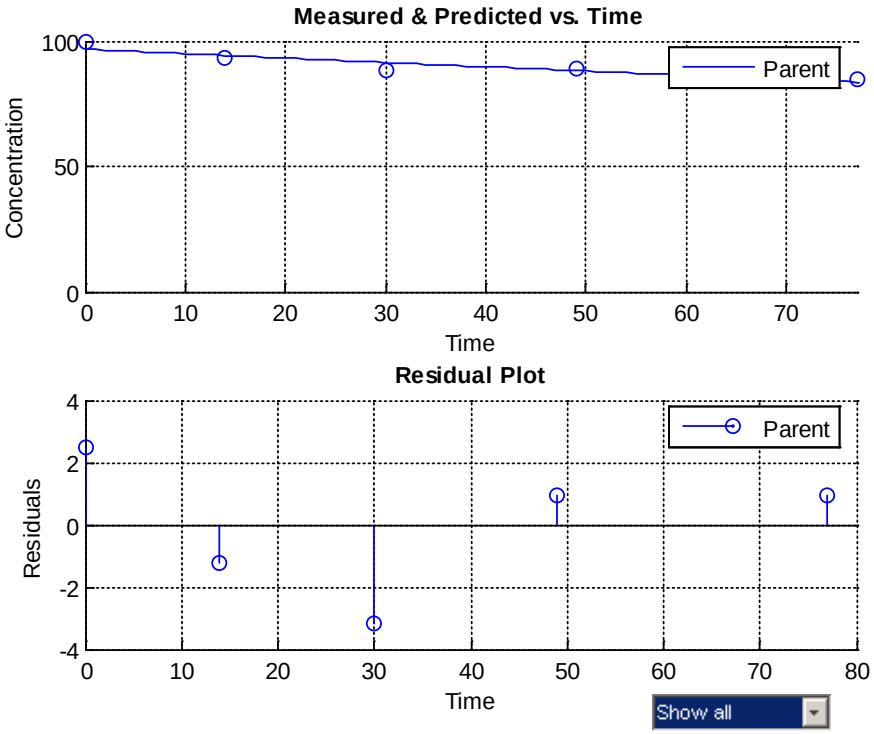
System A PYR label, total system degradation

SFO



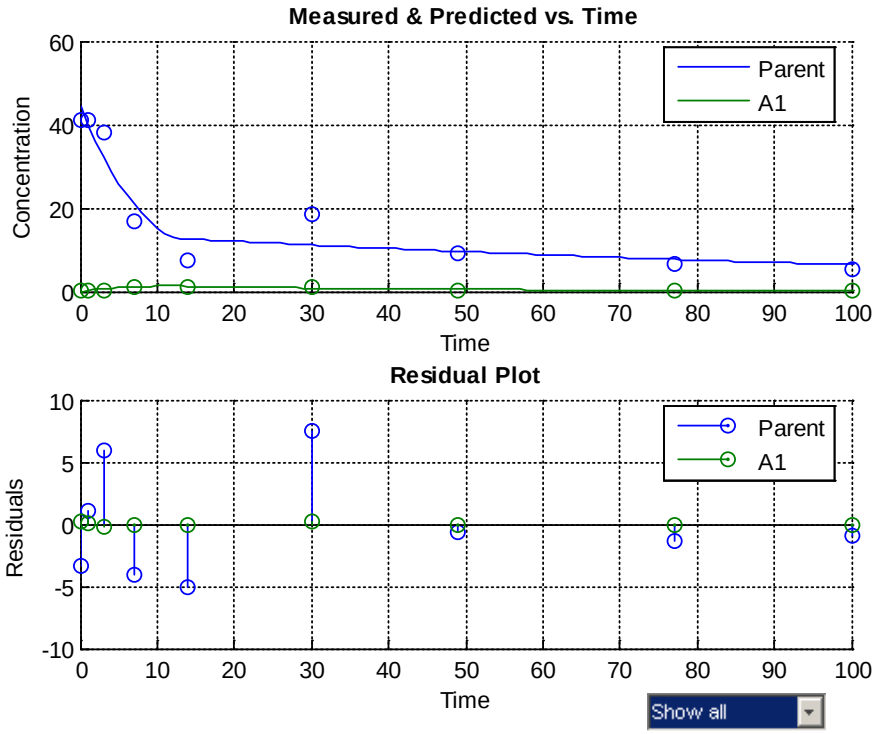
System A DCP label, total system degradation

SFO



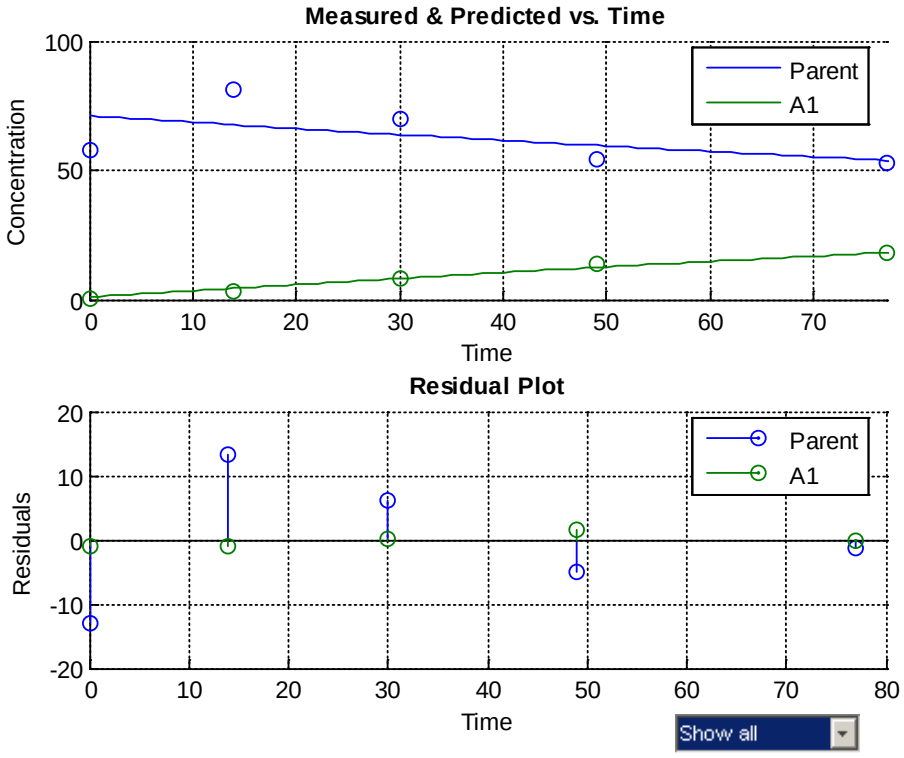
System B PYR label, dissipation in water

Best fit, HS-SFO



System B DCP label, dissipation in water

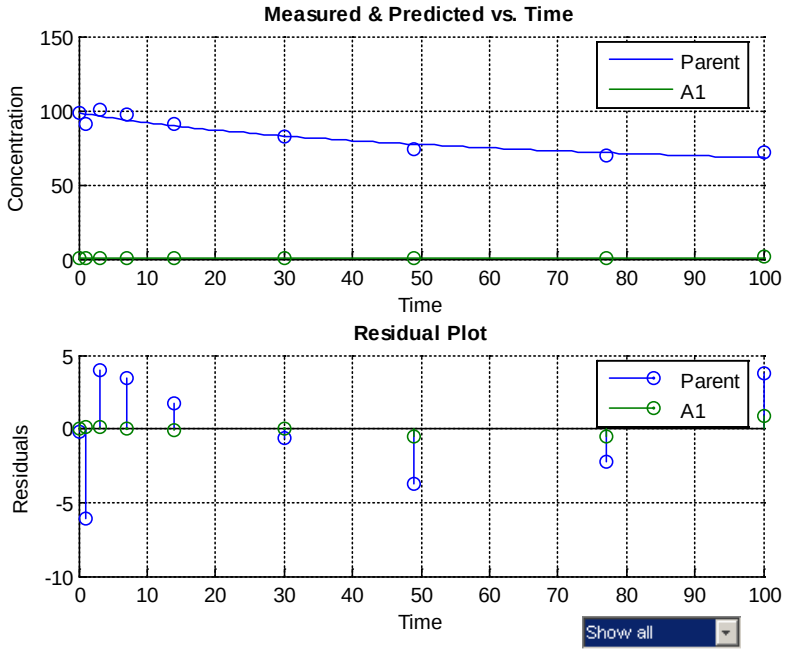
Best fit, SFO-SFO



The visual fir seems not very accurate. FOMC fit did not improve the fit. The statistical parameter of the fit show an acceptable chi^2 of 11.5. The t-test however show a p-value of 0.105 and thus above 0.05. Ikt can be concluded that there is no appropriate fit for the dissipation in water for the DCP label in system B.

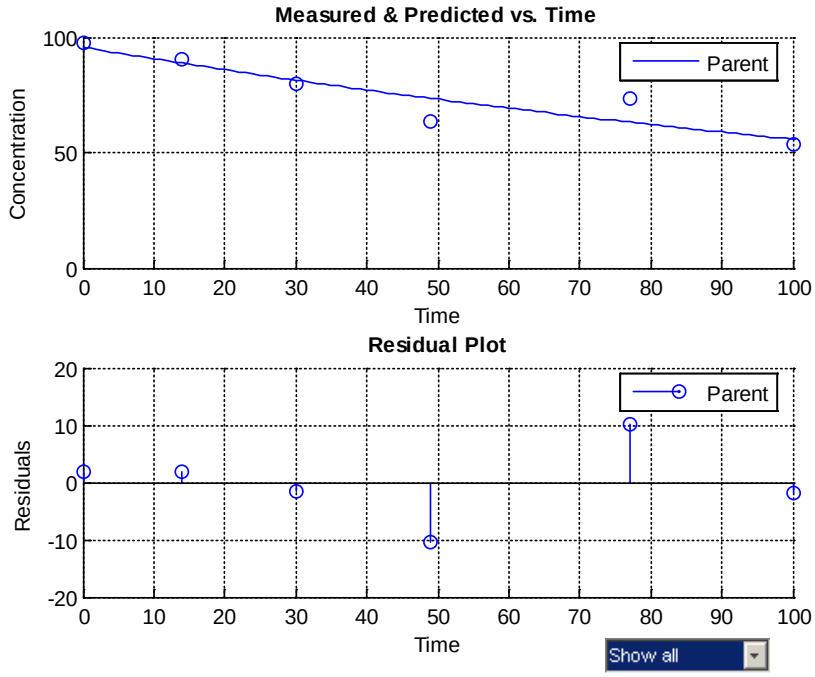
System B PYR label, total system degradation

SFO



System B DCP label, total system degradation

SFO



Despite the visual fit for the DCP label not being very favourable, the statistical parameters of the SFO fit are good with a chi^2 of 6.3 and a t-prob p-value of 0.0062. The SFO value is acceptable.

Conclusions

In two water/sediment systems, treated with [pyridyl-2,6-14C]-pyridalyl or [dichlorophenyl-U-14C]-pyridalyl at a concentration of 70 µg/L and incubated at 20°C in the dark for 100 days, pyridalyl degraded in the total water/sediment system with half-lives of 129-366 days (persistence and modelling). Pyridalyl dissipated from the water phase with half-lives of 6.5-11 days. The levels of parent pyridalyl reached a maximum in sediment of 78-85% AR on day 14-49, and pyridalyl dissipated from the sediment with half-lives of 121-244 days. The non-extractable fraction in sediment increased to a maximum of 10-15% AR, and CO_2 increased to 1-9% AR. The main metabolite was S-1812-DP, which reached maximum levels in water and sediment of 0.4-1.7% AR and 11-18% AR respectively. Besides pyridalyl and S-1812-DP, the following metabolites were found at low levels: S-1812-Ph-CH_2COOH (max. 1.9% AR in water and 4.2% AR in sediment), HTFP (max. 4.4% AR in water and 1.6% AR in sediment) and S-1812-DP-Me (max. 2.0% AR in water and 3.5% AR in sediment).

Guidelines & Limitations

The test concentration of 70 µg/L was a factor of 470 above the water solubility of pyridalyl (0.15 µg/L at 20°C). The water phase was not gently mixed after treatment. This may have lead to the formation of a surface film and/or inhomogeneous distribution of pyridalyl in the water phase. The presence of some non-dissolved pyridalyl (particulate matter) in the water-sediment systems may be responsible for the large difference between certain replicate values. It was reported that the test concentration of 70 µg/L was chosen in order to detect degradates at a level of 1% AR. Testing at or below the water solubility of pyridalyl is however not possible due to analytical limitations. The solubility of pyridalyl under the test conditions may be higher than 0.15 µg/L as the test medium contained 1% acetonitrile. The test systems were slightly agitated during incubation, which enhances dissolution. The measured concentrations of pyridalyl in the test water were 30 µg/L on day 0, declining to 10 µg/L on day 30-49, and these levels are far above the

solubility limit in water. The partitioning of pyridalyl into sediment was rapid and significant (up to 85% AR), but this is expected based on the adsorption studies with this substance (Koc ≥402000 L/kg). The high concentration of applied pyridalyl had no adverse effect on the microbial viability, which did not decline substantially during incubation. The study is therefore considered to be acceptable.

Study 2

Characteristics

reference	:	IIA 7.8.3/02, Study No. 1043.014.310	test system	:	outdoor microcosm
type of study	:	Fate in outdoor microcosms	treatment method	:	spray of water surface (spray-drift) or soil slurry (run-off)
year of execution		2002-2003	treatment rate		~1.4 µg/L (spray-drift); ~6.5 µg/L (run-off)
GLP statement	:	Yes	study duration	:	21 days
guideline	:	EWOFFT (1992), Hill *et al.* (1994), OECD (1996), SETAC (1991), WWF/RESOLVE (1992), HARAP (1998).	conclusion	:	see results
test substance	:	a) [dichlorophenyl-U-14C]-pyridalyl, lot no. RIS2002-008 (C-2002-032T) b) non-labelled S-1812, lot no. 980302 G	acceptability	:	acceptable
purity	:	a) chemical purity not reported, radiochemical purity ≥96.6%. b) 99.7% pure.			

The fate of [dichlorophenyl-U-14C]-pyridalyl formulated as 35 WP applied either via simulated spray-drift or run-off was investigated in outdoor microcosms in Switzerland.

Methods

Four weeks prior to application, four stainless steel cylinders (diameter ~2 m, surface area ~3.2 m^2, height ~ 1.5 m) were placed as enclosures into the same polyethylene coated concrete basin of 9 x 9 m and 1.5 m depth. Each enclosure represented one microcosm unit. The basin had been filled in December 2001 with ~25 cm of top layer sediment and ~ 1 m of water, collected from Lake Constance and containing a natural population of organisms and macrophytes. The basin and enclosures contained a natural population of the filamentous macrophyte *Chara fragilis* (density was determined prior to treatment to be 1254 g fresh weight per m^2). Prior to treatment each enclosure received 7 bundles of the macrophyte *Elodea canadensis*, each containing 100 g fresh weight. It was reported that care was taken that the load of introduced macrophytes did not exceed 50% of the bottom area and 25% of the water volume of a microcosm.

On 5 August 2002, two microcosms were treated with a simulated spray drift application, and the two remaining microcosms with a simulated run-off application. The first treatment was applied by spraying the water surface with 100 mL of an aqueous suspension containing ~4.7 mg of the radio-labelled test compound (~1:1 isotopically diluted) and 9.5 mg of 35 WP blank formulation (nominal concentration in water based on LSC analysis of treatment suspensions 1.4-1.5 µg/L, based on water volume of 3140 L). The run-off treatment was applied by distributing in small portions into the sub-surface water layer a 1:1 soil water slurry prepared from a batch of 24.5 kg d.w. of sandy loam soil (19/50/31% sand/silt/clay, pH 7.0, 3.0% oc, 50.2% MWC, 2 mm sieved), that had been treated with a nominal amount of 20.3 mg of the radio-labelled

test compound formulated as 35 WP (nominal concentration in water 6.5 µg/L based on water volume of 3140 L), mixed thoroughly and aged in the dark for 24 hours at ~20°C.

Water samples (~2 L per sample) from 3 different depths (top, middle, bottom) at 3 locations/enclosure were collected for chemical analysis at 2, 24 and 48 hours after treatment, and depth integrated water samples were collected from 4 locations/enclosure after 4, 7, 14 and 21 days. Two sediment samples per enclosure were collected with a corer (7x7 cm area) for chemical analysis after 2, 4, 7, 14 and 21 days. The top 2 cm and the 2-5 cm were pooled to one sample, each.

Water physico-chemical parameters (dissolved oxygen, pH, conductivity, alkalinity, hardness, temperature, turbidity, water level) were monitored in all microcosms on day -7, 0 (3 levels), 2, 4, 7, 14 (3 levels) and 21. Sediment TOC and water nitrogen and phosphorus were determined in a samples collected on day 0, and water DOC and sediment redox potential and pH in samples collected on day 0, 7, 14 and 21.

Water samples were filtered successively through 100 µm and 1 µm filters. The 100 µm filter was rinsed in water, the resulting water sample was discarded. The 1 µm filter was ultrasonicated in water, the resulting aqueous sample was filtered again (1 µm) and the filter was extracted with acetonitrile with 1% HCl followed by LSC of the extracts. The post-1µm filtration aqueous sample was acidified and partitioned with dichloromethane followed by LSC of both layers. All filters and the sample bottle were rinsed with acetonitrile and the rinses were analysed by LSC.

Sediment samples were centrifuged five times with Ludox AM-30 (colloidal silica suspension) and the separated benthic organisms were discarded. The post-centrifugation Ludox AM-30 samples contained <0.1% of sediment TRR. Post-centrifugation sediment samples were extracted twice with 50 mL of acetonitrile containing 1% HCl (180:0.5 v:v). Radioactivity in extracts was determined by LSC and in PES by combustion/LSC.

The dichloromethane extracts of all water samples and the sediment extracts at 0.1 and 0.65 µg a.s./L were concentrated and analysed by reversed phase HPLC with confirmation of compound identity by normal phase TLC. Identification was based on co-chromatography with the following reference compounds: pyridalyl, S-1812-DP, S-1812-Ph-CH_2COOH. The radioactivity levels in sediment extracts at 0.05 and 6.5 µg a.s./L were too low for chromatographic analysis.

Results

Physico-chemical properties

Total N and total P in water were <1 and <0.05 mg/L respectively. Dissolved oxygen, pH and temperature at top, middle and bottom water levels were comparable. The pH of the water was about 10 (pre-treatment), about 10 (post-treatment, spray-drift enclosures) and about 9.5 (post-treatment, run-off enclosures). Dissolved oxygen was 12-15 mg/L (pre-treatment) and 11-14 mg/L (post-treatment, spray-drift enclosures) but dropped post-treatment in run-off enclosures (minimum 7 mg/L on day 4, rising to 11-12 mg/L on day 21). The author of the report attributed the lower pH and dissolved oxygen values in run-off enclosures to increased turbidity due to introduction of the soil slurry causing reduced photosynthesis. Conductivity was 119-178 µSi/cm, alkalinity about 40-50 mg/L $CaCO_3$ and hardness about 50-60 mg/L $CaCO_3$. DOC increased from 4.9-5.6 mg C/L on day 0 to 7.0-8.6 mg C/L on day 7 (spray-drift enclosures) and from 5.4-8.9 mg C/L on day 0 to 13.1 mg C/L on day 7 (run-off enclosures, increase attributed to introduction of soil-slurry). Water temperature was 21-22°C pre-treatment but fell between day 0 and 7 from 22°C to 16°C, then

increasing to about 22°C after 14 and 21 days. Turbidity was increased in enclosures following introduction of the soil slurry compared to spray-drift enclosures, but by day 7 all enclosures had comparable turbidity. The redox potential of the sediment was always negative, and the sediment pH was 7-9. Sediment TOC in the 0-2 cm layer was 0.93 and 1.2%. The sediment texture was not reported.

Average global radiation increased from a low value of about 200 W/m^2 on the day of treatment to about 800 W/m^2 9 days later and remained at that level until study end. Average UV-global radiation increased from 5-10 W/m^2 on the day of treatment to about 35 W/m^2 9 days later and remained at that level until study end.

Residue analysis

Based on LSC analysis of the suspensions for treating the soil for run-off application, the amount of test substance applied was 21.5 and 19.9 mg pyridalyl (106% and 98% of target). Combustion analysis after ageing for one day showed that each soil portion contained 16.4 and 16.6 mg (81% and 82% of target). An explanation for the difference between the measurements before and after ageing was not provided. A desorption experiment was performed with 1-day aged soil, in which 1 g of soil was shaken by hand for one minute with 128 mL of basin water (proportion equivalent to 24.5 kg of soil and 3140 L of enclosure water). LSC analysis showed that the water layer contained only 2.2% of the radioactivity (corresponding with a concentration of 0.10 μg/L in the water).

It was reported that QC samples (n=28 for water, fortification level not specified, n=11 for sediment, fortification level 2-88 μg/kg) were analysed with each analytical batch, and that recoveries were acceptable (water 71-96%, except for 2 samples with 64-65%; sediment extraction efficiency 89-110%, recovery evaporation 78-95%).

Measurements at 3 different water depths indicated that 2 hours after application the radioactivity concentrations in the top, middle and bottom of the water column of the spray-drift enclosures were 2.7-2.8, 0.3-0.5 and 0.2-0.3 μg eq/L, but that they were homogenously distributed after 24 hours. The radioactivity concentrations in the water column of the run-off enclosures were homogenously distributed within 2 hours. The remaining results on distribution of radioactivity in the microcosms are shown in Tables 4.1.2.2-08 and -09.

In spray-drift enclosures, the radioactivity concentration in water fell from 1.13-1.14 μg eq/L immediately after application to 0.31 μg/L after 21 days. The highest radioactivity concentrations in sediment were reached on day 21 (7.3-12 μg eq/kg and 49-98 μg eq/kg in spray-drift and run-off enclosures respectively). The total recovery was variable and low (28-54% AR).

In run-off enclosures, the radioactivity concentration in water fell from 0.23-0.26 μg eq/L immediately after application to 0.17-0.18 μg/L after 21 days. The radioactivity concentration in sediment was 95-118 μg eq/kg after 2 days and showed the same pattern in both replicates (lowest level on day 7, followed by increase, 49-98 μg eq/kg after 21 days). The total recovery was variable and mostly low (22-96% AR, mean 57% AR). The author of the report stated that the variation between the sediment residues in run-off enclosures may be explained by (1) Deposition of the treated aged soil on the leaves of macrophytes on the sediment surface, and macrophyte coverage was variable between enclosures; (2) The fact that the surface layer was not homogeneous due to the treatment technique so that the quantity of contaminated soil on top of the 0-2 cm layer varied between samplings. This explanation is acceptable but the consequence is that the residue levels in sediment cannot be considered to be accurate values.

Possible other factors that may have contributed to the low and variable overall recoveries are (1) Adsorption of radioactivity to macrophytes (filamentous *A. chara* growing on sediment, and floating *E. canadensis*), and possibly variable macrophyte coverage; (2) Recovery of analytical procedures <100%.

The results on identification of radioactivity in the acetonitrile rinses and organic extracts of the water- and sediment are shown in Tables 4.1.2.2-10 and -11.

In spray-drift enclosures, the level of parent pyridalyl in water fell from 1.06-1.08 µg/L after 2 hours to 0.010-0.015 µg/L after 21 days, whilst those in sediment increased from 2.3-2.8 µg/kg after 2 days to 4.0-4.5 µg/kg after 21 days. S-1812-DP was found at maximum levels of 1.7-2.2% AR in water, and at low levels in sediment (0.6% AR). The level of S-1812-$PhCH_2COOH$ increased in water to 5.7-6.1% AR (not detected in sediment). Unidentified compounds were at the most 1.7% AR.
In run-off enclosures, the level of parent pyridalyl in water fell from 0.17-0.18 µg/L after 2 hours to 0.008 µg/L after 21 days, whilst those in sediment were 83-97µg/kg after 2 days and 41-83 µg/kg after 21 days. S-1812-DP was found at maximum levels of 0.7-1.0% AR in water, and was also found in sediment at levels up to 1.5-1.8% AR. The level of S-1812-$PhCH_2COOH$ increased in water to 1.2-1.3% AR (not detected in sediment). Unidentified compounds were <1% AR.

Note by RMS: levels of pyridalyl and metabolites in the whole system were not calculated since the recovery of radioactivity was <90% on most sampling days.

Table 4.1.2.2-08 Distribution of radioactivity (µg/L, % AR) in water and sediment of microcosms treated with [dichlorophenyl-U-14C]pyridalyl by simulated spray-drift application at nominal concentration 1.4-1.5 µg/L

		water[A]								sediment (0-2 cm)						sediment (2-5 cm)		system
		acetonitrile rinse		organic phase		aqueous phase		total		extract		non-extractable		total		total		
repl.	time	µg/L	% AR	µg/L	% AR	µg/L	% AR	µg/L	% AR	µg/kg	% AR	µg/kg	% AR	µg/kg	% AR	µg/kg	% AR	% AR
A	2 hr	0.08	5	1.0	74	0.03	2	1.13	82									
	24 hr	0.04	3	0.52	38	0.04	3	0.60	44									
	48 hr	0.02	2	0.33	24	0.05	4	0.41	29	2.81	7.5	1.66	4.5	4.47	12	na	na	41
	4 d	0.006	0.4	0.21	15	0.10	7	0.32	23									
	7 d	0.003	0.2	0.16	11	0.13	9	0.29	21	1.67	4.9	1.03	3.1	2.70	8.0	na	na	29
	14 d	0.001	0.1	0.13	10	0.18	13	0.32	23	2.08	5.8	1.53	4.3	3.61	10	na	na	33
	21 d	0.001	0.1	0.12	9	0.18	13	0.31	22	4.67	13	7.07	19	11.7	32	na	na	54
B	2 hr	0.08	5	1.04	69	0.03	2	1.14	76									
	24 hr	0.04	3	0.56	37	0.04	3	0.64	42									
	48 hr	0.02	2	0.35	23	0.06	4	0.43	29	2.34	6.6	0.58	1.6	2.92	8.2	na	na	37
	4 d	0.005	0.3	0.23	16	0.09	6	0.33	22									
	7 d	0.003	0.2	0.14	9	0.12	8	0.26	18	2.79	7.8	0.89	2.5	3.68	10	na	na	28
	14 d	0.002	0.1	0.16	11	0.18	12	0.34	23	1.42	3.2	0.79	1.8	2.21	5.0	na	na	28
	21 d	0.001	0.1	0.13	9	0.18	12	0.31	21	4.00	10	3.28	8.4	7.28	19	na	na	40

na = not analysed
(A) Solid particulate matter in water phase represented <0.01 µg/L in all samples

Table 4.1.2.2-09 Distribution of radioactivity (µg/L, % AR) in water and sediment of microcosms treated with [dichlorophenyl-U-14C]pyridalyl by simulated run-off application at nominal concentration 6.5 µg/L

		water[A]				sediment (0-2 cm)			sediment (2-5 cm)	
		acetonitrile rinse	organic phase	aqueous phase	total	extract	non-extractable	total	total[B]	system

repl.	time	µg/L	% AR	µg/L	% AR	µg/L	% AR	µg/L	% AR	µg/kg	% AR	µg/kg	% AR	µg/kg	% AR	µg/kg	% AR	% AR
A	2 hr	0.01	0.2	0.23	4.4	0.02	0.4	0.26	4.9									
	24 hr	0.01	0.2	0.18	3.5	0.03	0.6	0.23	4.3									
	48 hr	0.01	0.1	0.16	3.0	0.04	0.8	0.21	4.0	100	68	17.6	12	118	80	17.3	12	96
	4 d	0.002	0.05	0.13	2.5	0.06	1.1	0.19	3.6									
	7 d	0.001	0.03	0.10	1.9	0.07	1.3	0.17	3.2	35.2	24	4.98	3.4	40.2	28	2.30	2.3	33
	14 d	0.001	0.02	0.09	1.6	0.09	1.8	0.18	3.4	50.6	29	10.8	6.2	61.4	35	9.79	11	50
	21 d	0.001	0.01	0.09	1.7	0.08	1.6	0.17	3.3	85.0	65	13.3	10	98.3	76	9.70	11	90
B	2 hr	0.01	0.2	0.20	3.8	0.02	0.4	0.23	4.3									
	24 hr	0.01	0.2	0.20	3.8	0.03	0.6	0.24	4.6									
	48 hr	0.01	0.1	0.17	3.2	0.04	0.7	0.21	4.0	85.8	49	9.33	5.3	95.1	54	19.2	14	72
	4 d	0.002	0.04	0.12	2.2	0.05	0.9	0.17	3.2									
	7 d	0.001	0.03	0.13	2.5	0.06	1.2	0.19	3.7	18.0	13	3.79	2.7	21.8	16	2.29	2.6	22
	14 d	0.001	0.02	0.09	1.7	0.08	1.5	0.17	3.3	24.8	15	3.70	2.3	28.5	18	16.6	21	42
	21 d	0.001	0.01	0.09	1.8	0.09	1.6	0.18	3.4	41.5	29	7.27	5.1	48.7	34	11.6	12	49

na = not analysed

(A) Solid particulate matter in water phase represented <0.01 µg/L in all samples

(B) Of the radioactivity in the 2-5 cm sediment, 67-91% was extractable.

Table 4.1.2.2-10 Identification of radioactivity (µg/L, % AR) in water and sediment of microcosms treated with [dichlorophenyl-U-14C]pyridalyl by simulated spray-drift application at nominal concentration 1.4-1.5 µg/L

		water (organic extract + acetonitrile rinse)[A]								sediment (0-2 cm)			
		a.s.		S-1812-DP		S-1812-PhCH$_2$COOH		M1		a.s.		S-1812-DP	
repl.	time	ug/L	%AR	ug/L	%AR	ug/L	%AR	ug/L	%AR	ug/kg	%AR	ug/kg	%AR
A	2 hr	1.06	76.6	0.004	0.3	0.012	0.9	nd	nd				
	24 hr	0.52	37.6	0.029	2.1	0.013	0.9	nd	nd				
	48 hr	0.28	20.6	0.031	2.2	0.024	1.8	0.004	0.3	2.81	7.5		
	4 d	0.14	10.3	0.023	1.7	0.029	2.1	0.013	0.9				
	7 d	0.079	5.7	0.017	1.2	0.051	3.7	0.012	0.9	1.67	4.9		
	14 d	0.031	2.3	0.016	1.2	0.065	4.7	0.023	1.7	2.08	5.8		
	21 d	0.015	1.1	0.006	0.5	0.084	6.1	0.017	1.3	4.47	12.3	0.21	0.6
B	2 hr	1.08	72.1	nd	nd	0.006	0.4	nd	nd				
	24 hr	0.56	37.6	0.024	1.6	0.008	0.5	nd	nd				
	48 hr	0.34	22.5	0.026	1.7	0.006	0.4	nd	nd	2.34	6.6		
	4 d	0.17	11.6	0.025	1.7	0.029	1.9	0.010	0.6				
	7 d	0.054	3.6	0.024	1.6	0.053	3.5	0.011	0.7	2.79	7.8		
	14 d	0.037	2.5	0.011	0.7	0.084	5.6	0.019	1.3	1.42	3.2		
	21 d	0.010	0.6	0.010	0.7	0.086	5.7	0.024	1.6	4.00	10.3		

nd = not detected

(A) Remaining unidentified compounds were at the most 0.022 µg eq/L (1.6% AR), but represented 2 fractions, each <1% AR.

Table 4.1.2.2-11 Identification of radioactivity (µg/L, % AR) in water and sediment of microcosms treated with [dichlorophenyl-U-14C]pyridalyl by simulated run-off application at nominal concentration 6.5 µg/L

		water (organic extract + acetonitrile rinse)								sediment (0-2 cm)[A]			
		a.s.		S-1812-DP		S-1812-PhCH$_2$COOH		M1		a.s.		S-1812-DP	
repl.	time	ug/L	%AR	ug/L	%AR	ug/L	%AR	ug/L	%AR	ug/kg	%AR	ug/kg	%AR
A	2 hr	0.18	3.4	0.050	1.0	0.010	0.2	nd	nd				
	24 hr	0.15	2.9	0.031	0.6	0.010	0.2	nd	nd				
	48 hr	0.12	2.4	0.023	0.4	0.018	0.3	nd	nd	96.8	65.7	2.67	1.8
	4 d	0.074	1.4	0.017	0.3	0.027	0.5	0.012	0.2				
	7 d	0.047	0.9	0.016	0.3	0.032	0.6	0.004	0.1	34.7	23.7	0.57	0.4
	14 d	0.013	0.2	0.011	0.2	0.052	1.0	0.011	0.2	48.5	27.7	1.31	0.7
	21 d	0.008	0.1	0.006	0.1	0.063	1.2	0.01	0.2	82.7	63.7	1.88	1.4
B	2 hr	0.17	3.3	0.037	0.7	nd	nd	nd	nd				
	24 hr	0.16	3.0	0.034	0.7	0.019	0.4	nd	nd				

	48 hr	0.11	2.1	0.036	0.7	0.030	0.6	nd	nd	82.7	55.7	2.2	1.5
	4 d	0.055	1.1	0.021	0.4	0.031	0.6	0.013	0.2				
	7 d	0.067	1.3	0.018	0.3	0.034	0.7	0.013	0.3	17.4	12.4	0.48	
	14 d	0.012	0.2	0.008	0.2	0.065	1.2	0.008	0.2	24.8	15.3		
	21 d	0.008	0.2	0.005	0.1	0.069	1.3	0.011	0.2	41.0	28.5	0.42	0.3

nd = not detected
(A) An unidentified compound was at the most 0.89 μg eq/kg (0.6% AR), but represented 2 fractions, each <1% AR.

DT50 estimations

DT_{50} values for disappearance of pyridalyl and S-1812-DP from water were calculated according to SFO (single first-order) kinetics with ModelMaker v 4.0 software. Calculations for each scenario and compound were based on values of both replicates. The results are presented in the Table below. Pyridalyl disappeared from the water with SFO DT50 and DT90 values of 1.0-3.5 and 3.7-12 days. S-1812-DP disappeared from the water with SFO DT50 and DT90 values of 5.5-11 and 18-37 days.

Table 4.1.2.2-12 SFO half-lives for disappearance of pyridalyl and DP in the water of microcosms treated with [dichlorophenyl-U-14C]pyridalyl by simulated spray-drift or run-off application

scenario	com-pound	χ^2 (err)	r^2	DT50	DT90
spray-drift	pyridalyl	10.24	0.99	1.1	3.7
	DP	1.96	0.90	11	37
run-off	pyridalyl	8.39	0.97	3.5	12
	DP	10.83	0.86	5.5	18

Conclusions

In outdoor microcosms in Switzerland (1 m deep) treated with [dichlorophenyl-U-14C]-pyridalyl formulated as 35 WP, either via simulated spray-drift (nominal concentration 1.4-1.5 μg/L) or run-off (nominal concentration 6.5 μg/L), pyridalyl disappeared from the water with SFO DT50 and DT90 values of 1.0-3.5 and 3.7-12 days respectively. S-1812-DP was found at maximum levels of 2.2% AR in water and 1.8% AR in sediment. S-1812-DP disappeared from the water with SFO DT50 and DT90 values of 5.5-11 and 18-37 days respectively. The level of S-1812-$PhCH_2COOH$ (not detected in sediment) increased in water up to 6.1% AR (spray-drift) or 1.3% AR (run-off).

Guidelines & Limitations

The study is acceptable. The analytical results for the sediment in the run-off enclosures are not accurate (see above).

In run-off enclosures, the radioactivity concentration in water fell from 0.23-0.26 μg eq/L immediately after application to 0.17-0.18 μg/L after 21 days. The total recovery was variable and mostly low (22-96% AR, mean 57% AR). The author of the report stated that the variation between the sediment residues in run-off enclosures may be explained by (1) Deposition of the treated aged soil on the leaves of macrophytes on the sediment surface, and macrophyte coverage was variable between enclosures; (2) The fact that the surface layer was not homogeneous due to the treatment technique so that the quantity of contaminated soil on top of the 0-2 cm layer varied between samplings. This explanation is acceptable but the consequence is that the residue levels in sediment cannot be considered to be accurate values.

4.1.2.3 Field studies

STUDY 1

Characteristics

reference	:	IIA 7.3.1/01, Study No. 0333/210-D2149	location	:	France-S (2X), Italy (2X)
year of execution	:	2003-2006	dose	:	600 g a.s./ha
GLP statement	:	yes	residues	:	pyridalyl, S-1812-DP, S-1812-DP-Me, HTFP
guideline	:	Incl. SETAC (1995)	DT50	:	pyridalyl: 2.3-39 days
test substance	:	Pyridalyl 10 EW, lot SBM03/002/006	metabolites	:	S-1812-DP max. 9.7%, S-1812-DP-Me max. <5.3%, HTFP max. 12.9%
purity	:	10.4% w/w pyridalyl,	acceptability	:	acceptable
soils	:	loam (3X), sandy loam			

STUDY 2

Characteristics

reference	:	Jarvis T. 2007b (IIA 7.3.1/02)	study type	:	Position paper
year of execution	:	2007	incubation time	:	Not applicable
GLP statement	:	Not applicable	nominal concentration	:	Not applicable
guideline	:	Not applicable	temperature	:	Not applicable
test substance	:	pyridyl	DT50	:	Not applicable
purity	:	Not applicable	metabolites	:	Not applicable
soils	:	Not applicable	acceptability	:	acceptable

Study design

A study was conducted to determine the dissipation and accumulation of residues of pyridalyl and the levels of metabolites S-1812-DP, S-1812-DP-Me and HTFP in field soil following spraying of Pyridalyl 10 EW on bare soil at two locations in Southern France (trial 1 & 2) and two in Italy (trial 3 & 4), with a single spring application at nominal dose 600 g a.s./ha or with one spring application once a year for three consecutive years.

The trial sites represented agricultural land that had been used previously for commercial cropping, but had received no treatment with pyridalyl in the three years prior to the start of the trials. Each trial site had one non-treated control plot (108 m^2) and one treated plot (360 m^2). Each plot was divided into two halves. In trials 1 and 3, each half was used for studying dissipation (one application in 2003). In trials 2 and 4, one half was used for dissipation (one application in 2003), the other half for studying dissipation and accumulation (one application in 2003, one in 2004 and one in 2005). The properties of the 0-20 cm segment (not reported for deeper layers) and the application details are shown in Table 4.1.2.3-01 below.

No irrigation was applied during the trial, and plots were kept clear of vegetation by treatment with glyphosate and ferrous sulphate (France) or glyphosate, glufosinate and pendimethalin (Italy). In the Italian trials weed cover increased at the end of July 2003 from 5% to 70% at 10 August, following which herbicides were applied, which restored the site to bare soil early September 2003. It was reported that it was possible to collect the T1 + 3 months samples from bare soil. Meteorological conditions during the trial (max. and min. air temperature, humidity, daily rainfall and monthly soil temperature) were recorded at local meteorological stations within 45 km from the trial site. The meteorological data on rainfall and temperature

(monthly means or totals) together with the available data on long-term averages are shown in Table 4.1.2.3-02 to 4.1.2.3-04.

Table 4.1.2.3-01 Properties of study soils and application details

Trial number	**1**	**2**	**3**	**4**
Country Location	Southern France St Soulan, F-32220	Southern France Lombez, F-32220	Italy 22070 Vertimate con Minoprio	Italy 20089 Rozzano
Soil characteristics				
pH (H_2O)	6.93	6.92	5.64	5.89
pH (KCl)	6.44	6.25	4.55	4.89
pH ($CaCl_2$)	6.53	6.43	4.52	5.11
Organic carbon (%)	0.7	1.1	2.5	0.7
WHC at pF 2.5 (%)	21.16	19.99	24.59	21.13
WHC at pF 0 (%)	50.48	52.25	67.46	45.83
CEC (meq/ 100 g)	32.72	25.76	26.88	24.72
Sand 50-2000 µm (%)	37	35	45	60
Silt 2-50 µm (%)	37	41	41	27
Clay < 2 µm (%)	26	24	14	13
Classification (USDA)	loam	loam	loam	sandy loam
Microbial biomass (pre T1)	195 µg C/g dry soil (⇔ 2.8% OC)	198 µg C/g dry soil (⇔ 1.8% OC)	347 µg C/g dry soil (⇔ 1.4% OC)	246 µg C/g dry soil (⇔ 3.5% OC)
Microbial biomass (T1 + 12 months)	104 µg C/g dry soil (⇔ 1.5% OC)	119 µg C/g dry soil (⇔ 1.1% OC)	36 µg C/g dry soil (⇔ 0.1% OC)	35 µg C/g dry soil (⇔ 0.5% OC)
Microbial biomass (T2 + 12 months)	-	125 µg C/g dry soil (⇔ 1.1% OC)	-	80 µg C/g dry soil (⇔ 1.1% OC)
Application details				
Number of applications	1	3	1	3
Application date	28 May 2003	27 June 2003 & 29 June 2004 & 29 June 2005	21 May 2003	29 May 2003 & 27 May 2004 & 27 May 2005
Spray volume (L/ha)	608	598 & 593 & 617	597	611 & 599 & 558
Applied dose (g a.s./ha)	620	598 & 592 & 618	597	611 & 599 & 580

Table 4.1.2.3-02 Rainfall and temperature on a monthly basis for French trials 1 and 2 recorded at meteorological stations within 45 km from the trial sites

		recorded data during trials			long-term average		
		rainfall	air temperature (°C)		rainfall	air temperature (°C)	
month	year	(mm)	minimum	maximum	(mm)	minimum	maximum
jul	2003	2.5	17	30	62.0	15.1	25.9
aug	2003	57	18	34	48.0	16.3	27.8
sep	2003	51	13	25	67.8	11.3	23.9
oct	2003	60	8.5	18	44.1	9.1	19.9
nov	2003	53	5.9	15	67.8	4.2	13.0
dec	2003	100	3.3	11	64.1	2.9	10.7
jan	2004	124	3.2	9.9	61.3	1.9	10.1
feb	2004	9.0	0.9	11	57.0	2.8	11.6
mar	2004	69	3.3	13	42.4	4.3	15.5
apr	2004	101	6.6	15	68.0	6.1	16.8
may	2004	69	9.3	20	73.9	10.7	21.3
jun	2004	5.1	15	28	60.0	13.7	24.6
jul	2004	36	16	28	62.0	15.1	25.9
aug	2004	52	16	29	48.0	16.3	27.8
sep	2004	53	14	26	67.8	11.3	23.9
oct	2004	122	11	21	44.1	9.1	19.9
nov	2004	42	3.1	11	67.8	4.2	13.0
dec	2004	47	3.1	9.5	64.1	2.9	10.7
jan	2005	32	1.0	8.8	61.3	1.9	10.1
feb	2005	27	0.0	7.3	57.0	2.8	11.6
mar	2005	28	2.2	14	42.4	4.3	15.5
apr	2005	81	6.8	17	68.0	6.1	16.8
may	2005	49	10	22	73.9	10.7	21.3
jun	2005	13	15	35	60.0	13.7	24.6
jul	2005	18	16	29	62.0	15.1	25.9
aug	2005	85	15	35	48.0	16.3	27.8
sep	2005	116	13	23	67.8	11.3	23.9
oct	2005	47	15	35	44.1	9.1	19.9
nov	2005	63	3.0	12	67.8	4.2	13.0
dec	2005	51	-1.1	6.8	64.1	2.9	10.7
jan	2006	19	0.9	7.9	61.3	1.9	10.1

Table 4.1.2.3-03 Rainfall and temperature on a monthly basis for Italian trial 3 recorded at a meteorological station at the trial site

		recorded data during trials		
		rainfall	air temperature (°C)	
month	year	(mm)	minimum	maximum
may	2003	68.0	11.1	26.1
jun	2003	77.8	16.8	33.0
jul	2003	124.6	16.0	31.8
aug	2003	27.2	17.1	32.9
sep	2003	34.0	9.8	24.2
oct	2003	158.6	5.9	15.3
nov	2003	188.6	3.6	11.3
dec	2003	179.8	-1.2	8.2
jan	2004	53.2	-2.2	6.5
feb	2004	139.4	-1.7	9.1
mar	2004	77.2	1.5	11.9
apr	2004	183.6	5.6	17.2

may	2004	166.2	7.4	21.3
jun	2004	19.6	13.1	28.2
jul	2004	54.8	14.9	29.1
aug	2004	116.8	15.7	28.6
sep	2004	40.4	11.3	25.0
oct	2004	147.8	10.3	17.6
nov	2004	149.6	2.1	12.6
dec	2004	75.2	-0.7	8.6

Table 4.1.2.3-04 Rainfall and temperature on a monthly basis for Italian trial 4 recorded at meteorological stations within 18 km from the trial site

		recorded data during trials		
		rainfall	air temperature (°C)	
month	year	(mm)	minimum	maximum
may	2003	31.6	13.2	25.7
jun	2003	31.6	18.2	31.0
jul	2003	31.0	19.0	31.1
aug	2003	10.6	20.4	34.2
sep	2003	31.4	12.2	24.6
oct	2003	122.4	7.6	15.8
nov	2003	133.0	-	-
dec	2003	84.6	0.5	7.5
jan	2004	40.8	-0.7	5.7
feb	2004	48.4	-0.2	8.6
mar	2004	30.8	3.2	12.5
apr	2004	138.4	8.1	18.3
may	2004	66.8	11.5	21.7
jun	2004	7.0	16.0	27.6
jul	2004	61.4	18.2	28.3
aug	2004	27.0	17.7	29.6
sep	2004	76.4	13.9	25.6
oct	2004	102.2	10.9	18.3
nov	2004	114.8	4.2	12.2
dec	2004	85.0	0.7	8.0
jan	2005	4.4	-2.3	6.5
feb	2005	21.8	-2.9	7.7
mar	2005	18.6	0.9	13.3
apr	2005	82.4	7.3	17.3
may	2005	42.2	13.3	24.6
jun	2005	16.4	17.7	28.0
jul	2005	28.2	19.2	30.2
aug	2005	174.2	15.9	26.9
sep	2005	149.2	15.1	24.9
oct	2005	150.4	10.5	17.9
nov	2005	64.6	4.8	9.8
dec	2005	59.0	-2.5	5.2

To study dissipation, twenty soil cores (0-30 cm depth except samples taken immediately after treatment in trial 1 & 2: 0-10 cm) were removed from each treated plot pre first treatment (-0DAT), post first treatment (+0DAT) and at 7-8 time further points up to about 18 months after T1. To study accumulation, in trial 2 and 4 samples were taken in the same manner immediately after T2, 6 and 12 months after T2, immediately after T3 and 6 months after T3. Due to practical problems, minor deviations from the sampling procedure

(regarding the number of cores) occurred. These were considered to be without a negative impact on the study result.

The cores were cut into 0-10, 10-20 and 20-30 cm segments, which were combined per sample and stored frozen for periods which did not exceed the acceptable storage period determined in separate studies (see study 2 to 7 of this present section). Pyridalyl, S-1812-DP, S-1812-DP-Me and HTFP were analysed in all dissipation samples by method CLE 333/214-01V (evaluated in study 1 of section B.5.3). The accumulation samples were analysed for pyridalyl by the same method. Each sample was extracted with acidified acetonitrile/water. For determination of pyridalyl, S-1812-DP and S-1812-DP-Me, a portion of the extract was diluted with 1% NaCl solution and extracted with hexane. The hexane extract was cleaned up on a silica column and analysed in duplicate by GC/MS-CI or GC-ECD. For determination of HTFP, a portion of the initial extract was cleaned up on a silica column and analysed in duplicate by LC/MS-MS. The validation provided in study 1 and 3 of section B.5.3 was sufficient to support an LOQ in soil of 0.01 mg/kg for all four analytes. In the present study, concurrent validation was provided for each analyte by analysis of untreated soil samples of each soil fortified at 0.01 to 0.2 mg/kg (pyridalyl and metabolites). The analytical results for these samples (n=79 for pyridalyl, n= 40-43 for each metabolite) were acceptable (recoveries within 70-110% range with a few exceptions). Analytical results were not corrected for recovery. The LOD was about 0.0005-0.001 mg/kg for pyridalyl.

Results

Dissipation

At the Italian sites, biomass decreased by 86-90% between the samples taken pre-application and 12 months post-application. The author of the report suggested that this was due to the climatic conditions over the initial 12 month period. The weather in July and August was particularly dry with very high daily mean temperatures.

The maximum measured residues immediately after treatment represented 55-163% (mean 119%) of the PECsoil in the 0-10 cm layer calculated under the assumption that the soil density was 1.5 kg/dm^3 (no values for soil density were reported).

During the entire study, residues of all analytes in all segments from the control plots (analysed only immediately after T1, T2 and T3, and 12 months after T1), from the treated plots prior to treatment, and from the 10-20 cm and 20-30 cm segments of the treated plots after application were below the quantifiable limit (<0.01 mg/kg), except for pyridalyl on two occasions indicated in the tables below. Residues of pyridalyl and metabolites in the 0-10 cm segment of the treated plots are shown in Table 4.1.2.3-05 and 4.1.2.3-06. Residues of pyridalyl in the 0-10 cm layer were 0.19-0.63 mg/kg on the day of treatment and were detected up to 6 months (1 trial), 12 months (1 trial) and 18 months (2 trials). Residues after 1 year and after 18 months represented ≤22% and ≤10% respectively, of the initial measured residue. The maximum occurrence of the metabolites S-1812-DP, S-1812-DP-Me and HTFP in any trial represented 9.7%, <5.3% and 12.9% of the applied amount of pyridalyl.

Table 4.1.2.3-05 Dissipation trials 1 and 2: residues (duplicate mean, mg/kg) of Pyridalyl, S-1812-DP, S-1812-DP-Me and HTFP in bare field soil in Southern France (0-10 cm) treated with Pyridalyl 10 EW at 0.6 kg a.s./ha

trial 1 (application date 28 May 2003)					trial 2 (application date 27 June 2003)				
day	pyrida-lyl	S-1812-DP	S-1812-DP-Me	HTFP	day	pyrida-lyl	S-1812-DP	S-1812-DP-Me	HTFP

-0	<0.01	<0.01	<0.01	<0.01	-0	<0.01	<0.01	<0.01	<0.01
+0	0.50	<0.01	<0.01	<0.01	+0	0.19	<0.01	<0.01[D]	<0.01
2	0.28	<0.01	<0.01	<0.01	3	0.22	0.01	<0.01	0.01[E]
14	0.14	<0.01	<0.01	0.02	14	0.15	0.01	<0.01	0.01
30	0.12	<0.01	<0.01	0.01	31	0.11	0.01	<0.01	0.01
91	0.09	<0.01	<0.01	<0.01	91	0.07	<0.01	<0.01	<0.01
184	0.03	<0.01	<0.01	<0.01	189	0.04	<0.01	<0.01	<0.01
373	<0.01[C]	<0.01	<0.01	<0.01	368	0.02	<0.01	<0.01	<0.01
552	<0.01[A]	<0.01	<0.01	<0.01	544	<0.01[B]	<0.01	<0.01	<0.01

(A) The measured value of 0.004 mg/kg is used in calculation of degradation rate (LOD 0.0006 mg/kg).
(B) The measured value of 0.006 mg/kg is used in calculation of degradation rate (LOD 0.0005 mg/kg).
(C) A residue of 0.03 mg/kg was found in the 10-20 cm horizon.
(D) The residue of <0.01 mg/kg represented <5.3% of the highest measured residue of pyridalyl (including metabolite levels, corrected for differences in molecular mass), which was the highest level of formation of the metabolite in all 4 trails.
(E) The residue of 0.01 mg/kg represented 12.9% of the highest measured residue of pyridalyl (including metabolite levels, corrected for differences in molecular mass), which was the highest level of formation of the metabolite in all 4 trails.

Table 4.1.2.3-06 Dissipation trials 3 and 4: residues (duplicate mean, mg/kg) of Pyridalyl, S-1812-DP, S-1812-DP-Me and HTFP in bare field soil in Italy (0-10 cm) treated with Pyridalyl 10 EW at 0.6 kg a.s./ha

trial 3 (application date 21 May 2003)					trial 4 (application date 29 May 2003)				
day	**pyrida-lyl**	**S-1812-DP**	**S-1812-DP-Me**	**HTFP**	**day**	**pyrida-lyl**	**S-1812-DP**	**S-1812-DP-Me**	**HTFP**
-0	<0.01	<0.01	<0.01	<0.01	-0	<0.01	<0.01	<0.01	<0.01
+0	0.63[A]	0.01	<0.01	<0.01	+0	0.40	<0.01	<0.01	<0.01
3	0.27	0.02	<0.01	<0.01	4	0.47	0.01	<0.01	<0.01
14	0.10	0.03	<0.01	0.01	13	0.24	0.01	<0.01	<0.01
30	0.14	0.05[B]	<0.01	0.02	30	0.20	0.01	<0.01	0.01
98	0.09	0.02	<0.01	<0.01	90	0.10	<0.01	<0.01	<0.01
138	0.05	<0.01	<0.01	<0.01	138	0.05	<0.01	<0.01	<0.01
174	0.09	0.02	<0.01	<0.01	186	0.05	<0.01	<0.01	<0.01
370	0.08	0.01	<0.01	<0.01	364	0.09	0.01	<0.01	<0.01
554	0.05	na	na	na	546	0.04	na	na	na

na = not analysed
(A) A residue of 0.02 mg/kg was found in the 10-20 cm horizon.
(B) The residue of 0.05 mg/kg represented 9.7% of the highest measured residue of pyridalyl (including corrections for difference in molecular mass, and taking into consideration the residue of pyridalyl in the 10-20 cm segment, and the residue of S-1812-DP on the day of treatment, corrected for difference in molecular mass). This was the highest level of formation of the metabolite in all 4 trails.

Accumulation

Residues of pyridalyl in the 0-10 cm segment of the treated plots are shown in Table 4.1.2.3-07 (note: metabolite analysis was not performed on soil from the accumulation trials). The lowest points of the saw-tooth curve were 0.02, 0.03 and 0.02 mg/kg in trial 2 and 0.09, 0.02 and 0.02 mg/kg in trial 4 (plateau concentration 0.02 mg/kg, no evidence of accumulation). The upper limits of the saw-tooth curve were consistent in trial 4 (0.40, 0.44 and 0.39 mg/kg) but increased in trial 2 (0.19, 0.51 and 0. 64 mg/kg). The author of the report argued that these results also did not indicate accumulation because (1) The upper limits in trial 2 increased due to the low initial concentration of pyridalyl (0.19 mg/kg); this concentration was low not only relative to the concentration measured after T2 and T3, but also relative to the expected concentration following a dose of 600 g a.s./ha to bare soil (0.40 mg/kg). (2) There was no carry over of the pyridalyl residues from the previous years. This argumentation is acceptable.

Table 4.1.2.3-07 **Accumulation trials 2 and 4: residues (duplicate mean, mg/kg) of Pyridalyl in bare field soil in Southern France and Italy (0-10 cm) treated once yearly for three years with Pyridalyl 10 EW at 0.6 kg a.s./ha**

sampling time	trial 2 (T1 on 27 June 2003, T2 on 29 June 2004, T3 on 29 June 2005)	trial 4 (T1 on 29 May 2003, T2 on 27 May 2004, T3 on 27 May 2005)
<3 hours post T1	0.19	0.4
T1 + 6 months	0.04	0.05
T1 + 12 months	0.02	0.09
<3 hours post T2	0.51	0.44
T2 + 6 months	0.03	0.07
T2 + 12 months	<0.01	0.02
<3 hours post T3	0.64	0.39
T3 + 6 months	0.02	0.02

Degradation rates

DT50 values were calculated following the recommendations and procedures of the "Guidance document on estimating persistence and degradation kinetics from Environmental Fate studies on pesticides in EU registration" (SANCO/10058/2005 version 2.0). Calculations were based on the measurements in the table below. Where applicable, values <LOQ were set at 0.5x(LOD+LOQ) = 0.006 mg/kg. Any pyridalyl residues found in the deeper layers were added to the residue in the top 10 cm layer. Where applicable, day 0 concentrations for metabolites were added to the day 0 residue for pyridalyl, taking into consideration the difference in molecular mass between parent and metabolites. All calculations were performed with ModelMaker v 4.0 software (SFO = single first-order, FOMC = first-order multi-compartment, DFOP = Double-First-Order in Parallel mode).

Table 4.1.2.3-08 **Residues (duplicate mean, mg/kg) of Pyridalyl in bare field soil in Southern Europe (0-10 cm) treated with Pyridalyl 10 EW at 0.6 kg a.s./ha**

trial 1		trial 2		trial 3		trial 4	
day	pyridalyl (mg/kg)	day	pyridalyl (mg/kg)	day	pyridalyl (mg/kg)	day	pyridalyl (mg/kg)
0	0.50	0	0.19	0	0.66	0	0.40
2	0.28	3	0.22	3	0.27	4	0.47
14	0.14	14	0.15	14	0.10	13	0.24
30	0.12	31	0.11	30	0.14	30	0.20
91	0.09	91	0.07	98	0.09	90	0.10
184	0.03	189	0.04	138	0.05	138	0.05
373	0.03	368	0.02	174	0.09	186	0.05
552	0.006	544	0.006	370	0.08	364	0.09
				554	0.05	546	0.04

Endpoints for persistence

For persistence endpoints, SFO and FOMC were run first by the RMS (M0 free, all data, no weighting). FOMC gave a better fit than SFO in all cases, except for trial 3, where SFO and FOMC gave a comparable, but very poor fit. In case of field studies M0 fixed is less relevant due to uncertainty in sampling and analysis The persistence endpoints for the best fit model are summarised in Table 4.1.2.3-10.

In all cases the persistence endpoints were obtained from biphasic models, and the estimated DT90 persistence value were in trials 1, 2 and 3 reached within the experimental period, whereas the DT90 persistence in trial 4 (1045 days) represented about twice the experimental period of 546 days.

The Notfier provided a position paper with recalculated values for site 3 and site 4:
At **Site 3** the Notifier recalculated χ^2 error values for the FOMC approach to be 12.3% and the fit was based on Figure 3 it is the notifier's belief that the fit to FOMC is at least as good as that of DFOP, being 'fair'. Whilst it is acknowledged that the χ^2 error value is slightly smaller for the DFOP fit than for the FOMC approach, the FOMC χ^2 error value is still considered acceptable (<15% as cited in the FOCUS report). Therefore taking into account extreme nature of the DFOP breakpoint, it is concluded that the **FOMC DT_{90} value of 207 days** is the more appropriate for this site.
At **Site 4** the DT_{90} value of 1045 days provided by the DFOP approach is extrapolated beyond the duration of the study. Direct observations of the experimental data indicate that the "experimental DT_{90}" time (i.e. concentration reaches 10% of the maximum value, or 0.47 mg/kg) is between 138 and 546 days. The evaluator has subjectively rated the fits to both the FOMC and DFOP curves as "fair" but based on Figure 4 it is the notifier's belief that the fit to FOMC is superior to that of DFOP. The notifier believes that the χ^2 error values are not significantly different (19.8% for FOMC compared to 19.3% for DFOP). Therefore taking all data into account it is concluded that the **FOMC DT_{90} value of 282.6 days** is the more appropriate for this site.
The position paper was discussed in a meeting with the Notifier and the endpoints were agreed upon.

Endpoints for modelling
Endpoints for modelling were not calculated (contribution of photochemical degradation unknown, normalisation to standard moisture and temperature not possible in the absence of meteorological data measured on-site).

Table 4.1.2.3-09 Optimisation results of the Modelmaker runs for estimation of half-lives of pyridalyl in field soils in Southern Europe treated with Pyridalyl 10 EW at 0.6 kg a.s./ha

trial	model	M0[(B)]	χ^2 (err)	residuals	r^2	visual fit[(C)]	endpoint[(D)]
1	SFO[(A)]	-0.07	31.83	poor	0.852	1	
	FOMC[(A)]	0.00	9.19	fair	0.990	3	
	DFOP[(A)]	0.00	5.46	good	0.997	4	P
2	SFO[(A)]	0.00	14.31	good	0.940	3	
	FOMC[(A)]	+0.02	10.41	good	0.973	4	
	DFOP[(A)]	+0.02	10.55	good	0.976	5	P
3	SFO[(A)]	-0.02	36.72	poor	0.821	1	
	FOMC[(A)]	-0.02	38.81	fair	0.994	4	
	DFOP[(A)]	0.00	10.17	good	0.990	3	P
4	SFO[(A)]	+0.01	24.37	poor	0.863	2	
	FOMC[(A)]	+0.05	19.85	fair	0.920	3	
	DFOP[(A)]	+0.04	19.33	good	0.933	3	P

(A) M0 free.
(B Difference (A-B) between fitted value (A) and mean measured initial value (B)..
(C) Scale of 1 tot 5, where 1 = poor, 2 = fairly poor, 3 = fair, 4 = fairly good, 5 = good
(D). Endpoint selected for Persistence (P).

Table 4.1.2.3-10 Optimised fitting parameters and half-lives for pyridalyl in field soils in Southern Europe treated with Pyridalyl 10 EW at 0.6 kg a.s./ha

trial no.	parameter for	kinetics	Optimised fitting parameters	DT50	DT90
1	persistence	DFOP	$g = 0.693168$; $k_1 = 0.480722$; $k_2 = 0.00635628$; $M_0 = 0.499308$	2.6	176
2	persistence	DFOP(A)	$g = 0.536879$; $k_1 = 0.0411727$; $k_2 = 0.00437903$; $M_0 = 0.208956$	39	350
3	persistence	FOMC	$\alpha = 0.312121$; $\beta = 0.148777$; $M_0 = 0.660253$	1.2	207
4	persistence	FOMC	$\alpha = 0.803978$; $\beta = 18.0726$; $M_0 = 0.445394$	24.3	282.6

(A) χ^2 (err) for FOMC (10.41) was slightly lower than for DFOP, but r^2 for DFOP was slightly higher than for FOMC (0.97), and DFOP gave a slightly better visual fit. In particular, DFOP gave a better fit for the last data point, and hence a more accurate estimation of DT90. For FOMC, the DT50 and DT90 was 42 and 365 days respectively.

Conclusions

Following single spring treatment of bare soil at two locations in Southern France and two in Italy at 600 g a.s./ha, residues of pyridalyl dissipated with DT50 and DT90 values of 2.3-39 and 176-350 days, respectively. The maximum occurrence of the metabolites S-1812-DP, S-1812-DP-Me and HTFP in any trial represented 9.7%, <5.3% and 12.9% of the applied amount of pyridalyl. Following three consecutive annual spring treatments of bare soil at one location in Southern France and one in Italy at 600 g a.s./ha, there was no indication of accumulation of pyridalyl.

Guidelines & Limitations

The study is acceptable.

4.1.2.4 Hydrolysis

Reference: IIA 7.5/01, VP-22605

Test type: OPPTS 835.2110, EEC C.7

GLP: yes

Materials and methods

Test material: S01812 (pyridalyl), Batch no. RI97020, purity 98.6%

The rate of hydrolysis and hydrolytic products were examined in sterile aqueous buffers at pH 5, 7 and 9 over a 30-day period. The test systems were made up of buffer plus 10% acetronitriel to prepare the homogenous aqueous solution. The test concentration was approximately 4 µg/L.

Results:

No hydrolytic degradation was observed after 30 days incubation at 25°C in 0.01M buffer of pH5, pH 7 and pH 9 containing 10% acetonitrile. Pyridalyl represented an average of 96.8, 96.3 and 95.8% of the dose for the pH 5, pH 7 and pH9 buffer systems. The half-lives are estimated at >30 days and are likely greater than 1 year.

4.1.2.5 Photodegradation

Reference: IIA 7.6/01, Study No. 885W-2

Test type: EPA N:161-2
GLP: yes

Materials and methods
Test material: S01812, Batch no. 980302G, purity 99.7%
The study examined the photolytic breakdown rate of [^{14}C]S-1812 in sterile aqueous pH 7 buffer for up to 30 days. The study was carried out in accordance with test guideline EPA N:161-2.

Results:
The mean DT50 (photolysis, 25°C) for [pyridyl-2,6-^{14}C]-pyridalyl and [dichlorophenyl-U-^{14}C]-pyridalyl is 3.5 days under test conditions (Xenon light, 12 h light, 531 W/m2 for the 300-800 nm range). The photo-metabolites HTFP (max. 17.5% AR at the end of incubation) and S-1812-PYP (max. 63% AR on day 14, 57% AR on day 21 and 30) are stable to photolysis under the test conditions.

4.1.2.6 Sorption

STUDY 1

Characteristics

reference	:	IIA 7.4.1/01, Study No. VP-12140	soils	:	sandy loam (3X), sandy clay
year of execution	:	1999-2000	concentrations	:	0.01-0.1 µg/L
GLP statement	:	yes	temperature	:	room temperature
guideline	:	OPPTS 835.1220	$K_F$oc	:	402000-2060000 L/kg
test substance	:	[dichlorophenyl-U-^{14}C] pyridalyl; batch RIS98015	1/n	:	0.99-1.18
purity	:	radiochemical purity ≥99.1%	acceptability	:	acceptable

Study design
A batch equilibrium adsorption study with four soils was conducted on pyridalyl.

Soil properties are shown in Table 4.1.2.6-01. Test solutions were prepared at concentrations of 0.01, 0.025, 0.05, 0.075 and 0.1 µg/L by adding [dichlorophenyl-U-^{14}C] pyridalyl in acetonitrile to 0.01M $CaCl_2$ solution (final concentration acetonitrile 0.01% v/v). Prior to use in all experiments aliquots of 0.5 g soil were re-equilibrated with 1.0 mL of water in a stoppered 250 mL Erlenmeyer flasks by gently shaking for at least 24 hours. All experiments (pre-tests and main test) were conducted at a soil:water ratio of 1:400 (0.5 g of soil in 200 g of solution).

In a pre-test at 0.075 µg/L in all 4 soils, the minimum time required to reach equilibrium was determined to be 16 hours. In a screening test at 0.075 µg/L, two tubes with soil were equilibrated for 16 hours to check adsorption of pyridalyl on glass walls, which was found to be insignificant (not detectable in 7 tubes, 4.3% in remaining tube). Two-step desorption was studied in the pre-test but this information was not included in this summary.

In the main test to determine the Freundlich adsorption isotherms, aliquots (200 mL) of test solution were added to 250 mL Erlenmeyer flasks containing 0.5 g d.w. samples of 2-mm sieved re-equilibrated soils. Duplicate samples of each soil were prepared at each concentration. Treated slurries were shaken at room temperature for 16 hours. After equilibration, the supernatants were removed by centrifugation and extracted twice with hexane (procedure was shown in pre-test to quantitatively extract pyridalyl from 0.01M $CaCl_2$ solution). The combined hexane fractions were concentrated and analysed by LSC. In a pre-test at 0.075 µg/L in all 4 soils, HPLC analysis had shown that the radioactivity in the hexane extract from the adsorption steps, and that in the soil extracts after the second desorption step, consisted entirely of pyridalyl.

Mass balances were determined in the main adsorption test at 0.075 µg/L by determining the radioactivity in the centrifuged supernatants (LSC) and in the soil pellet (combustion/LSC). In this mass balance test, the supernatants were extracted with hexane and the radioactivity in both phases was quantified by LSC.

The amount of test material adsorbed to soil was calculated as the difference between the amount of test substance present in the application solutions and that in the hexane extracts. This method is supported by the available experimental data (quantitative extraction of radioactivity from supernatants by hexane, no degradation of pyridalyl). Freundlich adsorption isotherms were calculated by linear first order regression analysis of $\log C_{equilibrium}$ versus log(x/m).

To verify the results from the adsorption study, Koc values were estimated using a HPLC method (determination of retention factor for 16 reference compounds with published Koc values) and using two Molecular Fragment Constant Methods (method 1: Molecular Connectivity Indices by Meylan *et al.* (1992); method 2: Fragment Constant Method by Tao *et al.* (1999)). In addition, soil thin layer chromatography was performed (determination of mobility of pyridalyl in water on thin layers of all 4 soils).

Results

Mass balances ranged from 96-98% AR. Radioactivity in supernatants in the mass balance test represented 10-15% AR and that adsorbed to soil 82-88% AR. The extracted aqueous phases in the mass balance test contained no radioactivity.

The Freundlich adsorption parameters are given in Table 4.1.2.6-01. Adsorption Kf values were 3270-29900 L/kg (1/n values 0.99-1.18), and corresponding adsorption Koc values 402000-2060000 L/kg. Sorption in these four soils showed a relationship to organic carbon content (linear regression analysis of the Kf values of these 4 soil versus % oc gave $r^2 = 0.64$), where no relationship was observed with pH (r^2 0.10) or clay content (r^2 0.01).

Table 4.1.2.6-01 Adsorption coefficients for pyridalyl

origin of soil soil name	California Hanford	North Carolina Cecil	Washington Timmerman	California Elder
Texture(A)	sandy loam	sandy clay	sandy loam	sandy loam
%sand/silt/clay(A)	66/28/6	49/16/35	63/32/5	57/26/17
% moisture at 1/3 bar	12.4	20.2	17.1	21.5
pH(B)	6.0	5.1	6.7	6.1
% organic carbon	0.41	0.81	1.10	1.45
CEC [meq/100 g]	6.9	7.2	11.3	19.4
Adsorption Kf [L/kg]	6390	3270	9150	29900
Adsorption Koc [L/kg]	1570000	402000	832000	2060000
1/n	1.08	0.99	1.10	1.18
r^2	0.958	0.929	0.904	0.909

(A) According to USDA classification system.

(B) Medium in which pH was measured was not reported.

Verification of Koc values from adsorption study

The HPLC method gave a Koc estimate of 5600000 L/kg. This value was determined by extrapolation since the retention factor of pyridalyl (0.967) was above the highest retention factor of the reference standards (9.09). The Koc estimated using molecular structure analysis was 537000 L/kg (method 1) or 575000 L/kg (method 2). The above values are not reliable enough to be included in the List of Endpoints, but they confirm the order of magnitude of the Koc values determined in the adsorption test. The soil TLC results showed that pyridalyl did not move from the origin in al four soils, confirming the low mobility of pyridalyl in soil.

Conclusions

Adsorption Kf values were 3270-29900 L/kg (1/n values 0.99-1.18), and corresponding adsorption Koc values 402000-2060000 L/kg. Sorption in these four soils showed a relationship to organic carbon content (r^2 0.64), but not with pH (r^2 0.10) or clay content (r^2 0.01).

Guidelines & Limitations

The history of the test soils was not reported. The pH was not measured after adsorption but pyridalyl is not an ionisable substance. There are no further comments. The study is acceptable.

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

Study 1

Characteristics

reference	:	IIA 8.2.6.1/01 Study No 013648-1	species	: Bluegill sunfish (*Lepomis macrochirus*)
type of study	:	Bioconcentration and metabolism in fish	duration	: 49 days exposure, 57 days depuration
year of execution	:	2001-2002	nominal conc.	0.05 and 0.15 µg a.s./L
GLP	:	Yes	exposure method	: Flow-through
guideline	:	OPPTTS 850.1730		
test substance	:	[dichlorophenyl-U-14C]-pyridalyl, lot no. RIS2001-006	conclusion	: max BCF pyridalyl in whole fish 26858 L/kg wwt; CT50 for pyridalyl in whole fish 30-31 days; 60-76% clearance within 57 day depuration period.
purity	:	chemical purity not reported, radiochemical purity 98.5%	acceptability	: acceptable

Methods

Bluegill sunfish (*Lepomis macrochirus*) were exposed to [dichlorophenyl-U-^{14}C]-pyridalyl for 49 days in a flow-through system, followed by 57 days of depuration in clean water. Nominal concentrations of 0.05 and 0.15 µg/L, plus solvent control (DMF, 0.1 mL/L) were each tested in one replicate aquarium containing 65 fish at test initiation. An additional aquarium was set up at 0.15 µg/L to provide fish samples for metabolite identification. Based on the findings in preliminary tests, the fish were transferred from the high and low exposure aquaria to newly activated exposure aquaria after 18 days of exposure, in order to maintain homogeneous solutions and constant exposure concentrations. Fish biomass at study start was 0.5 g/L/24 hours.

Water quality parameters were: 16 h light (40-50 footcandles), 8 h dark; 22-24°C; dissolved oxygen >60% of saturation except day 15, high exposure (58%) and day 22, high exposure (45%, resolved by temporary gentle aeration); pH 6.3-7.5; TOC 32-46 mg/L during exposure and 0.69-2.5 mg/L during depuration (hence TOC mainly attributable to test article and solvent); hardness at test initiation 36 mg $CaCO_3$/L. No undissolved test substance was observed throughout exposure in the dilution system or the exposure aquaria.

Water samples (1 L) were removed from treated and control aquaria on day 0, 3, 7, 14, 21, 28, 35, 42 and 49 of uptake and 1, 3, 7, 14, 21, 28, 42 and 57 of depuration. Four fish were sampled from treated and control aquaria on day 3, 7, 14, 21, 28, 35, 42 and 49 of uptake and 1, 3, 7, 14 and 21 of depuration and three fish on day 28, 42 and 57 of depuration.

Water samples were extracted with dichloromethane. Fish samples were separated into edible and non-edible portions, which were separately extracted with chloroform/methanol. The chloroform/methanol extract was partitioned with saturated sodium chloride solution. The lipid content was determined by evaporation of the chloroform extracts and weighing of the dried residue. The methanol/water sample of the non-edible portion was partitioned with ethyl acetate. The residue from chloroform/methanol extracts was extracted with

acetone. Radioactivity in all extracts and fractions was quantified by LSC and in PES by combustion/LSC. Radioactivity in organic extracts of water samples, and in lipid fractions and representative ethyl acetate samples of fish was identified by HPLC. Compound identification was by co-chromatography with reference standards (S-1812 and S-1812-DP). The acetone extracts and aqueous fractions of fish contained <3% TRR and were not analysed by HPLC.

Validation of the extraction method of the water samples was provided by concurrent analysis of samples of control water fortified with pyridalyl (0.03-0.15 μg/L): recoveries ranged from 80 to 128%. Validation of the extraction method of the edible and non-edible fish samples was provided by concurrent analysis of samples of control fish fortified with pyridalyl (1 μg/g): it was reported that pyridalyl was quantitatively recovered.

Pyridalyl, S-1812-DP and a conjugate of S-1812-DP were isolated by HPLC from day 28 or 35 extracts of fish from the additional aquarium with exposure level 0.15 μg a.s./L, and pyridalyl was isolated by HPLC from day 35 high rate exposure water. The identity of the three compounds in the isolates was confirmed by reverse phase HPLC and silica TLC and, in the case of pyridalyl, by LC/MS.

Results

It was reported that the general fish population appeared healthy and exhibited normal behaviour throughout the study. During the exposure phase one mortality occurred in each of the solvent control, 0.05 and 0.15 μg/L aquaria. During depuration, six mortalities occurred in the 0.15 μg/L aquaria and one mortality in the 0.05 μg/L aquaria.

The mean daily radioactivity and pyridalyl concentrations in water and fish and the lipid content of fish during exposure and depuration are shown in Table B.9.2.3-01 and -02. It was reported that pyridalyl represented >90% of the radioactivity in extracts of water. More than 98% TRR in fish samples was extractable with chloroform/methanol. Pyridalyl represented at least 88% and 84%, respectively, of the TRR in extracts of fish samples of the low and high exposure, which also contained low levels of S-1812-DP and S-1812-DP conjugate (≤3.0% TRR and ≤2.1% TRR, respectively) and unidentified degradates (none likely to exceed 3% TRR).

The pyridalyl concentration in whole fish increased throughout exposure (first order regression coefficient R^2 0.98 for both exposure levels), and a steady-state situation was not reached within the 49 day exposure phase. The reported steady-state bioconcentration factor (BCF) values were calculated by dividing the mean ^{14}C or pyridalyl concentration in fish during day 35-49 by the mean ^{14}C or pyridalyl concentration in water during day 35-49. In addition, BCF values were calculated as k_1/k_2, where k_1 and k_2 are the uptake and depuration rate constant, estimated according to the methods outlined in Annex 6 of OECD 305 (1996) using non-linear parameter estimation methods and curve fitting with the computer program SigmaPlot™. The results for whole fish of all estimation methods are shown in Table 4.2.1-01 and -02.

Reported kinetic BCF values were about 50% higher than BCF values based on average day 35-49 concentrations in fish. The latter are considered to be inaccurate since a steady state of residues in fish was not reached within the exposure period.

The report did not provide any information to evaluate the accuracy of the kinetic parameters (e.g. plot of fitted and experimental values, confidence intervals, coefficient of correlation). Kinetic BCF values were therefore also estimated by the RMS based on the reported raw data according to the methods outlined in Annex 6 of OECD 305 (1996) using non-linear parameter estimation methods and curve fitting with the

computer program Modelmaker V 4.0. The k_2 value was first determined from the depuration curve, and implemented as a constant into the equation describing the uptake of residues (equation 2 in Annex 6 of OECD 305 (1996)). The estimates by the RMS produced BCF values which did not differ significantly from the reported value, and the fit was found to be acceptable in all cases. The reported kinetic BCF values for pyridalyl in whole fish (26858 and 22352 L/kg wwt at 0.05 and 0.15 µg a.s./L respectively) are acceptable.

The CT50 values for pyridalyl in whole fish were 30-31 days. CT90 values were not estimated since only 60-76% clearance was reached within 57 days of depuration.

Table 4.2.1-01 Mean radioactivity and pyridalyl concentrations in fish and test water and lipid content of fish from a bioconcentration experiment with [dichlorophenyl-U-14C] pyridalyl at nominal exposure concentration 0.05 µg a.s./L

		concn in water (µg eq./L)		**concn in fish (µg eq./g)**		**lipid**
phase	**day**	**14C**	**a.s.**	**14C**	**a.s.**	**content fish (% ww)**
uptake	0	0.044	0.043	0.000	0.000	2.72
	3	0.026	0.026	0.069	0.062	4.24
	7	0.026	0.025	0.134	0.118	3.87
	14	0.026	0.027	0.218	0.196	3.73
	21	0.032	0.033	0.339	0.316	4.77
	28	0.031	0.027	0.343	0.306	3.65
	35	0.030	0.026	0.432	0.396	4.93
	42	0.032	0.025	0.525	0.503	5.08
	49	0.030	0.028	0.561	0.543	5.12
depuration	1	nd	na	0.507	0.489	5.05
	3	nd	na	0.426	0.412	5.23
	7	nd	na	0.406	0.393	5.65
	14	nd	na	0.355	0.336	5.98
	21	nd	na	0.302	0.294	6.01
	28	nd	na	0.256	0.248	6.02
	42	nd	na	0.220	0.213	6.49
	57	nd	na	0.228	0.218	7.06
			% depuration	59	60	

nd = not detected (detection limit not reported); na = not applicable

Table 4.2.1-02 Mean radioactivity and pyridalyl concentrations in fish and test water and lipid content of fish from a bioconcentration experiment with [dichlorophenyl-U-14C] pyridalyl at nominal exposure concentration 0.15 µg a.s./L

		concn in water (µg eq./L)		**concn in fish (µg eq./g)**		**lipid**
phase	**day**	**14C**	**a.s.**	**14C**	**a.s.**	**content fish (% ww)**
uptake	0	0.15	0.14	0.000	0.000	3.02
	3	0.096	0.10	0.192	0.173	3.58
	7	0.095	0.088	0.334	0.279	2.44
	14	0.098	0.090	0.635	0.556	3.28
	21	0.10	0.11	0.949	0.853	4.76
	28	0.097	0.083	1.051	0.983	4.50
	35	0.10	0.11	1.379	1.305	4.98
	42	0.10	0.076	1.385	1.319	4.92
	49	0.10	0.10	1.677	1.610	5.83
depuration	1	nd	na	1.527	1.468	5.73
	3	nd	na	1.580	1.535	6.36
	7	nd	na	1.188	1.148	5.74
	14	nd	na	1.002	0.975	5.80

	21	nd	na	0.872	0.851	5.41
	28	nd	na	0.738	0.715	6.55
	42	nd	na	0.757	0.733	6.99
	57	nd	na	0.403	0.383	5.72
			% depuration	76	76	

nd = not detected (detection limit not reported); na = not applicable

Table 4.2.1-03 Bioconcentration factors (L/kg wwt) for radioactivity and pyridalyl in whole fish exposed to [dichlorophenyl-U-14C] pyridalyl at nominal exposure concentration 0.05 or 0.15 µg a.s./L

	0.05 µg/L nominal		**0.15 µg/L nominal**	
parameter	**14C**	**pyridalyl**	**14C**	**pyridalyl**
steady state BCF(A)	16323	18500	14800	14853
kinetic BCF	25476	26858	20769	22352
k_{uptake} (day^{-1})	615	600	508	515
$k_{depuration}$ (day^{-1})	0.02414	0.02234	0.02446	0.02304
CT50 (day)	29	31	28	30
lipid BCF(A)(B)	3239	3671	2824	2835

(A) Based on day 35, 42 and 49 average concentration in fish and water.
(B) Normalised to 1% fat; based on day 35, 42 and 49 average lipid content of fish.

Conclusion

BCF values for pyridalyl in whole fish 26858 and 22352 L/kg wwt at 0.05 and 0.15 µg a.s./L respectively (lipid BCF normalised to 1% fat 3671 and 2835 L/kg wwt); CT50 for pyridalyl in whole fish 30-31 days. CT90 values were not estimated (only 60-76% clearance within 57 days of depuration).

Guidelines and limitations

The study was in accordance with OECD 305.

Study 2

Characteristics

reference	:		species	:	Bluegill sunfish (*Lepomis macrochirus*)
type of study	:	Bioaccumulation in fish via food	duration	:	18 days
year of execution	:	2002-2003	nominal conc.		5 mg/kg food
GLP	:	No	exposure method	:	Single feeding
guideline	:	None.			
test substance	:	[Dichlorophenyl-U-14C]-pyridalyl, lot no. RIS2001-006 (C-2001-024-T-06)	conclusion	:	No reliable conclusion may be drawn
purity	:	Chemical purity not reported, radiochemical purity 99.5%	acceptability	:	**Not acceptable**

A study was conducted to determine the depuration rate constant of pyridalyl from bluegill sunfish that were fed a single dose of pyridalyl, and to use this parameter to estimate the bioaccumulation factor via food.

Methods

Bluegill sunfish (*Lepomis macrochirus*, bw about 20 g) were individually introduced into a 5 L aquarium and kept for the duration of the test under flow-through conditions in untreated water (total hardness about 60 mg/L, 29 renewals per day) under 16 hours light at 23±2°C. During the first two days of the three-day acclimation period each bluegill was fed 2 uncontaminated medaka (*Oryzias latipes*, bw about 0.2 g) daily, on the last day of acclimation they were not fed.

On the administration day (day 0), each bluegill was fed two medaka at a time, which had been injected with 2 µL of a solution of 0.5 µg/µL radiolabeled pyridalyl in fish oil (hence concentration in food 5 mg/kg medaka, test dose equivalent to 0.1 mg/kg bluegill sunfish). During the remainder of the study each bluegill was fed 2 uncontaminated medaka daily until the day before sampling.

Four fish were sampled on day 1 and 3 and 2 fish on day 5, 9 and 18 for chemical analysis. Faeces were collected from each aquarium 1-3 times a day and the cumulative faeces sample was analysed. Fish samples were separated into gut, gut contents, liver and gall bladder, edible and remaining non-edible portions. Fish portions and faeces were extracted 3X with acidified acetonitrile (0.5% HCl). Radioactivity in extracts was quantified by LSC. The extracts were concentrated and analysed by TLC. Compound identification was by co-chromatography with reference standards (S-1812 and S-1812-DP).

Liver and gall bladder extracts contained TLC origin material suspected to be conjugates and were therefore subjected to acid hydrolysis (6N HCl). The acid hydrolysate was partitioned with ethyl acetate followed by LSC and TLC analysis.

Results
It was reported that no abnormal fish behaviour and fish mortality were observed during the study. The results for distribution and identification of radioactivity in fish and faeces are shown in Table B.9.2.3-04.

The author of the report used the individual replicate data for whole body residues of pyridalyl to estimate the first order depuration rate constant using linear regression analysis of ln-transformed % AR data versus time. This gave the following results:
$k = 0.0564\ day^{-1}$
$C_0 = 33.7\%$ AR
DT50 = 12 days.
$r^2 = 0.584$

The estimates for C_0 and k were then used to estimate the cumulative concentrations in bluegill sunfish under repeated dose conditions and the bioaccumulation factors via food, according to the following equations:

C_{fish} initial = C_{food}*(DFC/100)*(C_0/100)

C_{fish} steady state = C_{fish} initial / (1-exp(-k*T)))

BAF = C_{fish} steady state / C_{food}

Where:

C_{fish} initial is the initial concentration in fish (mg/kg)

C_{food} = concentration in food (mg/kg): theoretical value is 5 mg/kg medaka

DFC = Daily Food Consumption as % of fish bw (2%)

C_0 = maximum efficiency of sorption from gut (%); estimated was 33.7%

C_{fish} steady state = steady state concentration in fish (mg/kg)

k = elimination rate constant; estimated was 0.0564 day^{-1}

T = administration interval (days), 1 day was taken as a normalized case.

BAF = Bioaccumulation Factor via Food

With DFC = 2% bw, C_{fish} steady state = 0.614 mg/kg and BAF = 0.12.

With DFC = 4-10% bw, C_{fish} steady state = 1.23-3.07 mg/kg and BAF = 0.25-0.61.

With DFC = 20% bw (reported to be a special case in a referenced paper by Carbonell of 2000), C_{fish} steady state = 6.15 mg/kg and BAF (1.23) exceeds 1 (BAF = 1 reached by day 35). The author of the report argued that such extremely high food consumption should not continuously occur under natural environmental conditions, particularly in the absence of growth dilution.

Evaluation by RMS.

When considering the plot of the pyridalyl residues in individual whole fish (see footnote (A) under Table 4.2.1-04), there is no clear trend of residue decline between day 1 and 9 (first order coefficient of correlation r^2 from linear regression analysis of ln-transformed % AR data versus time was only 0.20). The only data which suggests that there could be a true elimination in the 18-day test period is the day 18 data, but one of two replicate values was comparable to one replicate value determined after 3 days. Residue values from fish sampled on at least one additional sampling day well after day 18 would be required to confirm the claimed elimination rate. The depuration rate constant from this study, and therefore the BAFs based on this constant, are considered to be insufficiently reliable. In addition, no steady state concentration in fish was achieved with this study (only a single feeding event), wherefore a formula was constructed to estimate the steady state. RMS considered this is not an acceptable approach to derive a BAF and further this formula is considered too simple, containing only one k-value whilst dissipation from the fish constitutes more than one process (excretion, metabolism).

Table 4.2.1-04 Distribution and identification of radioactivity (% AR, replicate means) in fish and faeces following a single oral dose of 0.1 mg [dichlorophenyl-U-14C] pyridalyl/kg fish

	mean % AR on:				
fraction	day 1	day 3	day 5	day 9	day 18
Fish					
extractable	50.5	31.1	37.0	23.9	13.8
pyridalyl[(A)]	35.7	23.3	33.0	21.5	12.4
S-1812-DP	4.60	1.72	2.17	0.34	0.26
S-1812-DP conjugate	8.41	4.70	1.46	1.81	1.08
others	1.79	1.44	0.37	0.20	0.06
Gut content					
extractable	3.77	2.17	1.18	0.76	0.53
pyridalyl	0.64	0.09	0.04	0.04	0.08
S-1812-DP	1.34	0.39	0.86	0.16	0.22

S-1812-DP conjugate	1.67	1.43	0.15	0.50	0.20
others	0.11	0.26	0.13	0.05	0.02
Faeces (cumulative)					
extractable	29.0	28.8	20.7	27.3	44.4
pyridalyl	26.6	25.0	11.6	18.4	36.6
S-1812-DP	1.05	1.86	7.32	4.83	5.73
S-1812-DP conjugate	0.79	1.21	1.59	3.44	2.13
others	0.53	0.66	0.25	0.57	0.00

(A) Individual replicate values were 35.4, 25.5, 41.4 & 40.5% AR (day 1), 16.4, 24.4, 30.5 & 21.9% AR (day 3), 40.4 & 25.5% AR (day 5), 21.6 & 21.5% AR (day 9) and 8.71 & 16.2% AR (day 18).

Conclusion

The depuration rate and BAF values are insufficiently reliable (see above).

Guidelines and limitations

A guideline for this type of study is not available. The study was not conducted under GLP. The main problem identified in the study has been described above. In addition, the following comments can be made.

1. Medaka were injected with a very small volume (2 μL) of a solution of pyridalyl in fish oil. The report provided no evidence that such a small volume of an oily liquid could be dispensed in a reproducible manner (e.g. by quantifying radioactivity in 2 μL aliquots dispensed directly into scintillation vials before and after dosing). The fact that pyridalyl residues in fish early in the study were quite variable may be due to lack of biological variability (e.g. in resorption, metabolism and elimination rate), but possibly also due lack of reproducibility of treatment procedures.
2. No information was provided to assess the extraction efficiency (e.g. analysis of untreated fish samples fortified with pyridalyl). The radioactivity in PES from extracted fish was also not quantified.
3. No information was provided as to whether the bluegill consumed all of the offered medaka.

4.2.2 Bioaccumulation test with other organisms

Characteristics

reference	:	IIA 8.2.7/02 Study No SUM-0041	species	:	*Lumbriculus variegatus*
type of study	:	Bioconcentration in oligochaetae	duration	:	28 days exposure followed by 28 days depuration
year of execution	:	2003	exposure method	:	Spiked sediment
GLP	:	Yes	nominal conc.		1 mg a.s./kg dw sediment
guideline	:	Test method 100.3 (EPA, 2000), OECD 305 (1996)	conclusion	:	BCF 1.19 kg sediment dwt/kg worm wwt; CT50 46 days; CT90 not estimated (only about 30% clearance within 28 days of depuration).
test substance	:	[dichlorophenyl-U-14C]-pyridalyl, lot no. RIS2003-003	acceptability	:	acceptable
purity	:	chemical purity not reported, radiochemical purity 96.3%			

A study was conducted to determine the BCF of pyridalyl in oligochaetae (*Lumbriculus variegatus*) exposed for 28 days in sediment/water systems containing spiked sediment.

Methods

Natural sediment for the test, which was obtained from Strohs Folly Brook, Wareham, Massachusetts, USA and sieved through a 0.50-mm sieve, had the following characteristics: 15.8% oc, 84% sand, 14% silt, 2% clay and pH 5.9 (4 g/kg $CaCO_3$ was added prior to the test to buffer the system). Twenty g of dry sand was treated with [dichlorophenyl-U-^{14}C]-pyridalyl in acetone and after evaporation of the acetone the treated sand was mixed in with a batch of 4 kg wwt sediment (1.16 kg dw) to give a nominal 14C-pyridalyl concentration of 1 mg/kg dw. Aliquots of 200 g wwt sediment (58 g dw, volume about 100 mL) were dispensed into 1000 mL test beakers and 600 mL of untreated overlying water was added. Nineteen replicate beakers were established for the treatment level and seven replicates for the solvent (acetone) control. After one day of equilibration, each beaker received one gram of oligochaetae (adult *L. variegatus*), which were exposed for 28 days. On day 28, the oligochaetae mass in the six remaining test beakers was removed and divided into twelve replicate vessels which had been established one day earlier as described above using clean sediment and water, and a 28-day depuration phase was initiated. During the 28-day exposure and 28-day depuration period, the overlying water was renewed by adding two volume additions (i.e. 1200 mL) of untreated water per vessel per day using an intermittent delivery system. Water quality parameters were: 16 h light (500-1000 lux), 8 h dark; 22-25°C; dissolved oxygen ≥4.4 mg/L; pH 6.1-6.6; hardness 44-52 mg $CaCO_3$/L; alkalinity 24-34 mg $CaCO_3$/L; conductance 150-180 µS/cm; ammonia 0.19-0.51 mg N/L.

Immediately after preparation an aliquot of the treated sediment was removed for chemical analysis. One treated replicate beaker was removed for chemical analysis of sediment, oligochaetae, overlying water and pore water on day 0 of exposure and three treated beakers on day 3, 7, 14 and 28 of exposure. Three treated beakers were removed for chemical analysis of oligochaetae on day 1, 3, 7 and 28 of depuration. Water was separated by decanting and analysed by LSC. This step was followed by extraction with dichloromethane and analysis of the extract by LSC and HPLC, but the radioactivity levels were too low for quantification and compound identification. Pore water was separated from sediment by centrifugation and analysed by LSC. Post-centrifugation sediment was extracted with acetonitrile containing 1% HCl and the extracts were concentrated and analysed by LSC and HPLC. PES in sediment was quantified by combustion/LSC. Oligochaetae were purged for 6 hours in clean water prior to quantification of radioactivity by combustion/LSC. Compound identification in sediment extracts was by co-chromatography with reference standards (S-1812 and S-1812-DP).

Validation of the extraction method of the water and sediment samples was provided by concurrent analysis of samples of control water and sediment fortified with pyridalyl. Recoveries from sediment (fortification level 0.1-1 mg/kg) ranged from 96 to 109% (combustion/LSC) and 97-114% (extraction/HPLC). Recoveries from water by LSC (fortification level 0.01-0.4 mg/L) ranged from 100 to 112%.

Results

It was reported that no dead oligochaetae or adverse effects were observed in the treatment or control vessels.

Radioactivity levels in water, sediment and worm samples of the solvent control were <LOQ. The mean measured radioactivity concentration in treated soil determined prior to distribution into replicate test beakers was 0.93 mg/kg. The individual and mean radioactivity concentrations in water, sediment and worms of treated replicates during exposure and depuration are shown in Table 4.2.2-01. Pyridalyl represented >97% of the radioactivity in sediment extracts, and levels of S-1812-DP were <3%.

The radioactivity concentration in sediment was relatively stable during exposure, the mean concentration during exposure was 0.98 mg/kg. The mean radioactivity concentrations in overlying and pore water were low (≤0.19 and ≤1.4 µg/L respectively). The mean radioactivity concentrations in worms increased

throughout exposure. A steady state level had not been reached by day 28. On day 1, 3 and 7 of the depuration phase radioactivity levels in worms were higher than at the end of the uptake phase. An explanation for this phenomenon was not offered by the author of the report.

Table 4.2.2-01 Radioactivity concentrations in water, sediment and oligochaetae from a bioconcentration experiment with [dichlorophenyl-U-14C] pyridalyl at nominal exposure concentration 1 mg/kg dw sediment

		uptake					depuration			
concentration in:	**rep**	**day 0**	**day 3**	**day 7**	**day 14**	**day 28**	**day 1**	**day 3**	**day 7**	**day 28**
sediment (mg/kg dw)	A	*1.1*(A)	0.97	0.92	0.85	1.0				
	B		0.90	0.96	0.88	1.1				
	C		1.0	1.1	0.74	0.98				
	mean		*0.97*	*0.98*	*0.82*	*1.0*				
PES sed. (mg/kg dw)	A	*0.014*(A)	0.029	0.026	0.034	0.043				
	B		0.036	0.027	0.022	0.052				
	C		0.036	0.026	0.031	0.038				
	mean		*0.034*	*0.026*	*0.029*	*0.044*				
overlying water (µg/L)	A	*0.080*(A)	0.12	0.015	0.076	0.044				
	B		0.16	0.023	0.034	0.066				
	C		0.034	0.036	0.46	0.037				
	mean		*0.10*	*0.024*	*0.19*	*0.049*				
pore water (µg/L)	A	*0.36*(A)	1.5	0.44	0.47	0.11				
	B		1.1	0.47	0.38	0.12				
	C		1.6	0.49	0.44	0.10				
	mean		*1.4*	*0.47*	*0.43*	*0.11*				
worms (mg/kg wwt)	A	*nd*	0.11	0.17	0.18	0.11(B)	0.53	0.55	0.43	0.35
	B		0.050	0.18	0.088	0.27	0.47	0.49	0.38	0.32
	C		0.095	0.091	0.48	0.36	0.41	0.55	0.41	0.33
	mean		*0.085*	*0.15*	*0.25*	*0.32*	*0.47*	*0.53*	*0.41*	*0.33*

nd = <0.00031 mg/kg wwt.
(A) Only one sample was analysed on this day.
(B) The author of the report stated that this sample result was impacted by instrument difficulties on test day 28, and the result was excluded from mean calculation.

Calculation of bioconcentration factors

The author of the report calculated the BCF in two ways.

(1) The mean concentration in worms (reported value 0.31 mg/kg) on day 28 of uptake was considered to be a reasonable estimate of the steady state, although the steady state had apparently not been reached by day 28 of exposure. The BCF was then determined by dividing this value by the mean radioactivity concentration in sediment during exposure (0.98 mg/kg): BCF = 0.32 kg sediment dwt/kg worm wwt;

(2) In addition, BCF values were calculated as k_1/k_2, where k_1 and k_2 are the uptake and depuration rate constant. The depuration rate constant k_2 was determined from the depuration curve by a plot of $\ln(C_{worm})$ versus T. The uptake rate constant k_1 was determined as:

$k_1 = C_m * k_2 / (C_s * (1\text{-}\exp\text{-}(k_2*T))$,

where

C_m is one half of the steady state tissue concentration, predicted by linear regression of the natural log tissue concentration against uptake time; C_m = 0.95 mg/kg

C_s = mean sediment concentration (0.98 mg/kg)

k_2 = depuration rate constant

T = time to one half of the steady state tissue concentration predicted by the linear regression of natural log tissue concentration against uptake time; T = 28 days

This gave k_1 = 0.00453 day^{-1}, k_2 = 0.0142 day^{-1} en BCF = 0.32 kg sediment dwt/kg worm wwt.

Evaluation by RMS

As the concentration in worms increased throughout exposure (and part of depuration), it is not accepted that a steady state BCF is calculated (approach 1). Approach 2 is described in OECD 305 (1996), Annex 6, equation 1. This approach assumes that a smooth uptake curve is defined by the experimental data and that the midpoint of the smooth uptake curve can be identified accurately. In the present study a smooth uptake curve was not determined: linear regression of natural log tissue concentration against uptake time performed by author of report on mean values gave a poor fit (coefficient of correlation (r^2) value of 0.42). A justification for Cm = 0.95 mg/kg was not provided, and T = 28 days was reported to be empirically estimated without further explanations.

Kinetic BCF values were therefore also estimated by the RMS based on the data in the previous table (i.e. RA concentrations in sediment extracts and worms) according to the methods outlined in Annex 6 of OECD 305 (1996) using non-linear parameter estimation methods and curve fitting with the computer program Modelmaker V 4.0. The k_2 value was first determined from the depuration curve, and implemented as a constant into the equation describing the uptake of residues (equation 2 in Annex 6 of OECD 305 (1996)). Curve fitting was performed on individual replicate data, calculation of χ^2 error on replicate means (in agreement with the recommendations and procedures of the "Guidance document on estimating persistence and degradation kinetics from Environmental Fate studies on pesticides in EU registration" (SANCO/10058/2005 version 1.0)). This gave the following results:

k_2 = 0.0149112 day^{-1} (SFO, optimize error 0.00383414 day^{-1}, r^2 0.65, χ^2 error 6.52); depuration CT50 46 days; CT90 not estimated since only about 30% clearance was reached within 28 days of depuration.

k_1 = 0.0177786 day^{-1} (optimize error 0.00330452 day^{-1}, r^2 0.40, χ^2 error 22.27).

BCF = 1.19 kg sediment dwt/kg worm wwt.

According to OECD 305 (1996), the above method is the preferred means for obtaining uptake and depuration rate constants. The BCF value of 1.19 kg sediment dwt/kg worm wwt is therefore the endpoint from this study. The relatively low accuracy of the uptake rate constant is related to high variability between residues in replicate worm samples, in particular on day 14 and 28. The accuracy of the depuration rate constant is hampered by the fact that there were only 4 sampling points, and depuration during the first 3 sampling points was limited. By visual assessment the fit of the experimental versus the fitted $C_{worm}/C_{sediment}$ data however was found to be acceptable, and the optimize errors of the accumulation and depuration rate constants are relatively low. The study endpoint (BCF 1.19 kg sediment dwt/kg worm wwt) is therefore considered to be sufficiently reliable.

Conclusion

BCF 1.19 kg sediment dwt/kg worm wwt. CT50 46 days; CT90 not estimated (only about 30% clearance within 28 days of depuration).

Guidelines and limitations

1. In addition to the comments in the above summary, the following was noted. Residues in oligochaetae were determined by combustion analysis of the entire wet sample. No validation was provided to confirm the accuracy and precision of these measurements, which may be difficult in case of watery matrices. Residues in worms from different replicates were variable during uptake, but not during depuration. The variability may hence be related to factors other than analytical variation (e.g. variable amounts of sediment-containing gut contents).
2. Total radioactivity concentrations in sediment were also determined by combustion LSC, but the results were more variable than those determined by extraction, and slightly lower. The author of the report therefore based the endpoints on the radioactivity concentrations in sediment determined by extraction. This approach is acceptable and also followed in the above summary.

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

Study 1

Characteristics

reference	:	IIA 8.2.1.1/01 Study No. 13048.6206	species	:	Rainbow trout (*Oncorhynchus mykiss*)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	2000	nominal conc.	:	0, 0.094, 0.19, 0.37, 0.75 and 1.5 mg a.s./L
GLP statement	:	Yes			
guideline	:	FIFRA 72-1 OPPTS 850.1075	dosing method	:	Flow-through; stock solution in hydrogenated castor oil and
test substance	:	S-1812 (pyridalyl), Lot PS-98041G			DMF (1:1)
			acceptability	:	Acceptable
purity	:	93.7%	96 h-LC50	:	0.50 mg a.s./L (emulsion in water)
water solubility	:	0.15 µg/L at 20°C			<0.15 µg a.s./L (dissolved pyridalyl)

Methods

A 96-hour acute toxicity test in rainbow trout (*Oncorhynchus mykiss*) (2 replicates of ten fish each per concentration) was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test

concentrations of 0.094, 0.19, 0.37, 0.75 and 1.5 mg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. In the flow-through system, the stock solution in hydrogenated castor oil and DMF was delivered into a mixing chamber, which also received dilution water and was placed in an ultrasonic water bath where the contents were continuously sonicated and stirred to aid in the solubilisation of S-1812. The concentration of S-1812 in the solution in the mixing chamber was equivalent to the highest nominal test concentration (1.5 mg a.s./L) and was subsequently diluted (50% dilution factor) to provide the remaining nominal test concentrations (0.094, 0.19, 0.37 and 0.75 mg a.s./L). A similar system was used for the solvent control (solvent concentration 0.1 mL/L, equivalent to the concentration of solvent in the highest test concentration).

Results

Measured concentrations were 0.11, 0.19, 0.38, 0.74 and 1.7 mg a.s./L (representing 99-117% of nominal) at the start of the test and 0.10, 0.18, 0.37, 0.75 and 1.5 mg a.s./L (representing 95-106% of nominal) after 96 hours. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. Endpoints were based on mean measured concentrations (0.11, 0.19, 0.37, 0.75 and 1.6 mg a.s./L). Clinical signs observed in surviving fish included loss of equilibrium, darkened pigmentation and lethargy. The author of the report stated that observations of the physical characteristics of the test solutions (e.g. presence of precipitate, film on the solution's surface) were made and recorded, but the results of these observations were not reported.

The reported results are summarised in Table 4.3.1-01.

Table 4.3.1-01 The acute toxicity of S-1812 (pyridalyl) to fish

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	LC50 in mg a.s./L	NOEC mg a.s./L
Oncorhynchus mykiss	Flow-through 96 hours (93.7%), exposure to dilutions of the highest test concentration (prepared by dilution of a stock in hydrogenated castor oil and DMF)	95-117	24 h: >1.6(A) 48 h: 0.85(A) 72 h: 0.59(A) 96 h: 0.50(A)	24: 0.19(A) 48-96 h: <0.11(A)

(A) Based on mean measured concentrations

Remarks

The test was performed at concentrations far exceeding the water solubility of pyridalyl. The author of the report stated: *As the water solubility study of S-1812 was extremely low (less than 1 ppb), the Study Sponsor concluded that it should be optimised by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). The use of the 1:1 DMF:HCO-40 mixture yielded a water solubility approximating 30 mg/L. The Study Sponsor provided the details of this information to U.S. EPA, which subsequently approved the use of DMF:HCO-40 in this toxicity test.* The details were however not provided in the report of the present study. The test was performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by at least a factor of 627. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. The dissolved fraction of

pyridalyl in lower nominal concentrations may have been even lower than that in the highest test concentration, since these concentrations were prepared by dilution from the highest test concentration.

Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. The results of an Early Life Stage test in rainbow trout indicated that the LC50 of pyridalyl mixed with water in an oily emulsion is expected to be above the level of water solubility of pyridalyl (NOEC and LOEC respectively 24 and 49 µg a.s./L). For classification of the active substance, the endpoint should be based on the concentration of dissolved pyridalyl. Since the classification of pyridalyl (R50, highest risk category) would not be different when based on the reported LC50 (0.50 mg a.s./L) from that based on the dissolved fraction of pyridalyl (estimated as ≤0.15 µg a.s./L), the reported endpoint is also accepted for classification. For the dissolved fraction of active substance, the RMS set the LC50 at <0.15 µg a.s./L, based on the observation of more than 50% mortality at the highest test concentration, which is likely to have been at the solubility limit of ≤0.15 µg dissolved pyridalyl/L (worst-case).

Conclusion

96-hour LC50: 0.50 mg a.s./L (emulsion of dehydrogenated castor oil and DMF (1:1) in water; value to be used in classification for the present proposed application).

96-hour LC50 for dissolved pyridalyl: <0.15 µg a.s./L (value to be additionally included in the list of endpoints).

Guidelines and limitations

Comments have been discussed under “remarks” above.

Study 2

Characteristics

reference	:	IIA 8.2.1.2/01 Study No. 13048.6207	species	:	Bluegill sunfish (*Lepomis macrochirus*)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	2000	nominal conc.	:	0, 3.9, 6.5, 11, 18 and 30 mg a.s./L
GLP statement	:	Yes	dosing method	:	Flow-through; stock solution in
guideline	:	FIFRA 72-1, OPPTS 850.1075			hydrogenated castor oil and
test substance	:	S-1812 (pyridalyl),			DMF (1:1)
		Lot PS-98041G	acceptability	:	Acceptable
purity	:	93.7%	96 h-LC50	:	>24 mg a.s./L (emulsion in water)
water solubility	:	0.15 µg/L at 20°C			

Methods

A 96-hour acute toxicity test in bluegill sunfish (*Lepomis macrochirus*) (2 replicates of ten fish each per concentration) was conducted under flow-through conditions with S-1812 at nominal test concentrations of 3.9, 6.5, 11, 18 and 30 mg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. In the flow-through system, the stock solution in hydrogenated castor oil and DMF was delivered into a mixing chamber, which also received dilution water and was placed in an ultrasonic water bath where the contents were continuously sonicated and stirred to aid in the solubilisation of S-1812. The concentration of S-1812 in the solution in the mixing chamber was equivalent to the highest nominal test concentration (30 mg a.s./L) and was subsequently diluted (60% dilution factor) to provide the remaining nominal test concentrations (3.9, 6.5, 11 and 18 mg a.s./L). A similar system was used for the solvent control (solvent concentration 0.099 mL/L, equivalent to the concentration of solvent in the highest test concentration).

Results

Measured concentrations were 3.3, 6.1, 12, 18 and 30 mg a.s./L (representing 85-109% of nominal) and 2.4, 5.1, 8.6, 15 and 19 mg a.s./L (representing 62-83% of nominal) at test initiation and at the end of the test, respectively. The test solutions at the highest two concentrations were slightly cloudy during the test. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. Endpoints were based on mean measured concentrations (2.9, 5.6, 10, 16 and 24 mg a.s./L). No clinical signs were observed in any concentration, and mortality after 96 hours was limited to 0, 0, 5, 5, 0, 0 and 0% in the control, the solvent control and at 2.9, 5.6, 10, 16 and 24 mg a.s./L, respectively. The results are summarised in Table 4.3.1-02.

Table 4.3.1-02 The acute toxicity of S-1812 (pyridalyl) to fish

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	LC_{50} in mg a.s./L	NOEC mg a.s./L
Lepomis macrochirus	Flow-through 96 hours (93.7%)	85-109 (start) 62-83 (end)	24, 48, 72 & 96 h: >24(A)	24, 48, 72 & 96 h: 24(A)

(A) Based on mean measured concentrations

Conclusion

96-hour LC50: >24 mg a.s./L, based on mean measured concentrations (tested as an emulsion in water).

Guidelines and limitations

The test was performed at concentrations far exceeding the water solubility of pyridalyl. For discussion of the testing methodology, see the discussion included under “remarks” in the summary of study 1 above.

Study 3

Characteristics

reference	:	IIA 8.11.1/01 Study No. 12709.6200	species	:	Sheepshead minnow (*Cyprinodon variegatus*)
type of study	:	Acute toxicity study			
year of execution	:	2001	exposure duration	:	96 hours
GLP statement	:	Yes	nominal conc.	:	0, 3.9, 6.5, 11, 18 and 30 mg a.s./L
guideline	:	FIFRA 72-3 OPPTS 850.1075	dosing method	:	Flow-through; stock solution in hydrogenated castor oil and DMF (1:1)
test substance	:	S-1812 & S-1812 T.G. (pyridalyl), Lot PS-98041G & AS 1817d	acceptability	:	Acceptable
purity	:	93.7%	96 h-LC50	:	>32 mg a.s./L (emulsion in water)
water solubility	:	0.15 µg/L at 20°C			

Methods

A 96-hour acute toxicity test in the marine fish species sheepshead minnow (*Cyprinodon variegatus*) (2 replicates of ten fish each per concentration) was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 3.9, 6.5, 11, 18 and 30 mg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. In the flow-through system, the stock solution in

hydrogenated castor oil and DMF was delivered into a mixing chamber, which also received dilution water (natural filtered seawater) and was placed in an ultrasonic water bath where the contents were continuously stirred. The concentration of S-1812 in the solution in the mixing chamber was equivalent to the highest nominal test concentration (30 mg a.s./L) and was subsequently diluted (60% dilution factor) to provide the remaining nominal test concentrations (3.9, 6.5, 11 and 18 mg a.s./L). A similar system was used for the solvent control (solvent concentration 0.097 mL/L, equivalent to the concentration of solvent in the highest test concentration).

Results

Measured concentrations were 74-111% of nominal (74, 100, 100, 111 and 110% at 3.9, 6.5, 11, 18 and 30 mg a.s./L, respectively) at the start and 88-109% of nominal concentrations at the end of the test. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. Endpoints were based on mean measured concentrations. The solutions at the two highest test concentrations were reported to be cloudy with no visible undissolved material throughout the 96-hour exposure.

The reported results are summarised in Table 4.3.1.-03.

Table 4.3.1-03 The acute toxicity of S-1812 (pyridalyl) to fish

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	LC50 in mg a.s./L	NOEC mg a.s./L
Cyprinodon variegatus	Flow-through 96 hours (93.7%), exposure to dilutions of the highest test concentration (prepared by dilution of a stock in hydrogenated castor oil and DMF)	74-111	24, 48, 72 & 96 h: >32(A)	24, 48, 72 & 96 h: 32(A)

(A) Based on mean measured concentrations

Remarks

The test was performed at concentrations far exceeding the water solubility of pyridalyl. The author of the report stated: *As the water solubility study of S-1812 was extremely low (less than 1 ppb), the Study Sponsor concluded that it should be optimised by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). The use of the 1:1 DMF:HCO-40 mixture yielded a water solubility approximating 30 mg/L. Using data generated by Sumitomo Chemical Company, Ltd., the Study Sponsor provided the details of this information to U.S. EPA, which subsequently approved the use of DMF:HCO-40 in this toxicity test.* The details were however not provided in the report of the present study. The test was performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by at least a factor of 26000. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. For classification of the active substance, the endpoint should be based on the concentration of dissolved pyridalyl, and the result cannot be used for this purpose.

Conclusion

96-hour LC50: >32 mg a.s./L (emulsion of dehydrogenated castor oil and DMF (1:1) in water)).

Guidelines and limitations

See remarks.

4.3.2 Short-term toxicity to aquatic invertebrates

Study 1

Characteristics

reference	:	IIA 8.3.1.1/01 Study No. 13048.6208	species	:	*Daphnia magna*
type of study	:	Acute toxicity study	exposure duration	:	48 hours
year of execution	:	2000	nominal conc.	:	0, 3.2, 5.4, 9.0, 15 and 25 µg a.s./L
GLP statement	:	Yes	dosing method	:	Flow-through; stock solution in
guideline	:	FIFRA 72-2			hydrogenated castor oil and

		OPPTS 850.1010			DMF (1:1)
test substance	:	S-1812 (pyridalyl)	acceptability	:	Acceptable
		Lot PS-98041G	48 h-EC50	:	3.8 µg a.s./L (emulsion in water)
purity	:	93.7%			<0.15 µg a.s./L (dissolved pyridalyl)
water solubility	:	0.15 µg/L at 20°C			

Methods

A 48-hour acute toxicity test in *Daphnia magna* (2 replicates of ten daphnids each per concentration) was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 3.2, 5.4, 9.0, 15 and 25 µg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. In the flow-through system, the stock solution in hydrogenated castor oil and DMF was delivered into a mixing chamber, which also received dilution water. The concentration of S-1812 in the solution in the mixing chamber was equivalent to the highest nominal test concentration (25 µg a.s./L) and was subsequently diluted (60% dilution factor) to provide the remaining nominal test concentrations (3.2, 5.4, 9.0 and 15 µg a.s./L). A similar system was used for the solvent control (solvent concentration 1.0 µL/L, equivalent to the concentration of solvent in the highest test concentration).

Results

Measured concentrations were 2.5, 4.1, 7.2, 13 and 19 µg a.s./L (representing 76-87% of nominal) at test initiation and 1.9, 2.7, 5.6, 10 and 15 µg a.s./L (representing 50-67% of nominal) after 48 hours. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. Endpoints were based on mean measured concentrations (2.2, 3.4, 6.4, 11 and 17 µg a.s./L). The author of the report stated that observations of the physical characteristics of the test solutions (e.g. presence of precipitate, film on the solution's surface) were made and recorded, but the results of these observations were not reported.

The reported results are summarised in Table 4.3.2-01.

Table 4.3.2-01 The acute toxicity of S-1812 (pyridalyl) to invertebrates

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	EC50 in µg a.s./L	NOEC µg a.s./L
Daphnia magna	Flow-through 48 hours (93.7%), exposure to dilutions of the highest test concentration (prepared by dilution of a stock in hydrogenated castor oil and DMF)	start: 76-87 end: 50-67	24 h: >17(A) 48 h: 3.8(A)	24 h: 17(A) 48 h: <2.2(A)

(A) Based on mean measured concentrations

Remarks:

The test was performed at concentrations exceeding the water solubility of pyridalyl. The author of the report stated: *As the water solubility study of S-1812 was extremely low (less than 1 ppb), the Study Sponsor concluded that it should be optimised by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). The use of the 1:1 DMF:HCO-40 mixture yielded a water solubility approximating 30 mg/L. The Study Sponsor provided the details of this*

information to U.S. EPA, which subsequently approved the use of DMF:HCO-40 in this toxicity test. The details were however not provided in the report of the present study. The test was performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by at least a factor of 21. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. The dissolved fraction of pyridalyl in lower nominal concentrations may have been even lower than that in the highest test concentration, since these concentrations were prepared by dilution from the highest test concentration. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. For classification of the active substance, the endpoint should be based on the concentration of dissolved pyridalyl. Since the classification of pyridalyl would not be different when based on the reported EC50 (3.8 µg a.s./L) from that based on the dissolved fraction of pyridalyl (estimated as ≤0.15 µg a.s./L), the reported endpoint is also accepted for classification. For the dissolved fraction of active substance, the RMS set the EC50 at <0.15 µg a.s./L, based on the observation of more than 50% immobilisation at the highest test concentration, which is likely to have been at the solubility limit of ≤0.15 µg dissolved pyridalyl/L (worst-case).

Study 2

Characteristics

reference	:	IIA 8.3.1.1/02 Study No 1043.046.110	species	:	*Daphnia magna*
type of study	:	Higher Tier acute toxicity study	exposure duration	:	48 hours
year of execution	:	2005	nominal conc.	:	0, 0.88, 1.94, 4.27, 9.39, 20.7, 45.5
GLP statement	:	Yes			and 100 µg a.s./L
guideline	:	OECD 202, EEC C.2	dosing method	:	Static; stock solution in
test substance	:	a) S-1812 (pyridalyl), Lot PS-98041G			hydrogenated castor oil and DMF (1:1)
		b) [dichlorophenyl-^{14}C]S-1812, Lot RIS2005-009	acceptability	:	Acceptable
			48 h-EC50	:	34.6 µg a.s./L (emulsion in water)
purity	:	a) 93.7% b) radiochemical purity 99.3%			
water solubility	:	0.15 µg/L at 20°C			

Methods

A 48-hour higher tier acute toxicity test in *Daphnia magna* (4 replicates of five daphnids each per concentration) was conducted in natural water-sediment systems under static conditions with radiolabelled S-1812 (pyridalyl) at nominal test concentrations of 0.88, 1.94, 4.27, 9.39, 20.7, 45.5 and 100 µg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. One week before application, the test systems were prepared using 1.5 mm sieved sediment (natural sediment from an untreated area in Horn, Switzerland; 0.75% oc, 10.2% clay, 71.0% silt and 18.8% sand according to DIN classification) and 100 µm filtered natural water. The test systems were set up in 600 mL glass beakers filled with 2.5 cm sediment and 500 mL natural water (corresponding to a water depth of ca. 10 cm) after which the systems were allowed to equilibrate under constant slight aeration of the water phase. Pyridalyl, suspended in a solution containing solvent and distilled water, was applied in small circles into the surface water phase, and the test solutions were gently mixed for about 30 seconds using a glass rod. Measures were taken to minimise disturbance of the sediment. The solvent concentration (10 µL/L) was equal in all test concentrations and the solvent control. Directly after application, daphnids were introduced. Samples for analyses of actual exposure concentrations were taken at the start and the end of the test and analysed by LSC. Radiopurity of the three highest test concentrations was checked by direct HPLC.

Results

Measured concentrations were 0.76, 1.9, 4.1, 9.3, 20, 44 and 98 µg eq./L (representing 87-99% of nominal) at test initiation and 0.58, 1.5, 3.1, 7.9, 16, 38 and 80 µg eq./L (representing 66-84% of nominal) after 48 hours. Pyridalyl accounted for 96-97% and 94-96% of the radioactivity at the highest three concentrations after 0 and 48 hours, respectively. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. Endpoints were based on nominal concentrations, which is acceptable.

The results are summarised in Table 4.3.2-02.

Table 4.3.2-02 The acute toxicity of S-1812 (pyridalyl) to invertebrates in natural water-sediment systems

Species	Test type and duration	Actual concentration (as % of nominal)	Nominal concentration (µg a.s./L)	Mortality (%)	EC50 in µg a.s./L	NOEC µg a.s./L
Daphnia magna	Static, 48 hours, exposure in natural water-sediment systems (stock in hydrogenated castor oil and DMF)	start: 87-99 end: 66-84	0.88 1.94 4.27 9.39 20.7 45.5 100	0 0 0 5 0 95 95	24 h: >100(A) 48 h: 34.6(A)	24 h: 100(A) 48 h: 20.7(A)

(A) Based on nominal concentrations, analytically confirmed for initial concentrations

Conclusion

48-hour EC50: 34.6 µg a.s./L (tested as an emulsion in water).

Guidelines and limitations

The test was performed at concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by at least a factor of 6. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility.

Study 3

Characteristics

reference	:	IIA 8.11.1/02 Study No. 12709.6198	species	:	Mysid (*Americamysis bahia*)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	2001	nominal conc.	:	0, 0.41, 0.69, 1.2, 1.9 and 3.2 µg a.s./L
GLP statement	:	Yes			
guideline	:	FIFRA 72-3 OPPTS 850.1035	dosing method	:	Flow-through; stock solution in hydrogenated castor oil and
test substance	:	S-1812 & S-1812 T.G. (pyridalyl), Lot PS-98041G & AS 1817d	acceptability	:	DMF (1:1) Acceptable
purity	:	93.7%	96 h-LC50	:	1.0 µg a.s./L (emulsion in water)
water solubility	:	0.15 µg/L at 20°C			

Methods

A 96-hour acute toxicity test in the marine mysid *Americamysis bahia* (2 replicates of ten mysids (<24 hours old) each per concentration) was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 0.41, 0.69, 1.2, 1.9 and 3.2 µg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. In the flow-through system, the stock solution in hydrogenated castor oil and DMF was delivered into a mixing chamber, which also received dilution water (natural filtered seawater) and was placed in an ultrasonic water bath where the contents were continuously stirred. The concentration of S-1812 in the solution in the mixing chamber was equivalent to the highest nominal test concentration (3.2 µg a.s./L) and was subsequently diluted (60% dilution factor) to provide the remaining nominal test concentrations (0.41, 0.69, 1.2 and 1.9 µg a.s./L). A similar system was used for the solvent control (solvent concentration 2.0 µL/L, equivalent to the concentration of solvent in the highest test concentration).

Results

Measured concentrations were 88-96% and 88-100% of nominal concentrations at the start and the end of the test, respectively. Water quality parameters were pH (8.0-8.4), oxygen concentration (7.1-9.7 mg/L), temperature (24-26°C) and salinity (18-21‰). Endpoints were based on mean measured concentrations. The solutions were reported to be clear and colourless throughout the 96-hour exposure period.

The reported results are summarised in Table 4.3.2-03.

Table 4.3.2-03 The acute toxicity of S-1812 (pyridalyl) to mysid

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	LC50 in µg a.s./L	NOEC µg a.s./L
Americamysis bahia	Flow-through 96 hours (93.7%), exposure to dilutions of the highest test concentration (prepared by dilution of a stock in hydrogenated castor oil and DMF)	88-100	24 h: >3.0(A) 48 h: 1.8(A) 72 h: 1.2(A) 96 h: 1.0(A)	24 h: 1.8(A) 48 & 72 h: 0.62(A) 96 h: 0.40(A)

(A) Based on mean measured concentrations

Conclusion

96-hour LC50: 1.0 µg a.s./L (tested as an emulsion in water).

Guidelines and limitations

The test was performed at concentrations exceeding the water solubility of pyridalyl. The author of the report stated: *As the water solubility study of S-1812 was extremely low (less than 1 ppb), the Study Sponsor concluded that it should be optimised by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). The use of the 1:1 DMF:HCO-40 mixture yielded a water solubility approximating 30 mg/L. The Study Sponsor provided the details of this information to U.S. EPA, which subsequently approved the use of DMF:HCO-40 in this and other aquatic toxicity tests.* The details were however not provided in the report of the present study. The test was

performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by at least a factor of 3. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl.

Study 4

Characteristics

reference	:	IIA 8.11.1/03 Study No. 12709.6199	species	:	Eastern oyster (*Crassostrea virginica*)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	2001	nominal conc.	:	0, 0.38, 0.75, 1.5, 3.0 and 6.0 mg a.s./L
GLP statement	:	Yes			
guideline	:	FIFRA 72-3 OPPTS 850.1025	dosing method	:	Flow-through; stock solution in hydrogenated castor oil and DMF (1:1)
test substance	:	S-1812 (pyridalyl), Lot AS 1817d	acceptability	:	Acceptable
purity	:	93.7%	96 h-EC50	:	0.82 mg a.s./L (emulsion in water)
water solubility	:	0.15 µg/L at 20°C			

Methods

A 96-hour acute toxicity test in eastern oyster (*Crassostrea virginica*) (2 replicates of twenty oysters each per concentration) was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 mg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. In the flow-through system, the stock solution in hydrogenated castor oil and DMF was delivered into a mixing chamber, which also received dilution water (natural unfiltered seawater) and was placed in an ultrasonic water bath where the contents were continuously stirred. The concentration of S-1812 in the solution in the mixing chamber was equivalent to the highest nominal test concentration (6.0 mg a.s./L) and was subsequently diluted (50% dilution factor) to provide the remaining nominal test concentrations (0.38, 0.75, 1.5 and 3.0 mg a.s./L). A similar system was used for the solvent control (solvent concentration 20 µL/L, equivalent to the concentration of solvent in the highest test concentration).

Results

Measured concentrations were 84-96% and 79-97% of nominal concentrations at the start and the end of the test, respectively. Water quality parameters were pH (7.4-7.9), oxygen concentration (3.7-7.0 mg/L), temperature (21-22°C) and salinity (32‰). Endpoints were based on mean measured concentrations. The solutions at the highest test concentration and the solvent control were reported to have foam on the surface, but all mixing chamber solutions were clear and colourless with no visible evidence of undissolved test substance.

The reported results are summarised in Table 4.3.2-04.

Table 4.3.2-04 The acute toxicity of S-1812 (pyridalyl) to eastern oyster

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	EC50 in mg a.s./L	NOEC mg a.s./L
Crassostrea virginica	Flow-through 96 hours (93.7%), exposure to dilutions of the highest test concentration (prepared by dilution of a stock in hydrogenated castor oil and DMF)	79-97	96 h: 0.82(A)	96 h: <0.34(A)

(A) Based on mean measured concentrations

Conclusion
96-hour EC50: 0.82 mg a.s./L (tested as an emulsion in water).

Guidelines and limitations
The test was performed at concentrations exceeding the water solubility of pyridalyl. The author of the report stated: *As the water solubility study of S-1812 was extremely low (less than 1 ppb), the Study Sponsor concluded that it should be optimised by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). The use of the 1:1 DMF:HCO-40 mixture yielded a water solubility approximating 30 mg/L. The Study Sponsor provided the details of this information to U.S. EPA, which subsequently approved the use of DMF:HCO-40 in this and other aquatic toxicity tests.* The details were however not provided in the report of the present study. The test was performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by at least a factor of 2533. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl.

Conclusion
48-hour EC50: 3.8 µg a.s./L (emulsion of dehydrogenated castor oil and DMF (1:1) in water).
48-hour EC50 for dissolved pyridalyl: <0.15 µg a.s./L (value to be additionally included in the list of endpoints).

Guidelines and limitations
See remarks.

STUDY 5

Characteristics

reference	:	IIA 8.5.1/01	species	:	*Chironomus yoshimatsui*

type of study	:	Acute toxicity study	exposure duration	:	48 hours
year of execution	:	2004-2005	nominal conc.	:	0, 0.010, 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg/L
GLP statement	:	No			
guideline	:	ASTM 729	dosing method	:	Static; stock solution in hydrogenated castor oil and DMF (1:1)
test substance	:	a) S-1812 TG (pyridalyl), Lot PK-98062 b) [dichlorophenyl-^{14}C]S-1812, Lot YM020603-2 (C-2002-033T)	acceptability	:	Acceptable
			48 h-LC50	:	1.1 mg/L (emulsion in water)
purity	:	a) 92.4% b) radiochemical purity 97.1%			
water solubility	:	0.15 µg/L at 20°C			

Methods

A 48-hour acute toxicity test in *Chironomus yoshimatsui* (1 replicate containing an unspecified number of midges each per concentration) was conducted under static conditions with S-1812 (radiolabelled pyridalyl) at nominal test concentrations of 0.010, 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg/L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. The test was performed without sediment, under 16 hours of light and 8 hours of darkness. Samples for analyses of actual exposure concentrations were taken at the start and the end of the test and analysed by direct LSC. In addition, samples were extracted with ethyl acetate and analysed by LSC and TLC for quantification of S-1812 and metabolites. At test termination, midge larvae, separated into live and dead larvae, were homogenized and extracted with acidified acetonitrile for LSC and TLC analysis. The post-extraction residues were combusted for LSC analysis. As TLC analysis of the extracts of the midge samples indicated a significant amount of extracted ^{14}C at the origin, the fraction of the TLC origin was collected by scraping off and subjected to acid hydrolysis prior to extraction with ethyl acetate followed by TLC analysis.

Results

Mean measured pyridalyl concentrations were 0.0088, 0.032, 0.087, 0.32, 0.93, 3.0 and 8.8 mg a.s./L (87-100% of nominal). S-1812-DP was detected in the water at levels up to 2.2% of TRR.

Water quality parameters were: pH (7.4-7.9), dissolved oxygen concentration (7.9-8.7 mg/L) and temperature (22.6-23.3°C). Endpoints were based on mean measured pyridalyl concentrations, which is acceptable.

The results are summarised in Table 4.3.2-05.

Table 4.3.2-05 The acute toxicity of S-1812 (pyridalyl) to sediment-dwelling organisms

Species	Test type and duration.	Actual pyridalyl concentration (as % of nominal)	LC50 in mg a.s./L	NOEC mg a.s./L
Chironomus yoshimatsui	Static, 48 hours (stock in hydrogenated castor oil and DMF)	start: 81-97 end: 84-113	24 h: 6.8(A) 48 h: 1.1(A)	24 h: 0.93(A) 48 h: 0.32(A)

(A) Based on mean measured concentrations

Extraction of midge samples released 80-91% of the total radioactivity. Radioactivity levels and concentrations of pyridalyl and metabolites in midge extracts increased with increasing exposure concentration. S-1812, S-1812-DP and S-1812-DP conjugates accounted for 7.9-67%, 12-34% and 8.4-34% of TRR, respectively. A minor metabolite (<2% TRR) was S-1812-Ph-CH_2COOH. Results are presented in Table 4.3.2-06.

Table 4.3.2-06 Radioactivity levels and concentrations of S-1812 and its metabolites in midges after 48 hours of exposure

Test group	Contr.	Solv. Contr.	Mean measured concentration (mg a.s./L)								
			0.0088	0.032	0.087	0.32	0.93	0.93	3.0	8.8	8.8(A)
Live or dead midges	Live	Live	Live	Live	Live	Live	Live	Dead	Dead	Dead	Dead
TRR (mg eq./kg)	0.061	0.034	4.9	11	26	85	170	140	320	480	260
% of TRR											
S-1812	NA	NA	14	7.9	29	18	39	49	57	67	61
S-1812-DP	NA	NA	33	34	27	31	24	19	16	13	12
S-1812-DP conjugates	NA	NA	26	34	21	27	18	17	14	8.4	14
S-1812-Ph-CH_2COOH	NA	NA	1.5	1.8	1.6	1.9	1.1	ND	1.0	ND	1.4
Others	NA	NA	5.9	5.5	5.1	6.5	6.0	3.4	2.8	ND	2.4
mg a.s./kg											
S-1812	NA	NA	0.66	0.89	7.6	15	67	71	180	320	160
S-1812-DP	NA	NA	1.6	3.8	7.0	26	41	22	51	64	33
S-1812-DP conjugates	NA	NA	1.3	3.8	5.4	23	31	24	45	40	37
S-1812-Ph-CH_2COOH	NA	NA	0.072	0.20	0.42	1.6	2.0	ND	3.2	ND	3.7
Others	NA	NA	0.29	0.62	1.3	5.5	10	4.8	8.9	ND	6.3

ND Not detected
NA Not applicable
(A) Measured after 24 hours of exposure

Body burden

An attempt was made to determine the lethal body burden. Actual S-1812 concentrations in dead midges ranged from 71 to 320 mg a.s./kg and increased with the exposure concentration. The author of the report stated that this dependency was likely to be caused mainly by the uptake in excess beyond the lethal body burden level (i.e. overdosing). Thus, an accurate lethal body burden level would be estimated by the results at the lowest mortality level of 0.93 mg/L, in which the mortality was partial and the live and dead midges were analysed separately. Since the concentrations of S-1812 in live and dead midges at this concentration were similar (67 and 71 mg/kg), it was suggested that these levels approximated the acute lethal body burden level.

Conclusion

48-hour LC50: 1.1 mg a.s./L (tested as an emulsion in water).

Guidelines and limitations

1. The study was not conducted under GLP. The reported validation of the analytical method in midges was limited to recoveries at unspecified fortification levels, while no data on validation of the analytical method in water were reported. The test substance is however known to be stable under test conditions.
2. The number of midges per replicate was not reported. In the reported preliminary range-finding test, one replicate of seven midges each per concentration was used, but the reported mortality percentages suggest that at least 10 midges per concentration were used for the definitive test. The results of the range-finding test (14 and 43% mortality at 1.0 and 10 mg a.s./L) show that toxicity was not underestimated in the definitive test.
3. The test was performed at concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by at least a factor of 67. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility.

4.3.3 Algal growth inhibition tests

Study 1

Characteristics

reference	:	IIA 8.4/01 Study No. 12709.6207	species	:	*Pseudokirchneriella subcapitata*
type of study	:	Algal growth inhibition	exposure duration	:	96 hours
year of execution	:	2002	nominal conc.	:	0 and 0.20 mg a.s./L
GLP statement	:	Yes	dosing method	:	Static, stock in dehydrogenated castor
guideline	:	OECD 201			oil and DMF (1:1)
		EEC C.3, OPPTS 850.5400	acceptability	:	Acceptable
test substance	:	S-1812 TG (pyridalyl)	72h-EbC50	:	>0.20 mg a.s./L (emulsion in water)
		Lot AS 1817d (PS-98041G)	72h-ErC50	:	>0.20 mg a.s./L (emulsion in water)
purity	:	93.7%	72h NOEbC	:	0.20 mg a.s./L (emulsion in water)
			72h NOErC	:	0.20 mg a.s./L (emulsion in water)

Methods

A 96-hour toxicity test on green algae (*Pseudokirchneriella subcapitata*) (3 replicates per test group, each containing 1.0 x 10^4 cells/mL at the start) was conducted with S-1812 TG at a nominal test concentration of 0.20 mg a.s./L, with untreated and solvent (dehydrogenated castor oil and DMF, 1:1) control.

Results

The measured concentration was 0.16 mg a.s./L at test initiation (representing 80% of nominal), and 0.13 mg a.s./L at the end of exposure (representing 65% of nominal). Endpoints were based on mean measured concentrations (0-96 hours: 0.14 mg a.s./L). The RMS based the 72-hour endpoints on the nominal concentration, since the initial concentration in this static test was 80%. Water quality parameters were in accordance with the OECD guideline 201. The results are summarised in Table 4.3.3-1.

Table 4.3.3-1 The acute toxicity of S-1812 TG (pyridalyl) to algae

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	EC50 in mg a.s./L	NOEC mg a.s./L
Pseudokirchneriella subcapitata (green alga)	Static 96 hours (93.7%)	80 (start) 65 (end)	72h-EbC50 & 72h-ErC50: >0.20 (A)	72h-NOEbC & NOErC: 0.20(A)

(A) Based on nominal concentrations, analytically confirmed for initial concentrations

Remarks

The test was performed at a concentration exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by a factor of 1333. The tested solution is therefore likely to have been an emulsion rather than a true solution, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. For classification of the active substance, the endpoint should be based on the concentration of dissolved pyridalyl. The reported endpoint can therefore not be used for classification of dissolved pyridalyl.

Conclusion

72-hour EbC50 and ErC50 >0.20 mg a.s./L, 72-hour NOEbC and NOErC: 0.20 mg a.s./L (tested as an emulsion in water).

Guidelines and limitations

See remarks.

Study 2

Characteristics

reference	:	IIA 8.4/02 Study No 0109EAI	species	:	*Selenastrum capricornutum*
type of study	:	Algal growth inhibition	exposure duration	:	72 hours
year of execution	:	2002	nominal conc.	:	0 and 10 mg a.s./L

GLP statement	:	Yes	dosing method	:	Static, stock in dehydrogenated castor
guideline	:	JMAFF Notification No. 12 NohSan 8147			oil and DMF (1:1)
			acceptability	:	Acceptable
test substance	:	S-1812 TG (pyridalyl) PS-98041G	72h-EbC50	:	>10 mg a.s./L (emulsion in water)
			72h-ErC50	:	>10 mg a.s./L (emulsion in water)
purity	:	93.7%	72h NOEbC	:	10 mg a.s./L (emulsion in water)
			72h NOErC	:	10 mg a.s./L (emulsion in water)

Methods

A 72-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 replicates per test group, each containing 1.0 x 10^4 cells/mL at the start) was conducted with S-1812 TG at a nominal test concentration of 10 mg a.s./L, with untreated and solvent (dehydrogenated castor oil and DMF, 1:1) control.

Results

The measured concentration was 9.3 mg a.s./L (representing 93% of nominal) at test initiation and at the end of exposure. Endpoints were based on nominal concentrations. Water quality parameters were in accordance with the OECD guideline 201. The results are summarised in Table 4.3.3-2.

Table 4.3.3-2 The acute toxicity of S-1812 TG (pyridalyl) to algae

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	EC50 in mg a.s./L	NOEC mg a.s./L
Selenastrum capricornutum (green alga)	Static 72 hours (93.7%)	93	72h-EbC50 & 72h-ErC50: >10(A)	72h-NOEbC & NOErC: 10(A)

(A) Based on nominal concentrations

Remarks

The test was performed at a concentration exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by a factor of 66667. The tested solution is therefore likely to have been an emulsion rather than a true solution, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted with acetonitrile. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. For classification of the active substance, the endpoint should be based on the concentration of dissolved pyridalyl. The reported endpoint can therefore not be used for classification of dissolved pyridalyl.

Conclusion

72-hour EbC50 and ErC50: >10 mg a.s./L, 72-hour NOEbC and NOErC: 10 mg a.s./L (tested as an emulsion in water).

Guidelines and limitations

See remarks.

Study 3

Characteristics

reference	:		species	:	*Anabaena flos-aquae*
type of study	:	Algal growth inhibition	exposure duration	:	96 hours

year of execution	:	2002	nominal conc.	:	0 and 0.20 mg a.s./L
GLP statement	:	Yes	dosing method	:	Static, stock in dehydrogenated castor oil and DMF (1:1)
guideline	:	OPPTS 850.5400			
test substance	:	S-1812 TG (pyridalyl)	acceptability	:	**Not acceptable**
		Lot AS 1817d (PS-98041G)	72h-EC50	:	>0.14 mg a.s./L (emulsion in water)
purity	:	93.7%	72h NOEC	:	0.14 mg a.s./L (emulsion in water)

Methods

A 96-hour toxicity test on blue-green algae (*Anabaena flos-aquae*) (3 replicates per test group, each containing 1.0 x 10^4 cells/mL at the start) was conducted with S-1812 TG at a nominal test concentration of 0.20 mg a.s./L, with untreated and solvent (dehydrogenated castor oil and DMF, 1:1) control.

Results

The measured concentration was 0.16 mg a.s./L at test initiation (representing 80% of nominal), and 0.13 mg a.s./L at the end of exposure (representing 65% of nominal). Endpoints were based on mean measured concentrations. Water quality parameters were in accordance with the OECD guideline 201.
The results are summarised in Table 4.3.3-3.

The reported endpoint (EC50 >0.14 mg a.s./L) was based on a percent reduction of cell density (biomass and growth rate were not calculated). Cell counts showed high variation between replicates, and after 24 and 48 hours of exposure, no cells were counted in several replicates. Table 4.3.3-4 shows the individual data. The author of the report stated: *"Cell counts in the initial 72 hours were somewhat variable. This is due to the filamentous nature of this species. At test termination, the solutions were rapidly forced through a pipette multiple times to disrupt the filaments and improve the dispersement of cells.*" This procedure did however not reduce the variability in cell counts and reliable EC50 values cannot be determined. The study is therefore not acceptable.

Table 4.3.3-3 The acute toxicity of S-1812 TG (pyridalyl) to algae

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	EC50 in mg a.s./L	NOEC mg a.s./L
Anabaena flos-aquae (blue-green alga)	Static 96 hours (93.7%)	80 (start) 65 (end)	72h-EC50: >0.14$^{(A)(B)}$	72h-NOEC: 0.14$^{(A)(B)}$

(A) Based on mean measured concentrations
(B) Based on cell densities

Table 4.3.3-4 Cell counts (x 10^4 cells/ml) per replicate in the toxicity test

Group	replicate	24 hours	48 hours	72 hours	96 hours
Control	1	0.00$^{(A)}$	8.25	47.0	93
	2	0.00$^{(A)}$	13.50	59.5	83
	3	0.00$^{(A)}$	0.00$^{(A)}$	23.3	110
solvent control	1	0.00$^{(A)}$	5.00	23.5	80
	2	0.00$^{(A)}$	0.00$^{(A)}$	25.3	122
	3	0.00$^{(A)}$	0.00$^{(A)}$	49.0	204
0.14 mg a.s./L	1	0.00$^{(A)}$	0.00$^{(A)}$	8.0	80
	2	0.00$^{(A)}$	54.25	21.0	85
	3	0.00$^{(A)}$	0.00$^{(A)}$	69.8	177

(A) Cells were observed on slide, but not within the hemacytometer field

Conclusion
72-hour EC50 >0.14 mg a.s./L, 72-hour NOEC 0.14 mg a.s./L (tested as an emulsion in water). The result is not acceptable.

Guidelines and limitations
The study is not acceptable.

Study 4

Characteristics

reference	:	IIA 8.4/04 SUW-0017	species	:	*Navicula pelliculosa*
type of study	:	Algal growth inhibition	exposure duration	:	96 hours
year of execution	:	2002	nominal conc.	:	0 and 0.20 mg a.s./L
GLP statement	:	Yes	dosing method	:	Static, stock in dehydrogenated castor
guideline	:	OECD 201 EEC C.3, OPPTS 850.5400	acceptability	:	oil and DMF (1:1) Acceptable
test substance	:	S-1812 TG (pyridalyl) Lot AS 1817d (PS-98041G)	72h-EbC50 72h-ErC50	: :	>0.20 mg a.s./L (emulsion in water) >0.20 mg a.s./L (emulsion in water)
purity	:	93.7%	72h NOEbC 72h NOErC	: :	0.20 mg a.s./L (emulsion in water) 0.20 mg a.s./L (emulsion in water)

Methods
A 96-hour toxicity test on freshwater diatoms (*Navicula pelliculosa*) (3 replicates per test group, each containing 1.0 x 10^4 cells/mL at the start) was conducted with S-1812 TG at a nominal test concentration of 0.20 mg a.s./L, with untreated and solvent (dehydrogenated castor oil and DMF, 1:1) control.

Results
The measured concentration was 0.18 mg a.s./L at test initiation (representing 90% of nominal), and 0.17 mg a.s./L at the end of exposure (representing 85% of nominal). Endpoints were based on mean measured concentrations (0-96 hours: 0.18 mg a.s./L). The RMS based the 72-hour endpoints on the nominal concentration, since the initial concentration in this static test was >80%. Water quality parameters were in accordance with the OECD guideline 201. The results are summarised in Table 4.3.3-5.

Table 4.3.3-5 The acute toxicity of S-1812 TG (pyridalyl) to freshwater diatoms

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	EC50 in mg a.s./L	NOEC mg a.s./L
Navicula pelliculosa (freshwater diatom)	Static 96 hours (93.7%)	90 (start) 85 (end)	72h-EbC50 & 72h-ErC50: >0.20(A)	72h-NOEbC & NOErC: 0.20(A)

(A) Based on nominal concentrations, analytically confirmed for initial concentrations

Remarks:
The test was performed at a concentration exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by a factor of 1333. The tested solution is therefore likely to have been an emulsion rather than a true solution, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual

dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl.

Conclusion

72-hour EbC50 and ErC50 >0.20 mg a.s./L, 72-hour NOEbC and NOErC: 0.20 mg a.s./L (tested as an emulsion in water).

Guidelines and limitations:

See remarks.

Study 5

Characteristics

reference	:	IIA 8.11.1/04 Study No. 12709.6205	species	:	*Skeletonema costatum*
type of study	:	Algal growth inhibition	exposure duration	:	96 hours
year of execution	:	2002	nominal conc.	:	0 and 0.20 mg a.s./L
GLP statement	:	Yes	dosing method	:	Static, stock in dehydrogenated castor oil and DMF (1:1)
guideline	:	OPPTS 850.5400			
test substance	:	S-1812 TG (pyridalyl) Lot AS 1817d (PS-98041G)	acceptability 72h-EbC50	: :	Acceptable >0.15 mg a.s./L (emulsion in water)
purity	:	93.7%	72h-ErC50 72h NOEbC 72h NOErC	: : :	>0.15 mg a.s./L (emulsion in water) 0.15 mg a.s./L (emulsion in water) 0.15 mg a.s./L (emulsion in water)

Methods

A 96-hour toxicity test on marine diatoms (*Skeletonema costatum*) (3 replicates per test group, each containing 7.7 x 10^4 cells/mL at the start) was conducted under static conditions with S-1812 TG at a nominal test concentration of 0.20 mg a.s./L, with untreated and solvent (dehydrogenated castor oil and DMF, 1:1) control.

Results

The measured concentration was 0.15 mg a.s./L at test initiation (representing 73% of nominal), and 0.094 mg a.s./L at the end of exposure (representing 47% of nominal). Reported endpoints were based on the mean measured concentration (0-96 hours: 0.12 mg a.s./L). The RMS based the 72-hour endpoints on the initial measured concentration, since the initial concentration in this static test was <80%. Water quality parameters were in accordance with the OECD guideline 201. The results are summarised in Table 4.3.3-6.

Table 4.3.3-6 The acute toxicity of S-1812 TG (pyridalyl) to marine diatoms

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	EC50 in mg a.s./L	NOEC mg a.s./L
Skeletonema costatum (marine diatom)	Static 96 hours (93.7%)	73 (start) 47 (end)	72h-EbC50 & 72h-ErC50: >0.15(A)	72h-NOEbC & NOErC: 0.15(A)

(A) Based on initial measured concentrations

Remarks

The test was performed at a concentration exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by a factor of 1333. The tested solution is therefore likely to have been an emulsion rather than a true

solution, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. For classification of the active substance, the endpoint should be based on the concentration of dissolved pyridalyl. The reported endpoint can therefore not be used for classification of dissolved pyridalyl.

Conclusion
72-hour EbC50 and ErC50 >0.15 mg a.s./L, 72-hour NOEbC and NOErC: 0.15 mg a.s./L (tested as an emulsion in water).

Guidelines and limitations
OECD 201 states that the biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. In the present study, the average growth factor for the control was only 9.1. Since the tested species is known to be a slow grower, the study result is accepted.

4.3.4 *Lemna* sp. growth inhibition test

Study 1

Characteristics

reference	:	IIA 8.6/01 Study No. 12709.6208	species	:	Duckweed (*Lemna gibba*)
type of study	:	Duckweed growth inhibition test	exposure duration	:	7 days
year of execution	:	2002	nominal conc.	:	0 and 0.20 mg a.s./L
GLP statement	:	Yes	dosing method	:	Semi-static; stock solution in hydrogenated castor oil and DMF (1:1)
guideline	:	OPPTS 850.4400			
test substance	:	S-1812 TG (pyridalyl), Lot AS 1817d (PS-98041G)	acceptability	:	Acceptable
purity	:	93.7%	NOErC	:	0.17 mg a.s./L (emulsion in water)
water solubility	:	0.15 µg/L at 20°C	ErC50	:	>0.17 mg a.s./L (emulsion in water)

Methods
A 7-day toxicity test on the growth of duckweed (*Lemna gibba*) (3 replicates per concentration, each containing five plants with three fronds each) was conducted with S-1812 TG (pyridalyl) at a nominal test concentration of 0.20 mg a.s./L, with untreated control and solvent (dehydrogenated castor oil and DMF, 1:1) control. Test solutions were renewed every two days. Samples for analytical confirmation of the test concentration were taken from the fresh solution at the start of the test and from a 2-days aged solution at the end of the test.

Results
Measured concentrations were 0.18 mg a.s./L at test initiation (92% of nominal) and 0.16 mg a.s./L (78% of nominal) at the end of the test. Water quality parameters (light intensity, pH and temperature) were in accordance with the OECD 221 guideline.

Endpoints were based on mean measured concentrations, calculated from the concentration in the fresh solution prepared at the start of the test (first renewal interval) and the concentration in the aged solution at the end of the test (third renewal interval). No evidence was provided for the maintenance of the test concentration during the renewal intervals, or for the repeatability of the preparation of the fresh solution. In

algal growth inhibition tests (see section B.9.2.4), measured concentrations of S-1812, incubated under test conditions in static systems for 72-96 hours, were relatively stable in time (studies 1 and 3: 80% and 65% of nominal after 0 and 96 hours, respectively; study 2: 93% of nominal after 0 and 72 hours; study 4: 90% and 85% of nominal after 0 and 96 hours, respectively). Therefore, it is not considered likely that the measured concentration decreased by more than 20% during the renewal periods in the current duckweed test, and the measured concentrations are considered to be representative for all renewal intervals. The result is accepted.

The reported 7-day EC- and NOEC-values were based on frond density and growth rate (calculated from frond density). This is in agreement with OPPTS 850.4400, but not with the recommendations in OECD 221 (2006). According to the latter guideline, in addition to frond density, biomass should be measured at the end of the test (i.e. dry or wet weight, or total frond area). This latter parameter should then be used to determine the yield and corresponding EC- and NOEC values. The test was performed before the OECD guideline was published. Furthermore, no effects were observed on frond number or frond development (frond appearance). Therefore, the test is accepted.

The results are summarised in Table 4.3.4-1.

Table 4.3.4-1 The toxicity of S-1812 TG (pyridalyl) to aquatic plants

Species	Test type and duration. (purity of test substance)	Actual concentration (as % of nominal)	EC50 in mg a.s./L	NOEC in mg a.s./L
Lemna gibba (duckweed)	Semi-static 7 days (93.7%)	92 (start) 78 (end)	7d-E_rC_{50}: >0.17(A)	7d-NOE_rC: 0.17(A)

(A) Based on mean measured concentrations.

Conclusion

The 7-day ErC50 is >0.17 mg a.s./L; 7-day NOErC: 0.17 mg a.s./L (tested as an emulsion in water).

Guidelines and limitations

The test was performed at a concentration exceeding the water solubility of pyridalyl (0.15 µg/L at 20°C) by a factor of 1333. The tested solution is therefore likely to have been an emulsion rather than a true solution, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. The test is acceptable.

4.4 Chronic toxicity

4.4.1 Fish early-life stage (FELS) toxicity test

Characteristics

reference	:	IIA 8.2.4/01 Study No. 13048.6220	species	:	Rainbow trout (*Oncorhynchus mykiss*)
type of study	:	Chronic toxicity study (ELS)	exposure duration	:	89 days (60 days post-hatch)
year of execution	:	2001	nominal conc.	:	0, 6.3, 13, 25, 50 and 100 µg a.s./L
GLP statement	:	Yes	dosing method	:	Flow-through; stock solution in hydrogenated castor oil and DMF (1:1)
guideline	:	EPA 72-4, OECD 210, OPPTS 850.1400			
test substance	:	S-1812 (pyridalyl), Lot PS-98041G	acceptability	:	Acceptable
			NOEC	:	24 µg a.s./L (emulsion in water)
purity	:	93.7%			
water solubility	:	0.15 µg/L at 20°C			

Methods

A 89-day fish early life stage flow-through study was undertaken with rainbow trout (*Oncorhynchus mykiss*). Newly fertilised eggs (1 hour post fertilisation, two replicates/concentration, 2 incubation cups/replicate, 50 eggs/incubation cup) were exposed to pyridalyl (93.7% pure) at nominal concentrations of 6.3, 13, 25, 50 and 100 µg a.s./L plus control and solvent control (hydrogenated castor oil and DMF, 1:1). In the flow-through system, the stock solution in hydrogenated castor oil and DMF was delivered into a mixing chamber, which also received dilution water and was placed in an ultrasonic water bath where the contents were continuously sonicated and stirred to aid in the solubilisation of S-1812. The concentration of S-1812 in the solution in the mixing chamber was equivalent to the highest nominal test concentration (100 µg a.s./L) and was subsequently diluted (50% dilution factor) to provide the remaining nominal test concentrations (6.3, 13, 25, 50 and 100 µg a.s./L). A similar system was used for the delivery of solvent to the test solutions and the solvent control (final solvent concentration in all solutions 0.002 mL/L). On test day 19, ten live, viable embryos were impartially selected from each of the two incubation cups (20 embryos per replicate) and used to initiate the post-hatch phase of the exposure. The remaining viable embryos were maintained until test day 32 for assessment of hatching and survival. The total duration of the exposure period was 89 days. Samples for analytical confirmation of test concentrations were taken at least once weekly from each replicate. Behaviour and appearance of larvae was observed daily and survival was estimated at least twice weekly. Fish length, wet weight and dry weight were measured at 60 days post-hatch.

Results

Mean measured concentrations were 6.9, 12, 24, 49 and 96 µg a.s./L, representing 95 to 110% of nominal. Water quality parameters were according to the OECD 210 guideline.

Embryo viability and larval survival at hatch were not significantly affected in any of the test concentrations when compared to the pooled control group. Larvae began to exhibit signs of swim-up development and behaviour on day 39 (10 days post-hatch, details per group not reported). By day 46 (17 days post-hatch), all surviving larvae in the test solutions and the controls reached the swim-up stage. At the end of the test, survival was significantly reduced at the highest test concentration. Mean total length was not affected up to 49 µg a.s./L, but mean wet weight and dry weight were significantly reduced at this concentration. Results are summarised in the table below.

Table 4.4.1-01 Embryo viability, survival, fish length, wet weight and dry weight of rainbow trout exposed to pyridalyl

Mean measured concentration	Embryo viability (%)	Survival at hatch (%)	Larval survival (%)	Fish length (mm)	Wet weight (g)	Dry weight (g)
control	72	99	95	63.2	2.3	0.56
solvent-control	73	100	98	62.5	2.3	0.54
pooled control	73	100	96	62.9	2.3	0.55

6.9	75	99	98	62.5	2.3	0.54
12	75	99	93	63.1	2.3	0.56
24	76	99	98	62.2	2.2	0.53
49	77	96	98	61.9	2.1 *	0.51 *
96	74	99	83 *	61.5(A)	2.2 (A)	0.51 (A)

* Significantly different from pooled control at 5% level
(A) Data was excluded from statistical analysis due to a significant effect on larval survival

Conclusion
Based on reduced body weight, the NOEC was 24 µg a.s./L (tested as an emulsion in water).

Guidelines and limitations
The test was performed at concentrations exceeding the water solubility of pyridalyl. The author of the report stated: *As the water solubility study of S-1812 was extremely low (less than 1 ppb), the Study Sponsor concluded that it should be optimised by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). The use of the 1:1 DMF:HCO-40 mixture yielded a water solubility approximating 30 mg/L. The Study Sponsor provided the details of this information to U.S. EPA, which subsequently approved the use of DMF:HCO-40 in this toxicity test.* The details were however not provided in the report of the present study. The test was performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by at least a factor of 42.
The tested solutions, especially at the higher test concentrations, are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. The dissolved fraction of pyridalyl in lower nominal concentrations may have been even lower than that in the highest test concentration, since these concentrations were prepared by dilution from the highest test concentration. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl.

4.4.2 Chronic toxicity to aquatic invertebrates

Study 1

Characteristics

reference	:	IIA 8.3.2.1/01 Study N0. 13048.6221	species	:	*Daphnia magna*
type of study	:	Chronic toxicity study	exposure duration	:	21 days
year of execution	:	2000	nominal conc.	:	0, 0.98, 2.0, 3.9, 7.8 and 16 µg a.s./L
GLP statement	:	Yes			
guideline	:	FIFRA 72-4, OECD 211 and OPPTS 850.1300	dosing method	:	Flow-through, stock in DMF and hydrogenated castor oil (1:1)
test substance	:	S-1812 (pyridalyl), Lot PS-98041G	acceptability	:	Acceptable
purity	:	93.7%	NOEC	:	1.4 µg a.s./L (emulsion in water)

Methods
The chronic toxicity of S-1812 (pyridalyl) to *Daphnia magna* was assessed in a 21-day flow-through study. First instar daphnids (≤24 hours old, 40 per treatment, 10 daphnia per replicate vessel) were used to initiate the study. The nominal concentrations were 0.98, 2.0, 3.9, 7.8 and 16 µg a.s./L plus an untreated and a solvent control (hydrogenated castor oil and DMF, 1:1). In the flow-through system, the stock solution in

hydrogenated castor oil and DMF was delivered into a mixing chamber, which also received dilution water and was placed in an ultrasonic water bath where the contents were continuously stirred to aid in the solubilisation of S-1812. The concentration of S-1812 in the solution in the mixing chamber was equivalent to the highest nominal test concentration (16 µg a.s./L) and was subsequently diluted (50% dilution factor) to provide the remaining nominal test concentrations (0.98, 2.0, 3.9 and 7.8 µg a.s./L). A similar system was used for the solvent control (solvent concentration 1.0 µL/L, equivalent to the concentration of solvent in the highest test concentration).

Results

Mean measured concentrations were 0.93, 1.4, 2.7, 5.7 and 11 µg a.s./L (67-95% of nominal concentrations). Water quality parameters were in accordance with the OECD 211 guideline.

Survival of adult daphnia was statistically significantly reduced at and above 2.7 µg a.s./L, Concentrations of 2.7 µg a.s./L and above were therefore excluded from statistical analysis of other parameters, which, based on data from a limited number of survivors at these concentrations, appeared to be unaffected by treatment. Reproduction, as measured by the cumulative number of offspring per female daphnid, and time to first brood were not affected up to 1.4 µg a.s./L. Mean total body length after 21 days was significantly reduced at 0.93 and 1.4 µg a.s./L, but a clear dose response was not observed. Furthermore, body length was not affected in the preliminary test preceding the reported test (mean body length in preliminary test: 4.7, 5.0, 4.8, 4.8, 4.9, 4.9 and 4.8 mm for the control, solvent control, at 0.13, 0.25, 0.5, 1.0 and 2.0 µg a.s./L, respectively). In addition, mean body dry weight was not affected at any concentration. Therefore, the apparent effect on body length was considered an anomaly and not biologically relevant, and the NOEC for growth may be set at ≥1.4 µg a.s./L.

The NOEC for parental survival was 1.4 µg a.s./L, and the NOEC for reproduction was 11 µg a.s./L. The results are summarised in the Table below.

Table 4.4.3-01 Adult survival, reproduction, mean body length and mean body weight of *Daphnia magna* after exposure to S-1812 (pyridalyl)

Concentration (µg a.s./L)	Adult survival (%)	Reproduction(A)	Mean body length (mm)	Mean dry weight (mg)
control	100	82	4.6	0.68
solvent control	98	86	4.6	0.71
0.93	95	78	4.4*	0.59
1.4	93	83	4.4*	0.65
2.7	78*	86 (B)	4.4(B)	0.67(B)
5.7	38*	80(B)	4.5 (B)	0.57(B)
11	5*	96(B)	4.8(B)	0.82(B)

* Statistically significant at 5% level

(A) Cumulative number of offspring per female daphnid

(B) Not included in statistical analysis due to adverse effect on survival

Conclusion

The NOEC for parental survival, reproduction and growth was 1.4 µg a.s./L (tested as an emulsion in water).

Guidelines and limitations

The test was performed at concentrations exceeding the water solubility of pyridalyl. The author of the report stated: *As the water solubility study of S-1812 was extremely low (less than 1 ppb), the Study Sponsor concluded that it should be optimised by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). The use of the 1:1 DMF:HCO-40*

mixture yielded a water solubility approximating 30 mg/L. The Study Sponsor provided the details of this information to U.S. EPA, which subsequently approved the use of DMF:HCO-40 in this toxicity test. The details were however not provided in the report of the present study. The test was performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by at least a factor of 7. The tested solutions, especially at the higher test concentrations, are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. The dissolved fraction of pyridalyl in lower nominal concentrations may have been even lower than that in the highest test concentration, since these concentrations were prepared by dilution from the highest test concentration. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl.

Study 2

Characteristics

reference	:	IIA 8.5.2/05 Study No. 13048.6401	species	:	*Chironomus riparius*
type of study	:	Chronic toxicity study	exposure duration	:	28 days
year of execution	:	2003	nominal conc.	:	0, 0.38, 0.75, 1.5, 3.0, 6.0, 12 and 24 µg a.s./L
GLP statement	:	Yes			
guideline	:	OECD 219	dosing method	:	Static; stock solution in hydrogenated castor oil and DMF (1:1)
test substance	:	a) S-1812 (pyridalyl), Lot PS-98041G b) [dichlorophenyl-^{14}C]S-1812, Lot RIS2003-003	acceptability NOEC	: :	Acceptable 12 µg a.s./L (emulsion in water)
purity	:	a) 93.7% b) radiochemical purity 99.2%			
water solubility	:	0.15 µg/L at 20°C			

Methods

The chronic toxicity of radiolabelled pyridalyl to *Chironomus riparius* (1 day old, 1st instar larvae) was assessed in a 28-day water/sediment system under static conditions. Nominal test concentrations were 0.38, 0.75, 1.5, 3.0, 6.0, 12 and 24 µg a.s./L, with untreated and solvent control. The test was performed in 0.6 L glass beakers, with four replicates per treatment each containing 20 midge larvae and approximately 1.5 cm of sediment (artificial OECD 219 soil) and approximately 6 cm of overlying water. Additional replicates (with midges) were set up for analytical measurements on overlying water, sediment and pore water on days 0, 7 and 28. Midge larvae were added to the test system one day prior to the addition of test substance. Treatment was performed by spiking the water with an aliquot (1.0 mL) of a solution of the test substance in test water containing solvent (hydrogenated castor oil and DMF, 1:1). The final solvent concentration in all test solutions was 0.5 µL/L. Water samples were analysed by LSC. In addition, samples of overlying water of the highest concentration were extracted with methylene chloride and analysed by HPLC. Sediment samples were extracted with acidified acetonitrile, and extracts were analysed by LSC and those of the highest concentration by HPLC.

Results

Analytical results are presented in Table 4.4.3-02 below. S-1812 was the only compound identified in the overlying water. S-1812 was also the main compound in sediment, but S-1812-DP was additionally identified (16.3% AR on day 28).

Table 4.4.3-02 Measured radioactivity and pyridalyl concentrations in overlying water, sediment and pore water

Nominal conc. (µg a.s./L)	Overlying water			Pore water			Sediment		
	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28
	TRR (µg eq./L)								
0.38	0.52	0.10	0.051	<LOQ(B)	0.24	0.048	0.048	1.2	1.3
0.75	0.93	0.19	0.12	<LOQ(B)	0.55	0.13	0.11	2.3	2.3
1.5	1.8	0.25	0.18	<LOQ(B)	1.0	0.20	0.20	3.6	2.6
3.0	2.2	0.32	0.27	<LOQ(B)	1.3	0.31	0.34	6.2	5.7
6.0	7.3	0.93	1.0	<LOQ(B)	6.9	0.95	0.87	14	17
12	12	1.6	1.3	<LOQ(B)	9.0	1.4	1.2	26	27
24	25	3.2	2.6	<LOQ(B)	13	2.5	2.8	35	48
	Pyridalyl (µg a.s./L)								
24	25	2.7	<LOQ(A)	-	-	-	2.8	33	37

(A) LOQ = 0.0307 µg a.s./L
(B) LOQ = 0.0384 µg eq./L

Water quality parameters were in accordance with the OECD 219 guideline.

The only effect was a decrease of 11% in emergence rate at the highest concentration compared to the pooled controls (statistically significant). Mean development rate was not affected at any concentration. Effect concentrations were based on nominal concentrations, which is acceptable. The NOEC for emergence was 12 µg a.s./L, while the NOEC for development rate was 24 µg a.s./L.

The biological results are summarised in the table below.

Table 4.4.3-03 Measured initial concentrations in overlying water and effects of pyridalyl on emergence and development rate of *Chironomus riparius*

Measured initial conc. (µg eq./L)	Emergence (%)	Mean development rate		
		Male	Female	Male/female
Control	96	0.0653	0.0591	0.0619
Solvent control	90	0.0662	0.0581	0.0621
Pooled control	93	0.0657	0.0586	0.0620
0.52	94	0.0629	0.0561	0.0597
0.93	95	0.0660	0.0582	0.0625
1.8	96	0.0674	0.0574	0.0607
2.2	94	0.0670	0.0589	0.0631
7.3	94	0.0638	0.0599	0.0622
12	88	0.0666	0.0587	0.0621
25(A)	84*	0.0646	0.0587	0.0620

* Significantly different from control at 5% level
(A) S-1812 accounted for 100% AR; RA distribution not determined at lower concentrations

Conclusion

The overall NOEC was 12 µg a.s./L. This effect value was determined for an emulsion in water.

Guidelines and limitations

The test is acceptable.

Study 3

Characteristics

reference	:	IIA 8.11.1/05 Study No. 12709.6202	species	:	Mysid (*Americamysis bahia*)
type of study	:	Chronic toxicity study	exposure duration	:	28 days
year of execution	:	2002	nominal conc.	:	0, 0.063, 0.13, 0.25, 0.50 and 1.0 µg a.s./L
GLP statement	:	Yes			
guideline	:	FIFRA 72-4	dosing method	:	Flow-through; stock solution in hydrogenated castor oil and DMF (1:1)
test substance	:	S-1812 TG (pyridalyl), Lot AS 1817d (PS-98041G)			
purity	:	93.7%	acceptability	:	Acceptable
water solubility	:	0.15 µg/L at 20°C	NOEC	:	0.45 µg a.s./L (emulsion in water)

Methods

The chronic toxicity of S-1812 TG (pyridalyl) to the marine shrimp *Americamysis bahia* was assessed in a 28-day flow-through study. Mysids (≤24 hours old, 60 per treatment, 30 mysids per replicate vessel) were exposed to nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 µg a.s./L plus an untreated and a solvent-control (hydrogenated castor oil and DMF, 1:1). In the flow-through system, the stock solution in hydrogenated castor oil and DMF was delivered into a mixing chamber, which also received dilution water (artificial seawater) and was placed in an ultrasonic water bath where the contents were continuously stirred. The concentration of S-1812 in the solution in the mixing chamber was equivalent to the highest nominal test concentration (1.0 µg a.s./L) and was subsequently diluted (50% dilution factor) to provide the remaining nominal test concentrations (0.063, 0.13, 0.25 and 0.50 µg a.s./L). A similar system was used for the solvent control (solvent concentration 2.0 µL/L, equivalent to the concentration of solvent in the highest test concentration).

On day 13, males and females were paired and redistributed into glass pairing jars (5 pairs from each replicate, one pair per jar). The remaining mysids were pooled and placed in one of the initial retention chambers until study end. Survival and sub-lethal effects were assessed during the first 12 days of the study, reproduction and mortality of males and females were assessed after pairing (day 13) and body length and dry weight were assessed at the end of the test.

Results

Measured concentrations were 84-120% of nominal concentrations throughout the test period. Water quality parameters were pH (8.0-8.3), oxygen concentration (6.1-7.7 mg/L), temperature (24-26°C) and salinity (23-27‰). Endpoints were based on mean measured concentrations. The solutions were reported to be free of visible signs of undissolved test substance.

Results are summarised in Table 4.4.3-04. Based on significant effects on body length (males) and body weight (males and females) at 0.90 µg a.s./L, the NOEC was 0.45 µg a.s./L.

Table 4.4.3-04 Survival, body length, body weight and reproductive success of *Americamysis bahia* exposed to pyridalyl

Mean measured concentration (µg a.s./L)	survival %	Body length		Body weight		reproduction	
		♂	♀	♂	♀	Offspring/female/ reproductive day	% deviation from control[(A)]
Untreated control	77	7.5	7.5	0.88	1.0	1.11	-
Solvent control	77	7.6	7.6	0.84	1.0	0.92	-
Pooled control	77	7.6	7.6	0.86	1.0	NA[(B)]	-
0.066	87	7.7	7.7	0.87	1.1	1.29	+40
0.12	72	7.7	7.7	0.85	1.0	1.07	+16

0.24	78	7.8	7.6	0.88	1.1	1.13	+23
0.45	78	8.0	7.7	0.86	0.98	1.11	+21
0.90	78	7.4*	7.3	0.72*	0.86*	0.81	-12

* Significantly different from pooled control at 5% level
(A) Deviation from solvent control, calculated by RMS
(B) Not applicable: significant difference between untreated and solvent control

Conclusion

The overall NOEC for mysid mortality, reproduction and growth was 0.45 µg a.s./L (tested as an emulsion in water).

Guidelines and limitations

The highest three test concentrations exceeded the water solubility of pyridalyl (0.15 µg/litre at 20˚C). The nominal test concentration at the level of the NOEC was three times higher than the water solubility limit of pyridalyl. At this test concentration, the tested solution is therefore likely to have been an emulsion rather than a true solution, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl.

4.4.3 Chronic toxicity to algae or aquatic plants

See acute studies.

References

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IIA, 2.2/01	2002	Determination of density of S-1812 pure active ingredient. Koei Techno Services Co., Ltd., Study No. P01001. Sumitomo Chemical Co., Ltd. Report No. SUP-0021 GLP, Unpublished.
IIA, 2.2/02	2002	Physical and chemical properties of pyridalyl technical. Valent USA Corporation, Project Identification No. V-01-24264. Sumitomo Chemical Co., Ltd. Report No. SUP-0010 GLP, Unpublished
IIA, 2.3.1/01	2000	S-1812/V-1812 - Water solubility, vapor pressure, and octanol/water partition coefficient. Ricerca, LLC, Document No. 4067-98-0068-AS-001. Sumitomo Chemical Co., Ltd. Report No. SUP-0002 GLP, Unpublished
IIA, 2.4.1/01	2002a	Determination of physical state of pyridalyl. Sumika Chemical Analysis Service Ltd.), Study No. FIG01001. Sumitomo Chemical Co., Ltd. Report No. SUP-0015 GLP, Unpublished.
IIA, 2.9.1/01	2002	Hydrolysis of [^{14}C]S-1812 at pH 5, 7 and 9. Valent USA Corporation, Project Identification No. VP-22605 Sumitomo Chemical Co., Ltd. Report No. SUM-0015 GLP, Unpublished
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IIA, 5.2.2 / 01	1999b	Acute dermal toxicity of S-1812 technical in rats. Covance Laboratories, Project ID. 6311-218. Sumitomo Chemical Co., Ltd. Report No. SUT-0009 GLP, Unpublished.
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IIA, 5.6.10 / 02	2001c	Teratogenicity study in rats with S-1812. The Institute of Environmental Toxicology, Project ID. IET 00-0095. Sumitomo Chemical Co., Ltd..:SUT-0023 GLP, Unpublished.
IIA, 5.6.11 / 01	2001a	Teratogenicity study in rabbits with S-1812 preliminary Study. The Institute of Environmental Toxicology, Project ID. IET 00-0096. Sumitomo Chemical Co., Ltd. SUT-0022 GLP, Unpublished.
IIA, 5.6.11 / 02	2001b	Teratogenicity study in rabbits with S-1812. The Institute of Environmental Toxicology, Project ID. IET 00-0097. Sumitomo Chemical Co., Ltd. Report No. SUT-0024 GLP, Unpublished.

Annex point/ reference number	**Year**	**Title, Source, Company, Report No,** **GLP or GEP status (where relevant),** **Published or not**
IIA, 7.1.1/01, 7.2.1/01	2004a	[^{14}C]-Pyridalyl: Aerobic soil metabolism and degradation. Covance Laboratories Ltd, Report No. 0333/211-D2149. Sumitomo Chemical Co., Ltd. SUM-0043 GLP, Unpublished.
IIA, 7.1.1/02	2002a	Metabolism of [Propenyl-2-^{14}C]S-1812, [Pyridyl-2,6-^{14}C]S-1812 and [Dichlorophenyl-^{14}C] in aerobic soil. Valent, Project Identification No. 12152. GLP, Unpublished.
IIA 7.1.1/02	2012a	Further information on Unk-1, a metabolite present in a laboratory soil degradation study (Assaf, 2002) Sumitomo Chemical Co., Ltd. SUM-0073 Statement, Unpublished
IIA, 7.3.1/01, 7.3.3/01	2006a	Pyridalyl: The dissipation and accumulation of residues in soil in southern Europe (Final Report) Covance Laboratories Ltd, Report No. 0333/210-D2149. Sumitomo Chemical Co., Ltd. SUR-0115 GLP, Unpublished.
IIA, 7.4.1/01	2001	OPPTS 835.1220: Sediment and Soil adsorption/desorption isotherm. Valent, Project Identification No. VP-12140. Sumitomo Chemical Co., Ltd. SUM-0006 GLP, Unpublished.
IIA, 7.5/01	2002	Hydrolysis of [^{14}C]S-1812 at pH 5,7,and 9 Valent, Report No. VP-22605. Sumitomo Chemical Co., Ltd. SUM-0015 GLP, Unpublished (See IIA 2.9.1/01) No data is required for metabolites.
IIA, 7.6/01	2002a	Degradation study: Photodegradation of [^{14}C]S-1812 in sterilized buffer at pH 7 and synthetic humic water buffered at pH 7 by artificial light. PTRL West, Report No. 885W-2 Sumitomo Chemical Co., Ltd. SUM-0030 GLP, Unpublished. (See IIA 2.9.2/01)

Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not
IIA, 7.7/01	2003	Ready biodegradability of pyridalyl in a manometric respirometry test RCC Study No. 850273 Sumitomo Chemical Co., Ltd. SUM-0038 GLP, Unpublished.
IIA, 7.8.3/01	2004e	[^{14}C]-Pyridalyl: Aerobic degradation and retention in water-sediment systems. Covance Laboratories Ltd, Report No. 0333/212-D2149. Sumitomo Chemical Co., Ltd. SUM-0048 GLP, Unpublished.
IIA, 7.8.3/02	2003	[^{14}C]-Dichlorophenyl S-1812 formulated as 35 WP (350 g/kg): Environmental behaviour in outdoor natural water-sediment systems following realistic spray-drift and run-off entry into surface water. Springborn Smithers Labs. Study # 1043.014.310. Sumitomo Chemical Co., Ltd. SUM-0036 GLP, Unpublished.

Annex Point	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or unpublished
IIA, 8.2.1.1 /01	2000a	S-1812 - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. Springborn Laboratories Inc, Study No. 13048.6206. Sumitomo Chemical Co., Ltd. Report No. SUW-0007 GLP, Unpublished.
IIA, 8.2.1.2 /01	2000b	S-1812 - Acute toxicity to bluegill sunfish (*Lepomis macrochirus*) under flow-through conditions. Springborn Laboratories Inc, Study No. 13048.6207. Sumitomo Chemical Co., Ltd. Report No. SUW-0006 GLP, Unpublished.
IIA, 8.2.4 /01	2001	S-1812 - Early life-stage toxicity test with rainbow trout (*Oncorhynchus mykiss*). Springborn Laboratories Inc, Study No. 13048.6220. Sumitomo Chemical Co., Ltd. Report No. SUW-0010 GLP, Unpublished.
IIA, 8.2.6.1 /01	2002	Bioconcentration of [^{14}C]S-1812 by bluegill sunfish (*Lepomis macrochirus*). Ricerca, Document No. 013648-1. Sumitomo Chemical Co., Ltd. Report No. SUM-0011 GLP, Unpublished
IIA, 8.2.7 /02	2004	S-1812 - Bioconcentration and depuration study with oligochaetes (*Lumbriculus variegatus*). Springborn Smithers Laboratories Inc, Study No. 13048.6408. Sumitomo Chemical Co., Ltd. Report No. SUM-0041 GLP, Unpublished.
IIA, 8.3.1.1 /01	2000	S-1812 - Acute toxicity to water fleas (*Daphnia magna*) under flow-through conditions. Springborn Laboratories Inc, Study No. 13048.6208. Sumitomo Chemical Co., Ltd. Report No. SUW-0005 GLP, Unpublished.

Annex Point	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or unpublished**
IIA, 8.3.1.1 /02	2006b	S-1812: Acute immobilisation test with daphnids (*Daphnia magna*) in natural water-sediment system under static conditions. Springborn Smithers Laboratories (Europe), AG, Study No. 1043.046.110. Sumitomo Chemical Co., Ltd. Report No. SUW-0054 GLP, Unpublished.
IIA, 8.3.2.1 /01	2001	S-1812 - Full life-cycle toxicity test with water fleas, *Daphnia magna* under flow-through conditions. Springborn Smithers Laboratories Inc, Study No. 13048.6221. Sumitomo Chemical Co., Ltd. Report No. SUW-0008 GLP, Unpublished.
IIA, 8.4 /01	2002a	S-1812 TG - Toxicity to the freshwater green alga, *Pseudokirchneriella subcapitata*. Springborn Smithers Laboratories Inc, Study No. 12709.6207. Sumitomo Chemical Co., Ltd. Study No. SUW-0014 GLP, Unpublished.
IIA, 8.4 /02	2002	Growth inhibition test of S-1812 Technical Grade to algae (*Selenastrum capricornutum*). Sumika Technoservice Corporation, Study No. 0109EAI Sumitomo Chemical Co., Ltd. Report No. SUW-0024 GLP, Unpublished
IIA, 8.4 /04	2002c	S-1812 TG - Toxicity to the freshwater diatom, *Navicula pelliculosa*. Springborn Smithers Laboratories Inc, Study No. 12709.6206. Sumitomo Chemical Co., Ltd. Report No. SUW-0017 GLP, Unpublished.
IIA, 8.5.1 /01	2005	Acute toxicity test and determination of toxic body burden of [dichlorophenyl-^{14}C]S-1812 with midge (*Chironomus yoshimatsui*) larvae in static water. Sumitomo Chemical Co. Ltd., Study No. F-04050 Sumitomo Chemical Co., Ltd. Report No. SUW-0045 Non-GLP, Unpublished.
IIA, 8.5.2 /05	2003b	S-1812 - Sediment-water Chironomid toxicity test using spiked water. Springborn Smithers Laboratories Inc, Study No. 13048.6401. Sumitomo Chemical Co., Ltd. Report No. SUW-0029 GLP, Unpublished.
IIA, 8.6 /01	2002d	S-1812 TG - Toxicity to duckweed, *Lemna gibba*. Springborn Smithers Laboratories Inc, Study No. 12709.6208. Sumitomo Chemical Co., Ltd. Report No. SUW-0016 GLP, Unpublished.
IIA, 8.11.1 /01	2001	S-1812 - Acute toxicity to sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions. Springborn Laboratories Inc, Study No. 12709.6200. Sumitomo Chemical Co., Ltd. Report No. SUW-0011 GLP, Unpublished.

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IIA, 8.11.1 /02	2002a	S-1812 - Acute toxicity to mysids (*Americamysis bahia*) under flow-through conditions. Springborn Laboratories Inc, Study No. 12709.6198. Sumitomo Chemical Co., Ltd. Report No. SUW-0013 GLP, Unpublished.
IIA, 8.11.1 /03	2002	S-1812 - Acute toxicity to eastern oysters (*Crassostrea virginica*) under flow-through conditions. Springborn Laboratories Inc, Study No. 12709.6199. Sumitomo Chemical Co., Ltd. Report No. SUW-0012 GLP, Unpublished.
IIA, 8.11.1 /04	2002e	S-1812 TG - 96-hour toxicity test with the marine diatom, *Skeletonema costatum*. Springborn Smithers Laboratories Inc, Study No. 12709.6205. Sumitomo Chemical Co., Ltd. Report No. SUW-0021 GLP, Unpublished.
IIA, 8.11.1 /05	2002b	S-1812 TG - Life-cycle toxicity test with mysids (*Americamysis bahia*). Springborn Smithers Laboratories Inc, Study No. 12709.6202. Sumitomo Chemical Co., Ltd. Report No. SUW-0018 GLP, Unpublished.