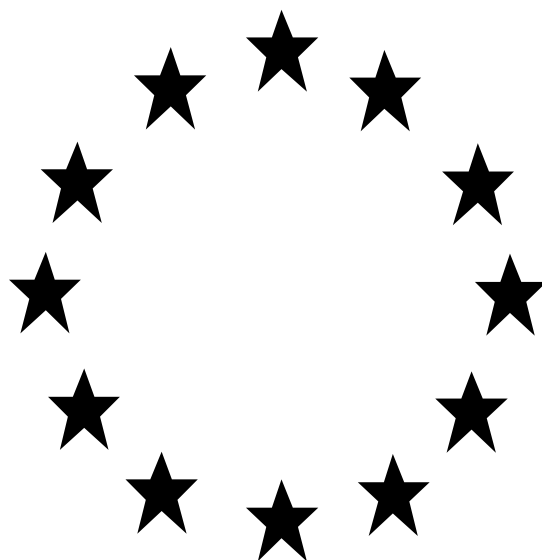




Draft Assessment Report (DAR)

- public version -

**Initial risk assessment provided by the rapporteur Member State
The Netherlands for the existing active substance**

PYRIDABEN

**of the third stage (part B) of the review programme
referred to in Article 8(2) of Council Directive 91/414/EEC**

Volume 3, Annex B, part 2, B.6

August 2007

B.6 Toxicology and metabolism

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (ANNEX IIA 5.1)

B.6.1.1. Toxicokinetic studies

STUDY 1

Characteristics

reference	: Rhenius, 1989a	exposure	: single by gavage
type of study	: Absorption, distribution and excretion	doses	: 3 mg/kg bw
year of execution	: 1988/1989	vehicle	: 1% Tween-80 at 2 mL
test substances	: Non-labelled pyridaben (NC-129), lot no. 129T8604, 99.6% pure. [3,6-pyridazinone-ring ¹⁴ C]-pyridaben: lot no. CP-928, chemical purity not reported, radiochemical purity ≥99.5%. [benzyl ring ¹⁴ C]-pyridaben: lot no. CP-927, chemical purity not reported, radiochemical purity ≥99.6%.	GLP statement	: yes
route	: oral	guideline	: OECD 417 (1981), MAFF (1985), EPA/FIFRA (1982)
species	: CD rat (180-200 g)	acceptability	: acceptable
group size	: see table 6.1.1-01		

Study design

The absorption, tissue distribution and excretion of ¹⁴C-pyridaben was investigated after a single oral dose of 3 mg/kg bw of 3,6-pyridazinone-ring¹⁴C-pyridaben or [benzyl ring¹⁴C]-pyridaben. All animals received food *ad libitum* throughout the study. Exposure and sampling of urine, cage wash, faeces, blood and tissues was as described in table 6.1.1-01.

Table 6.1.1-01 Experimental groups for each dose level

Group no.	No. of animals	Treatment	Sampling times (h after dosing)	Sacrifice time (h after last dose)
1	5 M & 5 F	Single oral dose at 3 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben (actual 2.8(M), 3.2(F) mg/kg)	0-12, 12-24 hours: urine, 0-24: faeces and expired air, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours: urine, faeces; 168: cage wash & carcass	168
2	3 M & 3 F	Single oral dose at 3 mg/kg [benzyl ring ¹⁴ C]-pyridaben (actual 2.3(M), 3.1(F) mg/kg)	0-12, 12-24 hours: urine, 0-24: faeces and expired air, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours: urine, faeces; 168: cage wash & carcass	168
3	5 M & 5 F	Single oral dose at 3 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben (actual 2.3(M), 3.0(F) mg/kg)	0.25, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120, 144, 168 hours: blood	168
4	3 M & 3 F	Single oral dose at 3 mg/kg [benzyl ring ¹⁴ C]-pyridaben (actual 2.7(M), 2.6(F) mg/kg)	0.25, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120, 144, 168 hours: blood	168
5	15 M & 15 F	Single oral dose at 3 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben (actual 2.4(M), 2.6(F) mg/kg)	n.a.	2, 24, 72, 168 ^(A)
6	12 M & 12 F	Single oral dose at 3 mg/kg [benzyl ring ¹⁴ C]-pyridaben (actual 2.0(M), 2.6(F) mg/kg)	n.a.	2, 24, 72, 168 ^(A)

n.a. = not applicable

(A) At 2, 24, 72 and 168 hours post-dose, the following tissues and organs were taken from three animals (group 5 five at 168 h): blood, liver, kidney, heart, lung, bone (femur), brain, fat (abdominal), skeletal muscle, testes (male), ovaries (female), spleen, adrenal glands, residual carcass, eyes, harderian gland, intestinal tract, intestinal tract contents, mesenteric lymph nodes, pancreas, salivary gland, skin, stomach, thymus, thyroid, uterus (female).

Radioactivity in urine, plasma and cage washings was quantified by LSC. Radioactivity in faeces (homogenised) was quantified by combustion/LSC. Tissues, organs, tissue and organ homogenates and blood were solubilised and radioactivity was quantified by LSC. Radioactivity in expired air was trapped with 2M NaOH and analysed by LSC.

RESULTS

Radiochemical analysis of the test compound post dosing showed that radiochemical purity of ^{14}C -pyridaben was between 99.1 and 100.2%. Achieved doses were 77-103 % of nominal, except for [benzyl ring ^{14}C]-pyridaben in group 5 in males: 67% of nominal.

Mass balance

After a single oral dose of 3 mg/kg bw after 168 hours the majority of administered radioactivity was excreted in faeces (80-87% AR), whilst radioactivity in urine accounted for 3-4% AR after dosing [3,6-pyridazinone-ring ^{14}C]-pyridaben and 11-15% after dosing [benzyl ring ^{14}C]-pyridaben (table 6.1.1-02. Radioactivity in expired air was <0.01% AR. Excretion was essentially completed (83-89% AR for [3,6-pyridazinone-ring ^{14}C]-pyridaben and 94-97% AR for [benzyl ring ^{14}C]-pyridaben) after 96 hours. Radioactivity in skin and residual carcass at 168 hours post-dose represented 0.1-0.8% AR. For [3,6-pyridazinone-ring ^{14}C]-pyridaben recovery was 84-90% AR and for [benzyl ring ^{14}C]-pyridaben) 96-98% AR. From the results of this study, oral absorption is estimated to be $\leq 15\%$.

Table 6.1.1-02 Excretion and retention of radioactivity (% AR) in rats after single oral exposure to ^{14}C -pyridaben

sample		3 mg/kg bw [3,6-pyridazinone-ring ^{14}C]-pyridaben		3 mg/kg bw [benzyl ring ^{14}C]-pyridaben	
		M	F	M	F
urine	0-12 h	1.4	1.3	4.6	4.6
	12-24 h	0.6	0.6	3.5	2.9
	24-48 h	0.9	0.4	3.3	2.3
	48-72 h	0.4	0.1	1.9	0.6
	72-96 h	0.2	0.04	0.8	0.4
	96-120 h	0.07	0.03	0.3	0.1
	120-144 h	0.04	0.02	0.2	0.08
	144-168 h	0.03	0.01	0.09	0.06
faeces	0-168 h	3.6	2.6	14.7	10.9
	0-24 h	43.8	45.3	52.1	56.3
	24-48 h	32.8	33.3	23.7	23.8
	48-72 h	4.3	0.8	3.8	2.7
	72-96 h	4.2	0.7	0.7	3.7
	96-120 h	0.9	0.09	0.3	0.3
	120-144 h	0.1	0.06	0.2	0.06
	144-168 h	0.2	0.04	0.06	0.06

	0-168 h	86.3	80.3	80.8	86.9
expired air	0-24 h	<0.01	<0.01	<0.01	<0.01
skin and carcass	168 h	0.02	0.02	0.2	0.4
cage wash	168 h	0.1	0.8	0.1	0.08
Recovery	168 h	90.1	83.7	95.8	98.3

Plasma pharmacokinetic parameters

The pharmacokinetic parameters are presented in Table 6.1.1-03. Peak ^{14}C -concentrations in blood were observed after 1-12 hours in both sexes. No differences between animals dosed with [3,6-pyridazinone-ring ^{14}C]-pyridaben and [benzyl ring ^{14}C]-pyridaben were found. Individual concentrations measured at the different time points differed substantially. The elimination half-life at 3 mg/kg bw as [3,6-pyridazinone-ring ^{14}C]-pyridaben (23 hours for both sexes) was longer than that for [benzyl ring ^{14}C]-pyridaben (9-10 hours).

Table 6.1.1-03 Pharmacokinetic parameters in blood of male and female rats after single oral exposure to ^{14}C -pyridaben

	3 mg/kg bw [3,6-pyridazinone-ring ^{14}C]-pyridaben		3 mg/kg bw [benzyl ring ^{14}C]-pyridaben	
	M	F	M	F
$C_{\max}$ (ng eq./ml)	28.8	33.9	40.3	36.4
$T_{\max}$ (h)	1-12	0.25-12	0.25-9	1-12
$T_{1/2}$ (h) ^(A)	23	23	9-10	9-10

(A) based on results at 12, 24 and 48 hours; no definitive pharmacokinetic parameters

Distribution in tissues

The distribution of radioactivity in tissues of rats sacrificed at 2, 24, 72 and 168 hours post-dose is presented in table 6.1.1-04 and 6.1.1-05. Ranges given below are for males and females together, unless indicated differently. In general a large inter-individual variation of radioactivity concentrations measured in the organs and tissues was observed.

Two hours post-dosing radioactivity was mainly found in the gastro-intestinal tract (83-85% AR in males and 73-83% AR in females including GI-contents). The [3,6-pyridazinone-ring ^{14}C]-pyridaben related radioactivity in the GI-tract decreased slightly more rapidly over 72 hours (mean values at 2, 24 and 72 hours: 83, 9.5 and 0.1% AR) than that of [benzyl ring ^{14}C]-pyridaben (mean values at 2, 24 and 72 hours: 78, 13 and 0.3% AR). In the liver [3,6-pyridazinone-ring ^{14}C]-pyridaben radioactivity was between 563 and 1150 ng eq./g (1.0-2.1 % AR) after 2 hours, and 62-101 ng eq./g (0.1-0.2% AR) after 24 hours. [Benzyl ring ^{14}C]-pyridaben related radioactivity was also identified in the liver after 2 to 72 hours (2 hours: 1250-1330 ng eq./g (2.0-2.9% AR); 24 hours 302-430 ng eq./g (0.8% AR); 72 hours: 54-109 ng eq./g (0.1-0.2% AR)).

In other organs radioactivity concentrations ranged between 256-392 ng eq./g (kidneys), 316-750 ng eq./g (pancreas), 407-700 ng eq./g (spleen), 1110 and 2170 ng eq./g (uterus), 896-3290 ng eq./g (ovary) and 849-1390 ng eq./g (lymph-nodes) after 2 hours and decreased rapidly thereafter. At 24 hours post-dose, radioactivity concentrations in the above mentioned tissues were on average 8-44% (for [3,6-pyridazinone-ring ^{14}C]-pyridaben) and 16-73% (for [benzyl ring ^{14}C]-pyridaben) of those at 2 hours. After 72 hours post-dose 1-7 % of 2 hours radioactivity was found. In males radioactivity in fat at 24 hours was higher (151-198%) than that at 2 hours.

Radioactivity in plasma decreased to 9-10% of that at 2 hours in both sexes after 72 hours (and to background ranges at 168 hours). Blood radioactivity decreased rapidly over 72 hours in both sexes treated with [benzyl ring-¹⁴C]-pyridaben to 0-5% of radioactivity at 2 hours (and to background ranges thereafter), but in animals treated with [3,6-pyridazinone-ring-¹⁴C]-pyridaben the decrease was slower (to 17-25% over 168 hours).

There were no remarkable differences between tissue levels and depletion rates of male and female rats.

Table 6.1.1-04 Distribution of radioactivity in tissues and organs [ng pyridaben equivalents/g and % AR] at 2, 24, 72 and 168 hours post dose of 3 mg/kg [3,6-pyridazinone-ring-¹⁴C]-pyridaben

tissue	2 hours				24 hours				72 hours				168 hours			
	male		female		male		female		male		female		male		female	
	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR
Blood (ng eq./mL)	16		30		14		14		11		11		4		5	
Plasma (ng eq./mL)	23		43		6		8		2		4		ND		ND	
Liver	563	1.0	1150	2.1	62	0.1	101	0.2	9	0.0	13	0.0	ND		2	0.0
GIT-content	30700	78.3	32100	66.4	2860	6.6	4670	7.5	32	0.1	83	0.1	2	0.0	7	0.0
Stomach	14400	3.3	27600	6.1	213	0.0	564	0.0	6	0.0	15	0.0	ND		ND	
Small intestine	4440	3.0	9830	6.5	417	0.3	526	0.3	21	0.0	27	0.0	1	0.0	1	0.0
Large intestine	2370	0.7	3010	1.1	1500	0.5	1770	0.4	28	0.0	42	0.0	2	0.0	6	0.0
Pancreas	316	0.0	750	0.1	153	0.0	191	0.0	2	0.0	7	0.0	ND		1	0.0
Spleen	407	0.0	700	0.1	45	0.0	51	0.0	ND		ND		ND		ND	
Kidney	256	0.1	327	0.1	64	0.0	63	0.0	7	0.0	8	0.0	ND		3	0.0
Testis	40	0.0			5	0.0			ND				ND			
Uterus			2170	0.2			263	0.0			8	0.0			1	0.0
Lung	143	0.0	208	0.1	17	0.0	19	0.0	2	0.0	4	0.0	ND		3	0.0
Heart	186	0.0	322	0.1	31	0.0	34	0.0	ND		ND		ND		ND	
Thymus	65	0.0	83	0.0	11	0.0	9	0.0	ND		ND		ND		ND	
Sal. Gland	209	0.0	141	0.0	23	0.0	27	0.0	ND		ND		ND		ND	
Brain	131	0.0	115	0.0	22	0.0	25	0.0	ND		ND		ND		ND	
Eye	17	0.0	29	0.0	11	0.0	5	0.0	ND		ND		ND		ND	
Lymph node	849	0.0	2390	0.1	346	0.0	437	0.0	33	0.0	33	0.0	3	0.0	9	0.0
Adrenals	116	0.0	186	0.0	87	0.0	48	0.0	6	0.0	7	0.0	ND		1	0.0
Thyroid	178	0.0	207	0.0	12	0.0	42	0.0	ND		63	0.0	ND		ND	
Harderian gland	53	0.0	101	0.0	23	0.0	25	0.0	2	0.0	2	0.0	ND		ND	
Ovary			3290	0.1			262	0.0			18	0.0			5	0.0
Muscle	53	0.0	104	0.0	18	0.0	33	0.0	3	0.0	2	0.0	ND		ND	
Fat	140	0.0	791	0.1	212	0.0	151	0.0	49	0.0	37	0.0	8	0.0	17	0.0
Skin	21	0.2	34	0.3	11	0.1	14	0.1	6	0.1	6	0.0	ND		ND	
Carcass	109	2.6	287	7.4	89	2.3	89	2.1	6	0.1	3	0.1	ND		ND	
Bone	7	0.0	14	0.0	ND		ND		1	0.0	ND		ND		ND	
total		89.2		90.9		9.9		10.7		0.3		0.2		0.0		0.0

ND results within background range

0.0 results <0.05%.

Table 6.1.1-05 Distribution of radioactivity in tissues and organs [ng pyridaben equivalents/g and % AR] at 2, 24, 72 and 168 hours post dose of 3 mg/kg [benzyl ring ¹⁴C]-pyridaben

tissue	2 hours				24 hours				72 hours				168 hours			
	male		female		male		female		male		female		male		female	
	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR
Blood (ng eq./mL)	35		41		8		11		ND		2		ND		ND	
Plasma (ng eq./mL)	52		65		12		18		5		6		ND		ND	
Liver	1250	2.9	1330	2.0	302	0.8	430	0.8	54	0.1	109	0.2	9	0.0	41	0.1
GIT-content	27400	77.0	37900	62.9	4070	8.9	8510	13.9	116	0.3	117	0.2	10	0.0	28	0.0
Stomach	4040	1.1	23800	4.2	511	0.1	1200	0.2	24	0.0	20	0.0	2	0.0	10	0.0
Small intestine	4820	3.3	8440	4.6	1140	0.9	1070	0.7	132	0.1	63	0.0	8	0.0	28	0.0
Large intestine	4100	1.4	2710	0.8	1290	0.4	3420	1.0	71	0.0	73	0.0	10	0.0	25	0.0
Pancreas	821	0.1	502	0.1	473	0.1	299	0.0	39	0.0	30	0.0	2	0.0	16	0.0
Spleen	384	0.0	250	0.0	96	0.0	65	0.0	8	0.0	4	0.0	ND		ND	
Kidney	392	0.2	318	0.1	93	0.0	132	0.0	12	0.0	10	0.0	ND		6	0.0
Testis	38	0.0			18	0.0			ND				ND			
Uterus			1110	0.1			379	0.0			16	0.0			10	0.0
Lung	221	0.1	214	0.1	26	0.0	73	0.0	4	0.0	14	0.0	ND		7	0.0
Heart	291	0.1	273	0.0	38	0.0	57	0.0	2	0.0	11	0.0	ND		5	0.0
Thymus	88	0.0	87	0.0	19	0.0	94	0.0	ND		2	0.0	ND		ND	
Sal. Gland	132	0.0	150	0.0	22	0.0	41	0.0	3	0.0	2	0.0	2	0.0	ND	
Brain	77	0.0	74	0.0	21	0.0	26	0.0	ND		ND		2	0.0	ND	
Eye	29	0.0	32	0.0	30	0.0	16	0.0	1	0.0	2	0.0	3	0.0	ND	
Lymph node	974	0.0	1390	0.0	690	0.0	304	0.0	65	0.0	33	0.0	11	0.0	32	0.0
Adrenals	217	0.0	193	0.0	146	0.0	117	0.0	14	0.0	31	0.0	ND		17	0.0
Thyroid	100	0.0	114	0.0	29	0.0	15	0.0	ND		ND		ND		ND	
Harderian gland	202	0.0	184	0.0	867	0.1	878	0.0	154	0.0	419	0.0	23	0.0	97	0.0
Ovary			896	0.0			143	0.0			23	0.0			24	0.0
Muscle	63	0.0	77	0.0	73	0.0	31	0.0	7	0.0	3	0.0	2	0.0	2	0.0
Fat	293	0.0	144	0.0	579	0.0	117	0.0	57	0.0	49	0.0	24	0.0	47	0.0
Skin	47	0.4	38	0.2	30	0.3	26	0.2	18	0.2	47	0.4	9	0.1	24	0.2
Carcass	115	3.5	161	3.6	68	2.2	108	2.4	10	0.3	8	0.2	0	0.0	6	0.1
Bone	7	0.0	10	0.0	ND		ND		ND		ND		ND		ND	
total		90.1		78.9		13.8		19.2		1.0		1.0		0		0.4

ND results within background range
0.0 results <0.05%.

Conclusions

The absorption, distribution and excretion of ¹⁴C-pyridaben was investigated after a single oral dose of 3 mg/kg bw. Oral absorption was found to ≤ 15%, but might have been higher because of the fact that some of the absorbed pyridaben might have been excreted via the entero-hepatic circulation (no bile-duct cannulation was applied). After 168 hours the majority of administered radioactivity was excreted in faeces (80-87% AR), whilst radioactivity in urine accounted for 3-4% AR after dosing [3,6-pyridazinone-ring ¹⁴C]-pyridaben and 11-15% after dosing [benzyl ring ¹⁴C]-pyridaben. Peak ¹⁴C-concentrations in blood were observed after 0.25-12 hours in both sexes. The elimination half-life at 3 mg/kg as [3,6-pyridazinone-ring ¹⁴C]-pyridaben (23 hours for both sexes) was longer than that for [benzyl ring ¹⁴C]-pyridaben (9-10 hours). Radioactivity was concentrated mainly in the gastro-intestinal tract and further widely distributed with highest (relative) amounts in liver,

kidney, pancreas, spleen, lymph nodes, uterus and ovary after 2 hours (fat after 24 hours). At 24, 72 and 168 hours post-dose, respectively, radioactivity concentrations in tissues were on average 8-44% ([3,6-pyridazinone-ring¹⁴C]-pyridaben) to 16-73% ([benzyl ring¹⁴C]-pyridaben), 1-7% and <1% of those after 2 hours. Blood and plasma concentrations decreased to background levels after 168 hours, except for blood levels of animals treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben (17-25% of 2 hours values). There were no remarkable differences between tissue levels and depletion rates of male and female rats. [3,6-pyridazinone-ring¹⁴C]-pyridaben and [benzyl ring¹⁴C]-pyridaben behave differently considering urinary excretion, liver concentrations and elimination from blood (higher for [benzyl ring¹⁴C]-pyridaben) and tissue elimination (slower for benzyl ring¹⁴C]-pyridaben).

Acceptability

The study is acceptable.

Remarks include the lack of accuracy of the achieved doses, variations between radioactivity levels in individual animals (tissues, organs, blood) and the omission of bile-duct cannulation from the study design.

STUDY 2

Characteristics

reference	: Hallifax, 1994a	exposure	: single by gavage
type of study	: Metabolism	doses	: 3 mg/kg bw
year of execution	: 1989-1991	vehicle	: 1% Tween-80 at 2 mL
test substances	: Non-labelled pyridaben (NC-129), lot no. 129T8604, 99.6% pure. [3,6-pyridazinone-ring ¹⁴ C]-pyridaben: lot no. CP-928, chemical purity not reported, radiochemical purity ≥99.5%. [benzyl ring ¹⁴ C]-pyridaben: lot no. CP-927, chemical purity not reported, radiochemical purity ≥99.6%.	GLP statement	: yes
route	: oral	guideline	: OECD 417 (1981), MAFF (1985), EPA/FIFRA (1982)
species	: CD rat (180-200 g)	acceptability	: Supplementary
group size	: see table 6.1.1-01, study 1		

Study design

The metabolism of ¹⁴C-pyridaben was investigated in urine and faeces (0-48 hour samples pooled per rat) and in liver samples collected 2 hours after dosing from 3 rats of the first four groups in the previous study (see Table 6.1.1-01), which had received a single oral dose of 3 mg/kg bw of 3,6-pyridazinone-ring¹⁴C]-pyridaben or [benzyl ring¹⁴C]-pyridaben.

Pooled samples of urine and faeces were divided into an 80% portion for extraction and a 20% portion for enzyme hydrolysis.

All radioactivity is indicated as percentage of the initially applied dose. In case metabolites could not be separated this is indicated by a slash (/) between metabolite codes. In case metabolites were separated, but were included into a larger fraction (of % AR) this is indicated by a “;” between metabolite codes.

Extraction

The 80% portion of urine was extracted with ethyl acetate and concentrated. The concentrated extract (pooled per rat) was analysed by TLC. From the aqueous phase (combined for each sex) 50% was acidified and 50% was made alkaline. Both portions were refluxed, extracted with ethyl acetate and analysed by TLC. Faeces (80% portion) and was mixed with methanol and centrifuged. Extracts were combined, concentrated, acid extracted with ethyl acetate and analysed by TLC. For the aqueous phase the procedure as described for urine was followed.

Liver was homogenised with methanol. The mixture was centrifuged and the combined extracts were concentrated and acid extracted with ethyl acetate. Samples were analysed by TLC as described for faeces. A second TLC analysis was performed on all individual animal samples. The remaining material at origin was removed with methanol and re-chromatographed with a different solvent system. In addition metabolites were identified by two-dimensional TLC in combined samples per sex.

Hydrolysis

The remaining portion of the samples of urine and faeces (20%) were pooled per sex and incubated with β -glucuronidase/sulphatase twice. The incubation medium was extracted with ethyl acetate and analysed by TLC. Distinction between enzymatic and non-enzymatic hydrolysis was possible by inclusion of a control sample incubated in absence of β -glucuronidase/sulphatase.

Results

Urine

Fractionation recovery of radioactivity in urine was 2.1-2.6% AR after treatment with 3 mg/kg bw [3,6-pyridazinone-ring- ^{14}C]-pyridaben (mass balance 92-93% of initial TRR). Total radioactivity in ethyl acetate extracts from urine of rats treated with [3,6-pyridazinone-ring- ^{14}C]-pyridaben showed mean concentrations of 1.4% AR and 1.2% AR in males and females, respectively. In the ethyl acetate fraction 5-6 unidentified metabolites (total 0.7-2.0% AR) were found. Methanol soluble metabolites (5-6 unidentified) extracted from the origin of the TLC plates accounted for 0.5-1.8% of AR. Aqueous extracts accounted for 1.3% AR (males) and 0.9% AR (females). Acid or alkali hydrolysed pooled aqueous samples ranged from 0.3-0.4% AR (in ethyl acetate extract). Except for PB-1(parent) in females (0.13% AR) none of the 7 metabolites could be identified.

Recovery of radioactivity in urine was 9.1-10.6% AR after treatment with 3 mg/ kg bw [benzyl ring- ^{14}C]-pyridaben (mass balance fractions 93-94% of initial TRR in urine). Total reactivity recovered in ethyl acetate extracts was 5.0 and 2.6% AR in males and females, respectively. Eleven metabolites were found in the ethyl acetate extracts: B-1, B-8, B-7, parent/PB-3 (females), PB-7/B-3 (females) and B-11/PB-12 (all <3.7% AR) were identified among these metabolites. In extracts from the aqueous fraction 5.6% AR (males) and 6.5% AR (females) was found, which was not further analysed. Methanol soluble metabolites (10-11 unidentified) and PB-7/B-3 (females 0.1% AR) accounted for <1.2% AR. In aqueous (acid or alkali hydrolysed) samples 14 metabolites were found in concentrations of 1.9-3.6% AR (males) and 0.7-3.7% AR

(females). Metabolites identified included B-1, B-7, B-8, B-11, PB-11, PB-7/B3, PB-4 and parent/PB-3 (all <0.8%).

Results of metabolite identification extracted with ethyl acetate and ethyl acetate/methanol are summarized in table 6.1.1-02 - 05.

In extracts from urine of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben followed by hydrolysis with β -glucuronidase/sulphatase 2.0-2.4% AR was recovered from ethyl acetate and extracts from the aqueous fraction. In the ethyl acetate extract 4 unidentified metabolites were found (total 1.2-1.3% AR) and in one male in addition PB1/PB-3 (0.04% AR) was identified. No difference in metabolite extraction between hydrolysed and non-hydrolysed control samples became apparent. Aqueous extracts were not further analysed. After treatment with [benzyl ring¹⁴C]-pyridaben 7.9-8.5% AR was recovered. Up to 8 metabolites were found in ethyl acetate extracts from hydrolysed samples (2.5% AR in females and 4.4% AR in males). Five were identified as B-1 (males), B-7, B-11, PB-3 (females), and PB-9 (females) and were present at concentrations <1.1% AR. No major differences in metabolite extraction pattern between hydrolysed and non-hydrolysed samples were found.

Metabolite identification using two-dimensional TLC gave the following results: P-9 after [3,6-pyridazinone-ring¹⁴C]-pyridaben treatment and B-1, B-3, B-7, B-8, B-11, B-15, PB-1 and PB-7 after [benzyl ring¹⁴C]-pyridaben. Figures of TLC plates were incorporated in the report, but no further details were included.

Faeces

Fractionation recovery of radioactivity in faeces was 46-50% AR after treatment with 3 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben (mass balance 60-64% of initial TRR in faeces). Total radioactivity in faecal ethyl acetate extracts from rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben showed mean concentrations of 16% AR (range 7-29%) and 21% AR (range 7-56%) in males and females, respectively. In the ethyl acetate fraction parent/PB-3, PB-9, PB-11 (one female), PB-12 (females) and 7-8 unidentified metabolites were found, but spots could not be separated accurately and ranges for individual metabolites were large. Methanol soluble metabolites (P-1 and 9 unidentified) accounted for 8.3% AR (males) and 10% AR (females). Aqueous extracts accounted for 8.7% AR (males) and 8.3% AR (females). In residue 21% AR was recovered. Acid or alkali hydrolysed pooled aqueous samples ranged from 4-7% AR (in ethyl acetate extract). Metabolites included PB-1/PB-3, PB-7 and P-1 (each <0.8% AR) and 2-5 unidentified metabolites (<2% AR).

Recovery of radioactivity in faeces after treatment with 3 mg/kg bw [benzyl ring¹⁴C]-pyridaben was 66-68% AR (mass balance fractions 85-87% of initial TRR in faeces). Total reactivity recovered in ethyl acetate extracts was 37 and 46% AR in males and females, respectively. Up to 16 metabolites were found in the ethyl acetate extracts: B-1, B-8, parent/PB-3, PB-7/B-3, PB-9/PB-11, B11/PB12 and D-1 (males) were identified among these metabolites. Methanol soluble metabolites (up to 5 unidentified and B-8 in one female (0.3%)) accounted for <16.2% AR. In extracts from the aqueous fraction 9.0% AR (males) and 4.1% AR (females) was found, which was not further analysed. In residue was 18-20% AR was recovered. In acid or alkali hydrolysed pooled aqueous samples 0.1-1.8% AR (males) and 0.1-2.4% AR (females) was recovered.

Metabolites identified included B-7, B-8, PB-12/B-7;B-11, PB-7/B-3 and parent/PB-3 (all <1.2%). In view of the poor separation no exact percentages per metabolite can be derived.

Results of metabolite identification extracted with ethyl acetate and ethyl acetate/methanol are summarized in table 6.1.1-02 - 05.

In extracts from faeces of rats treated with [3,6-pyridazinone-ring ^{14}C]-pyridaben followed by hydrolysis with β -glucuronidase/sulphatase 24% AR (males) and 37% AR (females) was recovered from ethyl acetate extracts. The aqueous fraction contained 6.8 and 7.9% AR in faeces of males and females, respectively. Residue was 16-26% AR. In the ethyl acetate extract PB-12/P-3 (1.6% AR in males), PB-1/PB-3 (8.2% AR males, 16.2% AR females), PB-9;PB-7 (males <2.9% AR), PB-12/P-3;PB-9;PB-7 (<4.8% in females) and 6-7 unidentified metabolites were found. No difference in metabolite extraction between hydrolysed and non-hydrolysed control samples became apparent. Aqueous extracts were not further analysed. After treatment with [benzyl ring ^{14}C]-pyridaben 25% AR (males) and 37% AR (females) was recovered from ethyl acetate extracts, 4.6-5.0% AR from aqueous extracts and 21% (males) and 4.5% AR (females) in residue. Nine to ten metabolites were found in ethyl acetate extracts from hydrolysed samples including 4-6 unidentified. The following metabolites were identified B-8;PB-12 (males 6.1% AR, females 7.8% AR), PB-9;PB-7/B-3 (males 3.2%, PB-9;B-1 (females 0.9%) and parent/PB-3 (males 9.7% AR and females 19% AR). No major differences in metabolite extraction pattern between hydrolysed and non-hydrolysed samples were found. In view of the poor separation no exact percentages per metabolite can be derived.

Metabolite identification using two-dimensional TLC gave the following results: PB-1, PB-4, PB-9, PB-11 and PB-12 after [3,6-pyridazinone-ring ^{14}C]-pyridaben treatment and B-1, B-8, B-15, PB-1, PB-3, PB-7, PB-9, PB-11 and PB-12 after [benzyl ring ^{14}C]-pyridaben. Figures of TLC plates were incorporated in the report, but no further details were included.

Liver

The total amount of radioactivity recovered in the liver of rats receiving a single oral dose of [3,6-pyridazinone-ring ^{14}C]-pyridaben or [benzyl ring ^{14}C]-pyridaben was 0.8-1.8% AR. Treatment with [3,6-pyridazinone-ring ^{14}C]-pyridaben gave mean recoveries of radioactivity of 0.38% AR (males and females) in ethyl acetate extracts, 0.24% AR (males) and 0.64% AR (females) in aqueous extracts and 0.27% AR (males) and 0.60% AR (females) in unextractable residue. Three unknown metabolites were found (<0.33% AR) in ethylacetate extracts.

For [benzyl ring ^{14}C]-pyridaben recoveries were 1.5% AR (males) and 0.91% AR (females) for ethyl acetate extracts, 0.34% AR (males and females) for aqueous extracts and residue accounted for 0.47% AR and 0.40% AR for males and females, respectively. B-1 accounted for 1.1% AR in males and 0.59% AR in females. In addition 5-7 unidentified metabolites were present (each <0.19% AR). Metabolite identification using two-dimensional TLC gave the following results: parent after [3,6-pyridazinone-ring ^{14}C]-pyridaben treatment and parent, B-1 and B-11 after [benzyl ring ^{14}C]-pyridaben. Figures of TLC plates were incorporated in the report, but no further details were included.

Conclusions

In 0-48 hour urine of rats treated with 3 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben, the metabolite pattern was essentially the same for both sexes (urinary concentration 2.1-2.6% AR). The only metabolite identified by two-dimensional TLC analysis was P-9, but the results of this identification were poorly reported. The parent compound was identified in urine after enzymatic and acid hydrolysis. Up to 7 unidentified metabolites were found, each accounting for <1.8% AR. After treatment with 3 mg/kg bw [benzyl ring¹⁴C]-pyridaben (total AR 6-11%) several metabolites were identified in urine: B-1, B-7, B-8, parent/PB-3 (females), PB-7/B-3 (females) and B-11/PB-12 (all <3.7% AR) in ethyl acetate extracts. After acid or alkaline hydrolysis in addition PB-11 was found (<0.8% AR) and after enzymatic hydrolysis PB-9 (in females ≤0.25% AR). Two-dimensional TLC confirmed parent B-1, B-3, B-7, B-8, B-11 and PB-7 and newly identified B-15. No major differences in the metabolites identified in both sexes were found.

In 0-48 hour faeces of rats treated with 3 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben 46-50% AR was recovered after fractioning. In ethyl acetate extracts parent/PB-3, PB-9, PB-12, P-1, PB-11 were identified and in acid and enzymatic hydrolysed fractions PB-7 and P-3 were found in addition. Recovery ranges between animals differed 4-8 fold. Two-dimensional TLC analysis identified parent, PB-4, PB-9, PB-11 and PB-12. Treatment with 3 mg/kg bw [benzyl ring¹⁴C]-pyridaben (total 66-68% AR in faeces fractions) gave B-1, B-8, parent/PB-3, PB-7/B-3, PB-9/PB-11, B-11/PB-12 and D-1 (males) in the ethyl acetate extracts and included B-7 after acid hydrolysis. Enzymatic hydrolysis did not show differences to non-enzymatic hydrolysis. Using two-dimensional TLC analysis one additional metabolite was identified, B-15 and parent B-1, B-8, PB-3, PB-7, PB-9, PB-11 and PB-12 were confirmed.

In liver collected from animals sacrificed 2 hours after a single dose of 3 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben or [benzyl ring¹⁴C]-pyridaben 3 unidentified metabolites (<0.33% AR) or B-1 (1.1% AR) and 5-7 unidentified metabolites (<0.19% AR) were found, respectively. Two dimensional TLC analysis identified parent, B-1 and B-11.

Acceptability

- (1) Metabolite identification was based on TLC only (no evidence of confirmation by MS or a second chromatographic method). HPLC confirmation was part of the protocol, but not performed in this study due to lack of a suitable method. Instead two-dimensional TLC analysis was used to confirm metabolite identity. This confirmation was incomplete and poorly reported.
- (2) Metabolite identification was insufficiently investigated due to incomplete separation on the TLC plates and evaluation of metabolites as groups, when spots could not be resolved by the linear analyser.
- (3) Mass balances were not maintained adequately. This means that not all radioactivity was recovered.
- (4) Overall evaluation by RMS. Considering the comments under point 1 - 3, the study is considered supplementary.

STUDY 3

Characteristics

reference	: Rhenius, 1989b	exposure	: single by gavage
type of study	: Absorption, distribution and excretion	doses	: 30 mg/kg bw
year of execution	: 1988/1989	vehicle	: 1% Tween-80 at 2 mL
test substances	: Non-labelled pyridaben (NC-129), lot no. 129T8604, 99.6% pure. [3,6-pyridazinone-ring ¹⁴ C]-pyridaben: lot no. CP-928, chemical purity not reported, radiochemical purity ≥99.5%. [benzyl ring ¹⁴ C]-pyridaben: lot no. CP-927, chemical purity not reported, radiochemical purity ≥99.6%.	GLP statement	: yes
route	: oral	guideline	: OECD 417 (1981), MAFF (1985), EPA/FIFRA (1982)
species	: CD rat (180-200 g)	acceptability	: acceptable
group size	: see table 6.1.1-06		

Study design

The absorption, tissue distribution and excretion of ¹⁴C-pyridaben was investigated after a single oral dose of 30 mg/kg bw of 3,6-pyridazinone-ring¹⁴C-pyridaben or [benzyl ring¹⁴C]-pyridaben. All animals received food *ad libitum* throughout the study. Exposure and sampling of urine, cage wash, faeces, blood and tissues was as described in table 6.1.1-06.

Table 6.1.1-06 Experimental groups for each dose level

Group no.	No. of animals	Treatment	Sampling times (h after dosing)	Sacrifice time (h after last dose)
1	5 M & 5 F	Single oral dose at 30 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben (actual 25.9(M), 30.2(F) mg/kg)	0-12, 12-24 hours: urine, 0-24: faeces and expired air, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours: urine, faeces; 168: cage wash & carcass	168
2	3 M & 3 F	Single oral dose at 30 mg/kg [benzyl ring ¹⁴ C]-pyridaben (actual 24.1(M), 25.2(F) mg/kg)	0-12, 12-24 hours: urine, 0-24: faeces and expired air, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours: urine, faeces; 168: cage wash & carcass	168
3	5 M & 5 F	Single oral dose at 30 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben (actual 26.5(M), 35.5(F) mg/kg)	0-12, 12-24 hours: urine, 0-24: faeces and expired air, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours: urine, faeces; 168: cage wash & carcass	168
4	3 M & 3 F	Single oral dose at 30 mg/kg [benzyl ring ¹⁴ C]-pyridaben (actual 27.2(M), 36.5(F) mg/kg)	0.25, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120, 144, 168 hours: blood	168
5	8 M & 8 F	Single oral dose at 30 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben (actual 29.1(M), 35.1(F) mg/kg)	n.a.	24, 168 ^(A)
6	6 M & 6 F	Single oral dose at 30 mg/kg [benzyl ring ¹⁴ C]-pyridaben (actual 25.5(M), 29.1(F) mg/kg)	n.a.	24, 168 ^(A)

n.a. = not applicable

(A) At 24 and 168 hours post-dose, the following tissues and organs were taken from 3 animals (group 5 five at 168 h): blood, liver, kidney, heart, lung, bone (femur), brain, fat (abdominal), skeletal muscle, testes (male), ovaries (female), spleen, adrenal glands, residual carcass, eyes, hardierian gland, intestinal tract, intestinal tract contents, mesenteric lymph nodes, pancreas, salivary gland, skin, stomach, thymus, thyroid, uterus (female).

Radioactivity in urine, plasma and cage washings was quantified by LSC. Radioactivity in faeces (homogenised) was quantified by combustion/LSC. Tissues, organs, tissue and organ homogenates and blood were solubilised and radioactivity was quantified by LSC. Radioactivity in expired air was trapped with 2M NaOH and analysed by LSC.

Results

Radiochemical analysis of the test compound post dosing showed that radiochemical purity of ^{14}C -pyridaben was between 99.1 and 100.2%. Achieved doses were 80-122% of nominal.

Mass balance

After a single oral dose of 30 mg/kg bw [3,6-pyridazinone-ring ^{14}C]-pyridaben the majority of administered radioactivity was excreted in faeces (87-97% AR) after 168 h, whilst radioactivity in urine accounted for 3-7% AR (Table 6.1.1-07). For [benzyl ring ^{14}C]-pyridaben faecal excretion was 74-78% AR and urinary excretion was 23% AR in males and 13% AR in females. Radioactivity in expired air was <0.01% AR. Excretion was essentially completed (91-100% AR for [3,6-pyridazinone-ring ^{14}C]-pyridaben and 88-96% AR for [benzyl ring ^{14}C]-pyridaben) after 96 hours. Radioactivity in skin and residual carcass at 168 hours post-dose represented 0.1-0.6% AR. For 3,6-pyridazinone-ring ^{14}C -pyridaben recovery was 95-100% AR and for [benzyl ring ^{14}C]-pyridaben) 92-99% AR. From the results of this study, oral absorption is estimated to be $\leq 24\%$.

Table 6.1.1-07 Excretion and retention of radioactivity (% AR) in rats after single oral exposure to ^{14}C -pyridaben

sample		30 mg/kg bw [3,6-pyridazinone-ring ^{14}C]-pyridaben		30 mg/kg bw [benzyl ring ^{14}C]-pyridaben	
		M	F	M	F
urine	0-12 h	0.74	0.51	2.14	1.96
	12-24 h	1.57	0.61	5.66	4.12
	24-48 h	2.70	1.41	8.23	3.67
	48-72 h	1.13	0.29	4.43	1.06
	72-96 h	0.36	0.07	1.79	0.51
	96-120 h	0.22	0.03	0.72	0.40
	120-144 h	0.14	0.02	0.31	0.34
	144-168 h	0.10	0.01	0.22	0.37
	0-168 h	6.9	2.9	23.5	12.5
faeces	0-24 h	27.7	37.8	13.3	28.3
	24-48 h	40.7	46.6	47.8	38.8
	48-72 h	11.1	10.1	8.70	7.82
	72-96 h	4.80	2.31	3.50	1.48
	96-120 h	1.66	0.24	0.53	0.38
	120-144 h	0.65	0.10	0.22	0.37
	144-168 h	0.13	0.05	0.25	0.31
	0-168 h	86.7	97.2	74.3	77.5
expired air	0-24 h	<0.01	<0.01	<0.01	<0.01
Skin and carcass	168 h	0.43	0.10	0.51	0.56
cage wash	168 h	0.69	0.04	0.63	1.70
Recovery	168 h	94.8	100.3	99.0	92.3

Plasma pharmacokinetic parameters

The pharmacokinetic parameters are presented in Table 6.1.1-08. Peak ^{14}C -concentrations in blood were observed after 24 hours in both sexes. No differences between animals dosed with [3,6-pyridazinone-ring ^{14}C]-pyridaben and [benzyl ring ^{14}C]-pyridaben were found. The elimination half-life at 30 mg/kg bw as [3,6-pyridazinone-ring ^{14}C]-pyridaben was longer (49-56 hours) than that for [benzyl ring ^{14}C]-pyridaben.

Table 6.1.1-08 Pharmacokinetic parameters in blood of male and female rats after single oral exposure to ^{14}C -pyridaben

	30 mg/kg bw [3,6-pyridazinone-ring ^{14}C]-pyridaben		30 mg/kg bw [benzyl ring ^{14}C]-pyridaben	
	M	F	M	F
$C_{\max}$ (ng eq./ml)	202	257	366	370
$T_{\max}$ (h)	24	24	24	24
$T_{1/2}$ (h) ^(A)	49-56		18-24	

(A) based on results at 12, 24 and 48 hours; no definitive pharmacokinetic parameters

Distribution in tissues

The distribution of radioactivity in tissues of rats sacrificed at 24 and 168 hours post-dose is presented in table 6.1.1-09 and 6.1.1-10. Ranges given below are for males and females together, unless indicated differently. In general a large inter-individual variation of radioactivity concentrations measured in the organs and tissues was observed.

Twenty-four hours post-dosing radioactivity was mainly found in the gastro-intestinal tract (49-50% AR in males and 47-55% AR in females including GI-contents). The ^{14}C -pyridaben related radioactivity in the GI-tract decreased rapidly over 168 hours to 0-0.2% AR.

In the liver [3,6-pyridazinone-ring ^{14}C]-pyridaben radioactivity was between 4330 and 5480 ng eq./g (0.8 and 0.7% AR) after 24 hours and decreased to 94-98 ng eq./g (0% AR) at 168 hours. For [benzyl ring ^{14}C]-pyridaben 24-hour concentrations in liver were 11900-15600 ng eq./g (1.8-3.1% AR). After 168 hours 485-494 ng eq./g (0.1% AR) was still present in the liver.

In other organs radioactivity concentrations were 9830-9800 ng eq./g (pancreas), 7810-11100 ng eq./g (uterus), 5740-11600 ng eq./g (ovary) and 19000-20600 ng eq./g (lymph nodes after [3,6-pyridazinone-ring ^{14}C]-pyridaben) and 5830-7370 ng eq./g (pancreas) and 9400-15300 ng eq./g (lymph nodes after [benzyl ring ^{14}C]-pyridaben) after 24 hours and decreased rapidly thereafter. In the Harderian glands 13700-16000 ng eq./g were found 24 hours after treatment with [benzyl ring ^{14}C]-pyridaben.

Radioactivity in plasma decreased to 0-1% of that at 24 hours in both sexes after 168 hours. Blood radioactivity decreased rapidly over 168 hours in both sexes to 0-1% of radioactivity at 24 hours.

In both sexes tissue levels of radioactivity were slightly higher after [benzyl ring ^{14}C]-pyridaben treatment. Depletion rates of were similar after both compounds.

Table 6.1.1-09 Distribution of radioactivity in tissues and organs [ng pyridaben equivalents/g and % AR] at 24 and 168 hours post dose of 30 mg/kg [3,6-pyridazinone-ring ¹⁴C]-pyridaben

tissue	24 hours				168 hours			
	male		female		male		female	
	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR
Blood (ng eq./mL)	405		399		132		162	
Plasma (ng eq./mL)	397		475		ND		5.4	
Liver	4330	0.8	5480	0.7	98	0.0	94	0.0
GIT- content	270000	40.6	439000	49.9	190	0.0	214	0.0
Stomach	96800	1.9	80300	1.2	26	0.0	38	0.0
Small intestine	53500	3.1	35600	2.1	75	0.0	95	0.0
Large intestine	9200	3.0	69200	2.2	162	0.0	196	0.0
Pancreas	9830	0.1	9800	0.1	37	0.0	97	0.0
Spleen	4650	0.0	3140	0.0	ND	ND	ND	ND
Kidney	3890	0.1	3190	0.1	67	0.0	102	0.0
Testis	1270	0.0			ND	ND		
Uterus			11100	0.1			37	0.0
Lung	749	0.0	1950	0.1	61	0.0	89	0.0
Heart	1250	0.0	1380	0.0	68	0.0	23	0.0
Thymus	452	0.0	441	0.0	ND	ND	ND	ND
Sal. Gland	844	0.0	578	0.0	9.4	0.0	ND	ND
Brain	497	0.0	501	0.0	ND	ND	ND	ND
Eye	287	0.0	292	0.0	3.2	0.0	ND	ND
Lymph node	20600	0.1	19000	0.0	116	0.0	181	0.0
Adrenals	2070	0.0	2690	0.0	24	0.0	259	0.0
Thyroid	1430	0.0	856	0.0	ND	ND	15	0.0
Harderian gland	1140	0.0	1190	0.0	5.0	0.0	7.5	0.0
Ovary			11600	0.0			117	0.0
Muscle	2080	0.0	1230	0.0	ND	ND	ND	ND
Fat	4250	0.0	4950	0.0	340	0.0	688	0.0
Skin	647	0.5	635	0.3	54	0.1	67	0.0
Carcass	1750	3.3	1870	3.2	46	0.1	45	0.1
Bone	52	0.0	ND	ND	ND	ND	ND	ND
Total		53.5		59.3		0.2		0.1

ND results within background range
0.0 results <0.05%.

Table 6.1.1-10 Distribution of radioactivity in tissues and organs [ng pyridaben equivalents/g and % AR] at 24 and 168 hours post dose of 30 mg/kg [benzyl ring ¹⁴C]-pyridaben

tissue	24 hours				168 hours			
	male		female		male		female	
	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR
Blood (ng eq./mL)	638		399		ND		ND	
Plasma (ng eq./mL)	1010		648		13		5.9	
Liver	15600	3.1	11900	1.8	485	0.1	494	0.1
GIT- content	225000	46.1	248000	40.7	266	0.0	430	0.1
Stomach	43700	0.8	98600	1.7	147	0.0	152	0.0
Small intestine	34300	2.2	42800	2.9	237	0.0	323	0.0
Large intestine	28000	0.8	52400	1.6	271	0.0	428	0.0
Pancreas	7370	0.1	5830	0.1	214	0.0	279	0.0
Spleen	2130	0.0	3750	0.0	25	0.0	ND	ND
Kidney	2960	0.1	2650	0.1	59	0.0	100	0.0
Testis	459	0.0			ND	ND		
Uterus			7810	0.1			165	0.0
Lung	4000	0.1	2130	0.1	55	0.0	50	0.0
Heart	3200	0.1	2040	0.0	23	0.0	18	0.0
Thymus	1260	0.0	936	0.0	19	0.0	22	0.0
Sal. Gland	1330	0.0	746	0.0	49	0.0	80	0.0
Brain	3140	0.0	829	0.0	ND	ND	ND	ND
Eye	520	0.0	432	0.0	6.4	0.0	21	0.0
Lymph node	9400	0.0	15300	0.0	439	0.0	356	0.0
Adrenals	2800	0.0	4590	0.0	74	0.0	234	0.0
Thyroid	3000	0.0	7420	0.0	ND	ND	ND	ND
Harderian gland	16000	0.1	13700	0.1	1750	0.0	1340	0.0
Ovary			5740	0.0			187	0.0
Muscle	1130	0.0	1220	0.0	25	0.0	19	0.0
Fat	5330	0.0	2850	0.0	1050	0.0	956	0.0
Skin	1120	0.9	607	0.4	795	0.9	475	0.3
Carcass	1730	3.8	1880	3.9	113	0.3	117	0.3
Bone	182	0.0	111	0.0	ND	ND	ND	ND
total		58.2		53.5		1.4		0.8

ND results within background range
0.0 results <0.05%.

Conclusions

The absorption, distribution and excretion of ¹⁴C-pyridaben was investigated after a single oral dose of 30 mg/kg bw. Oral absorption was found to ≤24%, but might have been higher because of the fact that some of the absorbed pyridaben might have been excreted via the entero-hepatic circulation (no bile-duct cannulation was applied). After 168 hours the majority of administered radioactivity was excreted in faeces 87-97% AR after [3,6-pyridazinone-ring ¹⁴C]-pyridaben and 74-78% AR after [benzyl ring ¹⁴C]-pyridaben, whilst radioactivity in urine accounted for 3-7% AR after dosing [3,6-pyridazinone-ring ¹⁴C]-pyridaben and 13-24% after dosing [benzyl ring ¹⁴C]-pyridaben. Peak ¹⁴C-concentrations in blood were observed after 24 hours in both sexes. The elimination half-life at 30 mg/kg as [3,6-pyridazinone-ring ¹⁴C]-pyridaben (49-56 hours for both sexes) was longer than that for [benzyl ring ¹⁴C]-pyridaben (18-24 hours). Radioactivity was concentrated mainly in the gastro-intestinal tract and further widely distributed with highest (relative) amounts in liver, lymph nodes,

uterus and ovary after 24 hours (harderian glands after [benzyl ring ^{14}C]-pyridaben). At 168 hours post-dose, radioactivity concentrations in tissues was on average 0.1-1.4% of AR. Blood and plasma concentrations decreased to 0-1% of those at 24 hours. There were no remarkable differences between tissue levels and depletion rates of male and female rats. In both sexes tissue levels of radioactivity were slightly higher after [benzyl ring ^{14}C]-pyridaben treatment.

Acceptability

The study is acceptable.

Remarks include variations between radioactivity levels in individual animals (urine, faeces, tissues, organs, blood) and the omission of bile-duct cannulation from the study design.

STUDY 4

Characteristics

reference	: Hallifax, 1994b	exposure	: single by gavage
type of study	: Metabolism	doses	: 30 mg/kg bw
year of execution	: 1989-1992	vehicle	: 1% Tween-80 at 2 mL
test substances	: Non-labelled pyridaben (NC-129), lot no. 129T8604, 99.6% pure. [3,6-pyridazinone-ring ^{14}C]-pyridaben: lot no. CP-928, chemical purity not reported, radiochemical purity $\geq 99.5\%$. [benzyl ring ^{14}C]-pyridaben: lot no. CP-927, chemical purity not reported, radiochemical purity $\geq 99.6\%$.	GLP statement	: yes
route	: oral	guideline	: OECD 417 (1981), MAFF (1985), EPA/FIFRA (1982)
species	: CD rat (180-200 g)	acceptability	: Supplementary
group size	: see table 6.1.1-06		

Study design

The metabolism of ^{14}C -pyridaben was investigated in urine and faeces (0-72 hour samples pooled per rat) and in liver samples collected 24 hours after dosing from 3 rats of the first four groups in the previous study (see Table 6.1.1-06), which had received a single oral dose of 30 mg/kg bw of 3,6-pyridazinone-ring ^{14}C -pyridaben or [benzyl ring ^{14}C]-pyridaben.

Pooled samples of urine and faeces were divided into an 80% portion for extraction and a 20% portion for enzyme hydrolysis.

All radioactivity is indicated as percentage of the initially applied dose. In case metabolites could not be separated this is indicated by a slash (/) between metabolite codes. In case metabolites were separated, but were included into a larger fraction (of % AR) this is indicated by a “;” between metabolite codes.

Extraction

The 80% portion of urine was extracted with ethyl acetate and concentrated. The concentrated extract (pooled per rat) was analysed by TLC. From the aqueous phase (combined for each sex) 50% was acidified and 50% was made alkaline. Both portions were refluxed, extracted with ethyl acetate and analysed by TLC.

Faeces (80% portion) and was mixed with methanol and centrifuged. Extracts were combined, concentrated, acid extracted with ethyl acetate and analysed by TLC. For the aqueous phase the procedure as described for urine was followed.

Liver was homogenised with methanol. The mixture was centrifuged and the combined extracts were concentrated and acid extracted with ethyl acetate. Samples were analysed by TLC as described for faeces. A second TLC analysis was performed on all individual animal samples. The remaining material at origin was removed with methanol and re-chromatographed with a different solvent system. In addition metabolites were identified by two-dimensional TLC in combined samples per sex.

Hydrolysis

The remaining portion of the samples of urine and faeces (20%) were pooled per sex and incubated with β -glucuronidase/sulphatase twice. The incubation medium was extracted with ethyl acetate and analysed by TLC. Distinction between enzymatic and non-enzymatic hydrolysis was possible by inclusion of a control sample incubated in absence of β -glucuronidase/sulphatase.

Results

Urine

Fractionation recovery of radioactivity in urine was 5.8% AR (males) and 2.2% AR (females) after treatment with 30 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben (mass balance 95% (males) and 79% (females) of initial TRR). Radioactivity in ethyl acetate extracts from urine of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben showed mean concentrations of 3.0% AR and 1.4% AR in males and females, respectively. In the ethyl acetate fraction 8-9 metabolites were found including P-3 (<0.06% AR) in females and parent, PB-3 (0.1-0.6% AR), P-1 and PB-11 (each <0.22% AR) in males. Methanol soluble metabolites (6 unidentified) extracted from the origin of the TLC plates accounted for 0.8-2.8% of AR. Aqueous extracts accounted for 2.8% AR (males) and 0.8% AR (females). Acid or alkali hydrolysed pooled aqueous samples contained $\leq$ 0.9% AR (in ethyl acetate extract). From the 5 (alkali hydrolysed) to 9 (acid hydrolysed) metabolites PB-12 (<0.13% AR) and very minor amounts of parent, PB-3 and PB-9 (each <0.06% AR) were identified.

Recovery of radioactivity in urine was 18.8% AR (males) and 11.6% (females) after treatment with 30 mg/kg bw [benzyl ring¹⁴C]-pyridaben (mass balance fractions 92-107% of initial TRR in urine). Total reactivity recovered in ethyl acetate extracts was 8.7 and 2.6% AR in males and females, respectively. Up to 15 metabolites were found in the ethyl acetate extracts: B-7, B-8, B-11, PB-12, PB-11 (one female), PB-7/B3 (females), B-1 (females) and parent (females) were identified among these metabolites. In extracts from the aqueous fraction 10.1% AR (males) and 7.9% AR (females) was found, which was not further analysed. Methanol soluble metabolites (up to 6 unidentified and B-8 in males and 2-3 unidentified in females) accounted for 2.6-4.3% AR in males and 0.2-0.3% AR in females. In aqueous (acid or alkali hydrolysed) samples 15 metabolites were found accounting for 2.8-6.5% AR (males) and 1.0-4.2% AR (females). Metabolites identified included B-1, B-3 (females), B-8, B-11 (males) and PB-12 (males)(individual all

estimated <0.5% AR). Due to poor separation on the TLC plates individual metabolites could not be accurately expressed as %TTR.

In extracts from urine of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben followed by hydrolysis with β -glucuronidase/sulphatase 2.5-5.5% AR was recovered from ethyl acetate and extracts from the aqueous fraction. In the ethyl acetate extract 4 unidentified metabolites were found (total 1.5-3.0% AR) and in addition PB1/PB-3 (males), P-1(males) and PB-12 (<0.14% AR) were identified. No difference in metabolite extraction between hydrolysed and non-hydrolysed control samples became apparent. Aqueous extracts were not further analysed. After treatment with [benzyl ring¹⁴C]-pyridaben 10-18% AR was recovered. Ten metabolites were found in ethyl acetate extracts from hydrolysed samples (11.3% AR in males and 4.1% AR in females). Six were identified as parent (males), B-1 (females), B-3 (males), B-7 (males), B-8, B-11 (males) and PB-12 (females) and were present at estimated individual concentrations <2.0% AR. No differences in metabolite extraction pattern between hydrolysed and non-hydrolysed samples were found.

Metabolite identification using two-dimensional TLC gave the following results: P-9 after [3,6-pyridazinone-ring¹⁴C]-pyridaben treatment and B-1/B-3, B-7, B-8, B-11, B-15, PB-1(females) and PB-12 (females) after [benzyl ring¹⁴C]-pyridaben. Figures of TLC plates were incorporated in the report, but no further details were included.

Faeces

Fractionation recovery of radioactivity in faeces was 61-91% AR after treatment with 30 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben (mass balance 60% (males) and 91% (females) of initial TRR in faeces). Total radioactivity in faecal ethyl acetate extracts from rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben showed mean concentrations of 35% AR (range 17-50%) and 62% AR (range 46-93%) in males and females, respectively. In the ethyl acetate fraction the parent accounted for 9.5-38% AR in males and for 26-82% AR in females. In addition low amounts of PB-9 (<2%), PB-4 (<2%), P-1 (males), PB-11 (one female) and PB-12 (males) and 8-9 unidentified metabolites were found. Methanol soluble metabolites included PB-12 (0.9% AR in females) and 8-12 unidentified (total accounted for 5.9% AR (males) and 6.3% AR (females)). Aqueous extracts accounted for 8.5% AR (males) and 11.4% AR (females). In residue was 17.5% AR (range 6-27%) was recovered. Acid or alkali hydrolysed pooled aqueous samples ranged from 2.8-5.8% AR (in ethyl acetate extract). Only PB-9 (<0.3% AR) and PB-4 (females 0.09% AR) were identified. Four to seven unidentified metabolites were extracted.

Recovery of radioactivity in faeces after treatment with 30 mg/kg bw [benzyl ring¹⁴C]-pyridaben was 57-60% AR (mass balance fractions 81% of initial TRR in faeces). Total reactivity recovered in ethyl acetate extracts was 34 and 29% AR in males and females, respectively. Up to 18 metabolites were found in the ethyl acetate extracts: parent, B-1, B-3, B-7, B-8 (males), B-11, PB-9, PB-11, PB-12 and D-1 were identified among these metabolites. Due to poor separation on the TLC plates individual metabolites could not be accurately expressed as %TTR. Methanol soluble metabolites (6-7 unidentified, B-11 in two males and B-8/PB-12) accounted for <6.2% AR. In extracts from the aqueous fraction 10.2% AR (males range 6-17%) and 19.4% AR (females range 8-29%) was found, which was not further analysed. In residue 12-13% AR

was recovered. In acid or alkali hydrolysed pooled aqueous samples 3.3-4.2% AR (males) and 8.6-9.6% AR (females) was recovered. Metabolites identified included parent (males), B-3, B-7, B-8, B-11 (females), PB-3 (females), PB-9 and PB-11 (all <2.5% AR, except PB-3 5.0-5.9%).

In extracts from faeces of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben followed by hydrolysis with β -glucuronidase/sulphatase 20-25% AR was recovered from ethyl acetate extracts. The aqueous fraction contained 21 and 36% AR in faeces of males and females, respectively. Residue was 12% AR. In the ethyl acetate extract PB-12/P-3 (1.6% in males), PB-1/PB-3 (8.2%AR males, 16.2% AR females), PB-9;PB-7 (males <2.9% AR), PB-12/P-3;PB-9;PB-7 (<4.8% in females) and 6-7 unidentified metabolites were found (total 1.2-1.3% AR). No difference in metabolite extraction between hydrolysed and non-hydrolysed control samples became apparent. Aqueous extracts were not further analysed. After treatment with [benzyl ring¹⁴C]-pyridaben 30-33% AR was recovered from ethyl acetate extracts, 8.6-14% AR from aqueous extracts and 9-10% in residue. Thirteen to fifteen metabolites were found in ethyl acetate extracts from hydrolysed samples including 7 unidentified. The following metabolites were identified: B-1, B-7, B-8 (males), B-11, PB-4 (females), PB-7 (females), PB-9 (males), PB-11 (males), PB-12 and D-1 (males). PB-12;B-7 was recovered in a concentration of 3.5-4.4% AR. Some fractions of unidentified metabolites were present for >15% AR. Due to poor separation on the TLC plates individual metabolites could not be accurately expressed as %TTR. No major differences in metabolite extraction pattern between hydrolysed and non-hydrolysed samples were found.

Metabolite identification using two-dimensional TLC gave the following results: PB-1, PB-9, PB-11 and PB-12 after [3,6-pyridazinone-ring¹⁴C]-pyridaben treatment and B-8, B-11, B-15 (males), PB-1, PB-7 (males), PB-9, PB-11 and PB-12 after [benzyl ring¹⁴C]-pyridaben. Figures of TLC plates were incorporated in the report, but no further details were included.

Liver

The total amount of radioactivity recovered in the liver of rats receiving a single oral dose of [3,6-pyridazinone-ring¹⁴C]-pyridaben was 0.6-0.8% AR and after [benzyl ring¹⁴C]-pyridaben was 1.5-4.0% AR. Treatment with [3,6-pyridazinone-ring¹⁴C]-pyridaben gave mean recoveries of radioactivity of 0.22% AR (males and females) in ethyl acetate extracts, 0.35% AR in aqueous extracts and 0.19% AR in unextractable residue. Up to 6 unknown metabolites were found (<0.11% AR) in ethylacetate extracts.

For [benzyl ring¹⁴C]-pyridaben recoveries were 1.7% AR (males) and 1.1% AR (females) for ethyl acetate extracts, 1.0 and 0.5% AR (males and females) for aqueous extracts and residue accounted for 0.38% AR and 0.25% AR for males and females, respectively. In males B-7/PB-12; PB-9 accounted for 1.3% AR and in females low amounts of B-7 and B-8 were reported. In addition 5-8 unidentified metabolites were present (each <0.6% AR).

Metabolite identification using two-dimensional TLC gave the following results: parent after [3,6-pyridazinone-ring¹⁴C]-pyridaben treatment and parent, B-1/B-3, PB-4 and B-11 after [benzyl ring¹⁴C]-pyridaben. Figures of TLC plates were incorporated in the report, but no further details were included.

Conclusions

In 0-72 hour urine of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben, the metabolite pattern was essentially the same for both sexes (total 1.4-3.0% AR). Metabolites identified included P-1, P-3, PB-3, PB-9 and PB-11. Only P-9 was identified by two-dimensional TLC, but the results of these analyses were poorly reported. The parent compound and PB-12 were identified in urine after alkali hydrolysis. Up to 9 unidentified metabolites were found. After treatment with [benzyl ring¹⁴C]-pyridaben (total AR 2.6-8.7%) several of the 15 metabolites found were identified: parent, B-1, B-7, B-8, B-11, PB-11, PB-12, PB-7/B3 in ethyl acetate extracts. After acid or alkaline and enzymatic hydrolysis no additional metabolites were identified. Two-dimensional TLC confirmed parent, B-1/B-3, B-7, B-8, B-11 and PB-12 and newly identified B-15. No major differences in the metabolites identified in both sexes were found.

In 0-72 hour faeces of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben 61-91% AR was recovered after fractioning. In ethyl acetate extracts parent (10-82% AR), PB-9 (<2% AR), PB-4 (<2% AR), P-1 and PB-12 and 8-9 unidentified metabolites were found. After hydrolysis very minor amounts of PB-4 were reported. The metabolite pattern after enzymatic hydrolysis was similar to that seen in non-hydrolysed samples. Recovery ranges between animals differed 2-4 fold. Two-dimensional TLC analysis identified parent, PB-9, PB-11 and PB-12. Treatment with [benzyl ring¹⁴C]-pyridaben (total 57-60% AR in faeces fractions) gave parent (up to 22% AR), B-1, B-3, B-7, B-8, B-11, PB-9, PB-11, PB-12 and D-1 in the ethyl acetate extracts and included PB-4 after enzymatic hydrolysis. Up to 4 unidentified metabolites were found. Using two-dimensional TLC analysis additional metabolites were identified: PB-7 and B-15 and parent, B-8, B-11, PB-9, PB-11 and PB-12 were confirmed. No differences between male and female metabolite profile were found.

In liver collected from animals sacrificed 24 hours after a single dose of 30 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben gave up to 6 unknown metabolites (each <0.11% AR). For [benzyl ring¹⁴C]-pyridaben treated rats B-7/PB-12; PB-9 accounted for 1.3% AR and in females only low amounts of B-7 and B-8 were reported. In addition 5-8 unidentified metabolites were present (each <0.6% AR). Two dimensional TLC analysis identified parent after [3,6-pyridazinone-ring¹⁴C]-pyridaben and parent, B-1/B-3, PB-4 and B-11 after [benzyl ring¹⁴C]-pyridaben.

Acceptability

- (1) Metabolite identification was based on TLC only. HPLC confirmation was part of the protocol, but not performed in this study due to lack of a suitable method. Instead two-dimensional TLC analysis was used to confirm metabolite identity. This confirmation was incomplete and poorly reported.
- (2) Metabolite identification was insufficiently investigated due to incomplete separation on the TLC plates and evaluation of metabolites as groups, when spots could not be resolved by the linear analyser.
- (3) Mass balances were not maintained adequately. This means that not all radioactivity was recovered.
- (4) Overall evaluation by RMS. Considering the comments under point 1 - 3, the study is considered supplementary

STUDY 5

Characteristics

reference	: Rhenius, 1989c	exposure	: repeated by gavage
type of study	: Absorption, distribution and excretion	doses	: 3 mg/kg bw
year of execution	: 1988/1989	vehicle	: 1% Tween-80 at 5 mL/kg bw
test substances	: Non-labelled pyridaben (NC-129), lot no. 129T8604, 99.6% pure. [3,6-pyridazinone-ring ¹⁴ C]-pyridaben: lot no. CP-928, chemical purity not reported, radiochemical purity ≥99.5%. [benzyl ring ¹⁴ C]-pyridaben: lot no. CP-927, chemical purity not reported, radiochemical purity ≥99.6%.	GLP statement	: yes
route	: oral	guideline	: OECD 417 (1981), JMAFF (1985), EPA/FIFRA (1982)
species	: CD rat (180-200 g)	acceptability	: acceptable
group size	: see table 6.1.1-11		

Study design

The absorption, tissue distribution and excretion of ¹⁴C-pyridaben was investigated after oral dosing with 3 mg/kg bw non-radiolabelled pyridaben for 14 days and a single dose of 3 mg/kg bw of 3,6-pyridazinone-ring ¹⁴C-pyridaben or [benzyl ring ¹⁴C]-pyridaben on day 15. All animals received food *ad libitum* throughout the study. Exposure and sampling of urine, cage wash, faeces, blood and tissues was as described in table 6.1.1-11.

Table 6.1.1-11 Experimental groups for each dose level

Group no.	No. of animals	Treatment	Sampling times (h after last dosing)	Sacrifice time (h after last dose)
1	5 M & 5 F	14 day 3 mg/kg unlabelled pyridaben and a single oral dose at 3 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben on day 15 (actual 1.9(M), 1.9(F) mg/kg)	0-12, 12-24 hours: urine, 0-24: faeces and expired air, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours: urine, faeces; 168: cage wash & carcass	168
2	3 M & 3 F	14 day 3 mg/kg unlabelled pyridaben and a single oral dose at 3 mg/kg [benzyl ring ¹⁴ C]-pyridaben on day 15 (actual 2.8(M), 2.9(F) mg/kg)	0-12, 12-24 hours: urine, 0-24: faeces and expired air, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours: urine, faeces; 168: cage wash & carcass	168
3	5 M & 3 F	14 day 3 mg/kg unlabelled pyridaben and a single oral dose at 3 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben on day 15 (actual 2.4(M), 2.3(F) mg/kg)	0.25, 1, 2, 3, 4, 6, 9, 12, 24, 48, 72, 96, 120, 144, 168 hours: blood	168
4	3 M & 3 F	14 day 3 mg/kg unlabelled pyridaben and a single oral dose at 3 mg/kg [benzyl ring ¹⁴ C]-pyridaben on day 15 (actual 2.6(M), 2.8(F) mg/kg)	0.25, 1, 2, 3, 4, 6, 9, 12, 24, 48, 72, 96, 120, 144, 168 hours: blood	168
5	5 M & 5 F	14 day 3 mg/kg unlabelled pyridaben and a single oral dose at 3 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben on day 15 (actual 1.9(M), 1.9(F) mg/kg)	n.a.	168 ^(A)
6	3 M & 3 F	14 day 3 mg/kg unlabelled pyridaben and a single oral dose at 3 mg/kg [benzyl ring ¹⁴ C]-pyridaben on day 15 (actual 2.5(M), 3.0(F) mg/kg)	n.a.	168 ^(A)

n.a. = not applicable

(A) At 168 hours post-dose, the following tissues and organs were taken from three animals (group 5 five at 168 h): blood, liver, kidney, heart, lung, bone (femur), brain, fat (abdominal), skeletal muscle, testes (male), ovaries (female), spleen, adrenal glands, residual carcass, eyes, hardier gland, intestinal tract, intestinal tract contents, mesenteric lymph nodes, pancreas, salivary gland, skin, stomach, thymus, thyroid, uterus (female).

Radioactivity in urine, plasma and cage washings was quantified by LSC. Radioactivity in faeces (homogenised) was quantified by combustion/LSC. Tissues, organs, tissue and organ homogenates and blood were solubilised and radioactivity was quantified by LSC. Radioactivity in expired air was trapped with 2M NaOH and analysed by LSC.

Results

Radiochemical analysis of the test compound post dosing showed that radiochemical purity of ^{14}C -pyridaben was between 97.3 and 100.2%. Achieved doses were 83-100 % of nominal for [benzyl ring ^{14}C]-pyridaben, 63% for [3,6-pyridazinone-ring ^{14}C]-pyridaben in group 1 and 5 and 77-80% in group 3. No information on actual dose levels of the unlabelled compounds was available.

One female in group 4 died on day 11 of the study (considered un-related to treatment).

Mass balance

After repeated oral dosing of 3 mg/kg bw after 168 hours the majority of administered radioactivity was excreted in faeces (93-95% AR), whilst radioactivity in urine accounted for 4% AR after dosing [3,6-pyridazinone-ring ^{14}C]-pyridaben (Table 6.1.1.-12). For [benzyl ring ^{14}C]-pyridaben radioactivity in faeces accounted for 83-85% AR and urine for 11-19% AR. Radioactivity in expired air was <0.07% AR. Excretion was essentially completed (91-100% AR for [3,6-pyridazinone-ring ^{14}C]-pyridaben and 88-96% AR for [benzyl ring ^{14}C]-pyridaben) after 96 hours. Radioactivity in skin and residual carcass at 168 hours post-dose represented 0.1-0.8% AR. For [3,6-pyridazinone-ring ^{14}C]-pyridaben recovery was 98-99% AR and for [benzyl ring ^{14}C]-pyridaben) 95-105% AR. From the results of this study, oral absorption is estimated to be $\leq 19\%$.

Table 6.1.1-12 Excretion and retention of radioactivity (% AR) in rats after repeated oral exposure to ¹⁴C-pyridaben

sample		3 mg/kg bw [3,6-pyridazinone-ring ¹⁴ C]-pyridaben		3 mg/kg bw [benzyl ring ¹⁴ C]-pyridaben	
		M	F	M	F
urine	0-12 h	1.6	1.7	3.9	4.0
	12-24 h	0.6	0.7	3.5	3.0
	24-48 h	0.9	0.7	6.0	2.6
	48-72 h	0.6	0.4	2.7	0.7
	72-96 h	0.3	0.1	1.3	0.3
	96-120 h	0.2	0.07	0.7	0.2
	120-144 h	0.1	0.05	0.3	0.1
	144-168 h	0.05	0.03	0.2	0.09
0-168 h		4.4	3.7	18.6	11.0
faeces	0-24 h	59.4	60.3	36.7	58.4
	24-48 h	19.3	25.6	29.5	20.8
	48-72 h	6.7	7.0	9.9	3.2
	72-96 h	0.6	1.4	3.6	0.7
	96-120 h	3.4	0.3	2.1	0.2
	120-144 h	2.5	0.3	1.1	0.08
	144-168 h	0.9	0.3	1.7	0.06
0-168 h		92.8	95.2	84.6	83.3
expired air	0-24 h	<0.07	<0.07	<0.05	<0.05
Skin and carcass	168 h	0.3	0.07	0.7	0.4
cage wash	168 h	0.1	0.07	0.6	0.1
Recovery	168 h	97.5	99.0	104.5	94.8

Plasma pharmacokinetic parameters

The pharmacokinetic parameters are presented in Table 6.1.1-13. Peak ¹⁴C-concentrations in blood were observed between 1-2 and 12 hours in both sexes. No differences between animals dosed with [3,6-pyridazinone-ring ¹⁴C]-pyridaben and [benzyl ring ¹⁴C]-pyridaben were found. Individual concentrations measured at the different time points differed substantially. The elimination half-life at 3 mg/kg bw as [3,6-pyridazinone-ring ¹⁴C]-pyridaben (60-70 hours for both sexes) was longer than that for [benzyl ring ¹⁴C]-pyridaben (12 hours).

Table 6.1.1-13 Pharmacokinetic parameters in blood of male and female rats after repeated oral exposure to ¹⁴C-pyridaben

	3 mg/kg bw [3,6-pyridazinone-ring ¹⁴ C]-pyridaben		3 mg/kg bw [benzyl ring ¹⁴ C]-pyridaben	
	M	F	M	F
C _{max} (ng eq./ml)	43	41	38	65*
T _{max} (h)	2-12	2-12	1-12	1-12
T _{1/2} (h) ^(A)	60-70		12	12

* High value might be due to a higher dose given to the female animals

(A) no definitive pharmacokinetic parameters

Distribution in tissues

The distribution of radioactivity in tissues of rats sacrificed at 168 hours post-dose is presented in table 6.1.1-14. In general a large inter-individual variation of radioactivity concentrations measured in the organs and tissues was observed.

Minor amounts of radioactivity were found in the gastro-intestinal tract 168 hours after the last dose with [3,6-pyridazinone-ring¹⁴C]-pyridaben. In the study with [benzyl ring¹⁴C]-pyridaben radioactivity was mainly recovered from the GI-tract, liver, pancreas, eye (males), lymph nodes, Harderian gland and skin (all $\leq$ 0.1% AR). Small amounts were present in fat after both compounds. There were no clear differences between males and females. After [benzyl ring¹⁴C]-pyridaben more radioactivity was recovered from the GI-tract and tissue levels were slightly higher than after [3,6-pyridazinone-ring¹⁴C]-pyridaben.

Conclusions

The absorption, distribution and excretion of pyridaben was investigated after a repeated oral dosing with 3 mg/kg bw and a single dose of radiolabelled ¹⁴C-pyridaben. Oral absorption was found to $\leq$ 19%, but might have been higher because of the fact that some of the absorbed pyridaben might have been excreted via the entero-hepatic circulation (no bile-duct cannulation was applied). After 168 hours the majority of administered radioactivity was excreted in faeces (93-95% AR), whilst radioactivity in urine accounted for 4% AR after dosing [3,6-pyridazinone-ring¹⁴C]-pyridaben. For [benzyl ring¹⁴C]-pyridaben radioactivity in faeces accounted for 83-85% AR and urine for 11-19% AR. Peak ¹⁴C-concentrations in blood were observed between 1-2 and 12 hours after both compounds in both sexes. The elimination half-life at 3 mg/kg as [3,6-pyridazinone-ring¹⁴C]-pyridaben (60-70 hours) was longer than that for [benzyl ring¹⁴C]-pyridaben (12 hours). Radioactivity 168 post-dosing was low and concentrated mainly in the gastro-intestinal tract (concentrations higher after [benzyl ring¹⁴C]-pyridaben). There were no remarkable differences between tissue levels of male and female rats.

Acceptability

The study is acceptable.

Remarks include the lack of accuracy of the achieved doses, variations between radioactivity levels in individual animals (tissues, organs, blood) and the omission of bile-duct cannulation from the study design.

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Table 6.1.1-14 Distribution of radioactivity in tissues and organs [ng pyridaben equivalents/g and % AR] at 168 hours post dose of 3 mg/kg [3,6-pyridazinone-ring¹⁴C]-pyridaben and [benzyl ring¹⁴C]-pyridaben

tissue	3 mg/kg bw [3,6-pyridazinone-ring ¹⁴ C]-pyridaben				3 mg/kg bw [benzyl ring ¹⁴ C]-pyridaben			
	male		female		male		female	
	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR
Blood (ng eq./mL)	6.3		5.7		ND		ND	
Plasma (ng eq./mL)	ND		ND		ND		1.2	
Liver	1.2	0.0	ND	ND	18.0	0.0	30.8	0.1
GIT- content	4.8	0.0	3.5	0.0	16.9	0.0	13.3	0.0
Stomach	ND	ND	ND	ND	ND	ND	4.1	0.0
Small intestine	0.9	0.0	1.5	0.0	16.1	0.0	10.6	0.0
Large intestine	1.4	0.0	1.1	0.0	17.9	0.0	12.5	0.0
Pancreas	ND	ND	ND	ND	3.4	0.0	7.9	0.0
Spleen	ND	ND	ND	ND	ND	ND	ND	ND
Kidney	1.1	0.0	2.2	0.0	ND	ND	1.9	0.0
Testis			ND	ND			ND	ND
Uterus	ND	ND			ND	ND		
Lung	ND	ND	ND	ND	ND	ND	2.1	0.0
Heart	ND	ND	ND	ND	ND	ND	ND	ND
Thymus	ND	ND	ND	ND	ND	ND	ND	ND
Sal. Gland	ND	ND	ND	ND	ND	ND	ND	ND
Brain	ND	ND	ND	ND	ND	ND	ND	ND
Eye	ND	ND	ND	ND	91.8	0.0	0.5	0.0
Lymph node	0.6	0.0	5.3	0.0	25.6	0.0	21.3	0.0
Adrenals	ND	ND	ND	ND	ND	ND	9.5	0.0
Thyroid	ND	ND	ND	ND	ND	ND	ND	ND
Harderian gland	ND	ND	ND	ND	213	0.0	66.9	0.0
Ovary			2.7	0.0			10.9	0.0
Muscle	ND	ND	ND	ND	ND	ND	ND	ND
Fat	10.7	0.0	11.0	0.0	27.7	0.0	29.4	0.0
Skin	ND	ND	1.9	0.0	24.6	0.2	18.8	0.1
Carcass	ND	ND	ND	ND	4.6	0.1	1.5	0.0
Bone	ND	ND	ND	ND	ND	ND	ND	ND
Total		0.0		0.0		0.3		0.2

ND results within background range
0.0 results <0.05%.

tissue	3 mg/kg bw [3,6-pyridazinone-ring ¹⁴ C]-pyridaben				3 mg/kg bw [benzyl ring ¹⁴ C]-pyridaben			
	male		female		male		female	
	ng/g	% AR	ng/g	% AR	ng/g	% AR	ng/g	% AR
Blood (ng eq./mL)	6.3		5.7		ND		ND	
Plasma (ng eq./mL)	ND		ND		ND		1.2	
Liver	1.2	0.0	ND	ND	18.0	0.0	30.8	0.1
GIT- content	4.8	0.0	3.5	0.0	16.9	0.0	13.3	0.0
Stomach	ND	ND	ND	ND	ND	ND	4.1	0.0
Small intestine	0.9	0.0	1.5	0.0	16.1	0.0	10.6	0.0
Large intestine	1.4	0.0	1.1	0.0	17.9	0.0	12.5	0.0
Pancreas	ND	ND	ND	ND	3.4	0.0	7.9	0.0
Spleen	ND	ND	ND	ND	ND	ND	ND	ND
Kidney	1.1	0.0	2.2	0.0	ND	ND	1.9	0.0
Testis			ND	ND			ND	ND
Uterus	ND	ND			ND	ND		
Lung	ND	ND	ND	ND	ND	ND	2.1	0.0
Heart	ND	ND	ND	ND	ND	ND	ND	ND
Thymus	ND	ND	ND	ND	ND	ND	ND	ND
Sal. Gland	ND	ND	ND	ND	ND	ND	ND	ND
Brain	ND	ND	ND	ND	ND	ND	ND	ND
Eye	ND	ND	ND	ND	91.8	0.0	0.5	0.0
Lymph node	0.6	0.0	5.3	0.0	25.6	0.0	21.3	0.0
Adrenals	ND	ND	ND	ND	ND	ND	9.5	0.0
Thyroid	ND	ND	ND	ND	ND	ND	ND	ND
Harderian gland	ND	ND	ND	ND	213	0.0	66.9	0.0
Ovary			2.7	0.0			10.9	0.0
Muscle	ND	ND	ND	ND	ND	ND	ND	ND
Fat	10.7	0.0	11.0	0.0	27.7	0.0	29.4	0.0
Skin	ND	ND	1.9	0.0	24.6	0.2	18.8	0.1
Carcass	ND	ND	ND	ND	4.6	0.1	1.5	0.0
Bone	ND	ND	ND	ND	ND	ND	ND	ND
Total		0.0		0.0		0.3		0.2

ND results within background range
0.0 results <0.05%.

STUDY 6

Characteristics

reference	: Hallifax, 1994c	exposure	: 15 days by gavage
type of study	: Metabolism	doses	: 3 mg/kg bw
year of execution	: 1989-1992	vehicle	: 1% Tween-80 at 2 mL
test substances	: Non-labelled pyridaben (NC-129), lot no. 129T8604, 99.6% pure. [3,6-pyridazinone-ring ¹⁴ C]-pyridaben: lot no. CP-928, chemical purity not reported, radiochemical purity ≥99.5%. [benzyl ring ¹⁴ C]-pyridaben: lot no. CP-927, chemical purity not reported, radiochemical purity ≥99.6%.	GLP statement	: yes
route	: oral	guideline	: US EPA 85-1
species	: CD rat (180-200 g)	acceptability	: Supplementary
group size	: see table 6.1.1-11		

Study design

The metabolism of ¹⁴C-pyridaben was investigated in urine and faeces (0-48 hour samples pooled per rat) and in liver samples collected 2 hours after dosing from 3 rats of the first four groups in the previous study (see Table 6.1.1-11), which had received 3 mg/kg bw of non-radiolabelled pyridaben for 14 days followed by a single dose of [3,6-pyridazinone-ring¹⁴C]-pyridaben or [benzyl ring¹⁴C]-pyridaben (3 mg/kg bw) on day 15. Pooled samples of urine and faeces were divided into an 80% portion for extraction and a 20% portion for enzyme hydrolysis.

All radio activity is indicated as percentage of the initially applied dose. In case metabolites could not be separated this is indicated by a slash (/) between metabolite codes. In case metabolites were separated, but were included into a larger fraction (of % AR) this is indicated by a “;” between metabolite codes.

Extraction

The 80% portion of urine was extracted with ethyl acetate and concentrated. The concentrated extract (pooled per rat) was analysed by TLC. From the aqueous phase (combined for each sex) 50% was acidified and 50% was made alkaline. Both portions were refluxed, extracted with ethyl acetate and analysed by TLC. Faeces (80% portion) and was mixed with methanol and centrifuged. Extracts were combined, concentrated, acid extracted with ethyl acetate and analysed by TLC. For the aqueous phase the procedure as described for urine was followed.

A second TLC analysis was performed on all individual animal samples. The remaining material at origin was removed with methanol and re-chromatographed with a different solvent system. In addition metabolites in urine and faeces were identified by two-dimensional TLC in combined samples per sex.

Hydrolysis

The remaining portion of the samples of urine and faeces (20%) were pooled per sex and incubated with β-glucuronidase/sulphatase twice. The incubation medium was extracted with ethyl acetate and analysed by TLC. Distinction between enzymatic and non-enzymatic hydrolysis was possible by inclusion of a control sample incubated in absence of β-glucuronidase/sulphatase.

Results

Urine

Fractionation recovery of radioactivity in urine was 3.3-3.4% AR after 14-day treatment with 3 mg/kg bw pyridaben followed by a single dose of 3 mg/kg bw [3,6-pyridazinone-ring ^{14}C]-pyridaben on day 15 (mass balance 107-109% of initial TRR). Total radioactivity in ethyl acetate extracts from urine of rats treated with [3,6-pyridazinone-ring ^{14}C]-pyridaben showed mean concentrations of 1.4% AR and 1.6% AR in males and females, respectively. In the ethyl acetate fraction 5-8 unidentified metabolites (total 0.9-2.3% AR) were found and very small amounts of PB-11 (one female), P-1 (females) and PB-12 were identified (<0.10% AR). Methanol soluble metabolites (16 unidentified and PB-12 (males) and P-3) extracted from the origin of the TLC plates accounted for 0.6-1.9% of AR. Aqueous extracts accounted for 1.9% AR (males) and 1.7% AR (females). Acid or alkali hydrolysed pooled aqueous samples ranged from 0.4-0.6% AR (in ethyl acetate extract). PB-12, P-3, P-1 and PB-9 (<0.06% AR) and 11 unidentified were found.

Recovery of radioactivity in urine was 10-14% AR after treatment with 3 mg/kg bw unlabelled pyridaben for 14 days followed by 3 mg/kg bw [benzyl ring ^{14}C]-pyridaben on day 15 (mass balance fractions 107-108% of initial TRR in urine). Total reactivity recovered in ethyl acetate extracts was 6.8 and 2.4% AR in males and females, respectively. Fourteen metabolites were found in the ethyl acetate extracts: B-1, B-3 (females), B-7, B-8, B-11 and PB-4 (females) (all <2.3% AR) were identified among these metabolites. In extracts from the aqueous fraction 7.5% AR (males) and 8.0% AR (females) was found, which was not further analysed. Methanol soluble metabolites (13 unidentified) and B-1, B-3, B-7/B-8/B-11, PB-12 (one female) and parent/PB-4 (one female) (all <0.3% AR) accounted for <0.7% AR. In aqueous (acid or alkali hydrolysed) samples 15 metabolites were found in concentrations of 1.8-4.4% AR (males) and 0.8-3.9% AR (females). Metabolites identified included parent, B-1 (females), B-3, B-7 (females), B-8, PB-3 (males), PB-4 (females) and PB-7 (all <0.3%).

Due to poor separation on the TLC plates individual metabolites could not be accurately expressed as %TTR.

In extracts from urine of rats treated with [3,6-pyridazinone-ring ^{14}C]-pyridaben followed by hydrolysis with β -glucuronidase/sulphatase 3.0-3.1% AR was recovered from ethyl acetate and extracts from the aqueous fraction. In the ethyl acetate extract PB-12 (males), PB-3 (females) and 5 unidentified metabolites were found (total 1.6-1.8% AR). No major differences in metabolite extraction between hydrolysed and non-hydrolysed control samples became apparent. Aqueous extracts were not further analysed. After treatment with [benzyl ring ^{14}C]-pyridaben 9.4-12.8% AR was recovered. Ten metabolites were found in ethyl acetate extracts from hydrolysed samples (8.0 % AR in males and 3.1% AR in females). Six were identified as B-1, B-3/PB-7 (females), B-7 (females) B-8 (females), PB-3 and PB-11 (males) and were present at concentrations <2.2% AR. No major differences in metabolite extraction pattern between hydrolysed and non-hydrolysed samples were found.

Metabolite identification using two-dimensional TLC gave the following results: none after [3,6-pyridazinone-ring ^{14}C]-pyridaben treatment and parent, B-1 (males), B-1/B-3 (females), B-8, B-11 and PB-4 (females) after

[benzyl ring-¹⁴C]-pyridaben. Figures of TLC plates were incorporated in the report, but no further details were included.

Faeces

Fractionation recovery of radioactivity in faeces was 45-54% AR after 14-day treatment with 3 mg/kg bw pyridaben followed by a single dose of 3 mg/kg bw [3,6-pyridazinone-ring-¹⁴C]-pyridaben (mass balance 79-86% of initial TRR in faeces). Total radioactivity in faecal ethyl acetate extracts from rats treated with [3,6-pyridazinone-ring-¹⁴C]-pyridaben showed mean concentrations of 36% AR (range 14-44%) and 37% AR (range 31-44%) in males and females, respectively. In the ethyl acetate fraction parent (females), PB-3 (males), PB-9 (females), PB-11, PB-12 (males), P-3 (females) and 7-8 unidentified metabolites were found. Methanol soluble metabolites (P-3 (males), PB-7 (males), PB-12 (females) and 11-13 unidentified) accounted for 8.7% AR (males) and 11.5% AR (females). Aqueous extracts accounted for 9.1% AR (males) and 16.7% AR (females). In residue 11% AR (males range 4-22%) and 18% AR (females range 6-27%) was recovered. Acid or alkali hydrolysed pooled aqueous samples ranged from 4-11.5% AR (in ethyl acetate extract). Metabolites included parent (males), PB-3 (females), PB-4 (females), PB-7, PB-9, PB-12, P-1 (females) and P-3 (each <4% AR) and 5-7 unidentified metabolites. Spots poorly resolved by linear analyzer. Recovery of radioactivity in faeces after 14-day treatment with 3 mg/kg bw pyridaben followed by a single dose of 3 mg/kg bw [benzyl ring-¹⁴C]-pyridaben was 35-42% AR (mass balance fractions 65-79% of initial TRR in faeces). Total reactivity recovered in ethyl acetate extracts was 29 and 26% AR (range 23-64%) in males and females, respectively. Up to 12 metabolites were found in the ethyl acetate extracts: parent (12.6-42% AR), B-1, B-8, B-11, PB-9 and PB-11 were identified among these metabolites. Twenty-one methanol soluble metabolites accounted for <15.6% AR. Parent/PB-4, PB-7, PB-12 and B-8 were identified (each <<4% AR). In extracts from the aqueous fraction 13% AR (males) and 8.8% AR (females) was found, which was not further analysed. In residue was 7.7-12% AR was recovered. In acid or alkali hydrolysed pooled aqueous samples 10-69% AR (males) and 13-74% AR (females) was recovered. Metabolites identified included parent, B-1 (females), B-7, B-8, B-11, PB-3, PB-9 (females), PB-11 and PB-12. Due to poor separation on the TLC plates individual metabolites could not be accurately expressed as %TTR.

In extracts from faeces of rats treated with [3,6-pyridazinone-ring-¹⁴C]-pyridaben followed by hydrolysis with β -glucuronidase/sulphatase 23% AR (males) and 38% AR (females) was recovered from ethyl acetate extracts. The aqueous fraction contained 4.8 and 13.2% AR in faeces of males and females, respectively. Residue was 42% AR. In the ethyl acetate extract PB-3, PB-9, PB-12 and P-3 (males) and 4-5 unidentified metabolites were found. No difference in metabolite extraction between hydrolysed and non-hydrolysed control samples became apparent. Aqueous extracts were not further analysed. After treatment with [benzyl ring-¹⁴C]-pyridaben 31% AR (males) and 38% AR (females) was recovered from ethyl acetate extracts, 3.8-4.8% AR from aqueous extracts and 33% (males) and 24% AR (females) in residue. Seven to eight metabolites were found in ethyl acetate extracts from hydrolysed samples including 3-4 unidentified. The following metabolites were identified B-8, B-11 (males), PB-3 (18-22% AR) and PB-11. No major differences in metabolite extraction pattern between hydrolysed and non-hydrolysed samples were found.

Metabolite identification using two-dimensional TLC gave the following results: parent, PB-9, PB-11 and PB-12 after [3,6-pyridazinone-ring¹⁴C]-pyridaben treatment and parent, B-8, B-11, PB-9, PB-11 and PB-12 after [benzyl ring¹⁴C]-pyridaben. Figures of TLC plates were incorporated in the report, but no further details were included.

Conclusions

In 0-48 hour urine of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben (total of AR 3.3-3.4%) the metabolite pattern was essentially the same for both sexes. No metabolites were identified by two-dimensional TLC analysis. PB-12 was identified in all urinary fractions. In addition P-1 and P-3 were identified in methanol soluble and acid hydrolysed fractions. Up to 16 unidentified metabolites were found. After treatment with [benzyl ring¹⁴C]-pyridaben (total AR 10-14%) several metabolites were identified: B-1, B-3, B-7, B-8, B-11 and PB-4 (all <2.3% AR) in ethyl acetate extracts. After acid or alkaline hydrolysis in addition parent, PB-3 and PB-7 were found (<0.3% AR) and after enzymatic hydrolysis PB-11 (<2.2 % AR). Two-dimensional TLC confirmed parent, B-1 (males), B-1/B-3 (females), B-8, B-11 and PB-4 (females). No major differences in the metabolites identified in both sexes were found.

In 0-48 hour faeces of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben 45-54% AR was recovered after fractioning. In ethyl acetate extracts parent, PB-3, PB-7 (methanol), PB-9, PB-11, PB-12 and P-3 were identified and in acid hydrolysed fractions of females P-1 and PB-4 were found. In enzymatic hydrolysed fractions no additional identified metabolites were recovered. Recovery ranges between animals differed up to 5-fold. Two-dimensional TLC analysis identified parent, PB-9, PB-11 and PB-12. Treatment with [benzyl ring¹⁴C]-pyridaben (total 35-42% AR in faeces fractions) gave parent (12.6-42% AR), B-1, B-8, B-11, PB-4 (methanol), PB-7 (methanol), PB-9, PB-11 and PB-12 (methanol) in the ethyl acetate extracts and included B-7 and PB-3 after non-enzymatic hydrolysis. Enzymatic hydrolysis gave only PB-3 (18-22% AR). Using two-dimensional TLC analysis parent, B-8, B-11, PB-9, PB-11 and PB-12 were confirmed.

Acceptability

- (1) Metabolite identification was based on TLC only (no evidence of confirmation by MS or a second chromatographic method). HPLC confirmation was part of the protocol, but not performed in this study due to lack of a suitable method. Instead two-dimensional TLC analysis was used to confirm metabolite identity. This confirmation was incomplete and poorly reported.
- (2) Metabolite identification was insufficiently investigated due to incomplete separation on the TLC plates and evaluation of metabolites as groups, when spots could not be resolved by the linear analyser.
- (3) Mass balances were not maintained adequately. This means that not all applied radioactivity could be recovered.
- (4) Overall evaluation by RMS. Considering the comments under point 1 - 3, the study is considered supplementary.

STUDY 7

Characteristics

reference	: Hallifax, 1994d	exposure	: single by gavage
type of study	: Metabolism	doses	: 30 mg/kg bw
year of execution	: 1994	vehicle	: 1% Tween-80
test substances	: Non-labelled pyridaben (NC-129), lot no. 129T8604, 99.6% pure. [3,6-pyridazinone-ring ¹⁴ C]-pyridaben: lot no. CP-1505, chemical purity not reported, radiochemical purity ≥98.9%. [benzyl ring ¹⁴ C]-pyridaben: lot no. CP-1504, chemical purity not reported, radiochemical purity ≥98.0%.	GLP statement	: yes
route	: oral	guideline	: US EPA 85-1
species	: CD rat (180-220 g)	acceptability	: Partly acceptable
group size	: see table 6.1.1-15		

Study design

The excretion, distribution and metabolism of ¹⁴C-pyridaben was investigated in rats after a single oral dose of 30 mg/kg bw of 3,6-pyridazinone-ring¹⁴C-pyridaben or [benzyl ring¹⁴C]-pyridaben. All animals received food *ad libitum* throughout the study. Exposure and sampling of urine, cage wash, faeces, blood and tissues was as described in table 6.1.1-15.

Table 6.1.1-15 Experimental groups for each dose level

Group no.	No. of animals	Treatment	Sampling times mass balance (h after dosing)	Samples used for metabolite identification
1	15 M & 15 F	Single oral dose at 30 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben	0-24, 24-48, 48-72: urine, faeces and cage wash and debris 72: organs (liver, GI-tract, skin) and carcass	0-24, 24-48, 48-72: urine, and faeces
2	15 M & 15 F	Single oral dose at 30 mg/kg [benzyl ring ¹⁴ C]-pyridaben	0-24, 24-48, 48-72: urine, faeces and cage wash 72: organs (liver, GI-tract, skin) and carcass	0-24, 24-48, 48-72: urine, and faeces
3	5 M & 5 F	Single oral dose at 30 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben	0-24: urine, faeces, bile, organs and carcass, cage wash and debris	0-24: bile
4	5 M & 5 F	Single oral dose at 30 mg/kg [benzyl ring ¹⁴ C]-pyridaben	0-24: urine, faeces, bile, organs and carcass, cage wash and debris	0-24: bile
5	5 M & 5 F	Single oral dose at 30 mg/kg [3,6-pyridazinone-ring ¹⁴ C]-pyridaben	24: plasma, liver, kidney	24: plasma, liver, kidney
6	5 M & 5 F	Single oral dose at 30 mg/kg [benzyl ring ¹⁴ C]-pyridaben	24: plasma, liver, kidney	24: plasma, liver, kidney

For the mass balance studies dosing suspensions, urine, bile, plasma and cage wash were analysed by LSC. Faeces, carcass, skin (two samples), liver, kidney and gastro-intestinal tract were homogenised and solubilised prior to LSC.

Samples of urine, faeces, bile, plasma, liver and kidney (taken at the different time-points) were pooled per sex and extracted with methanol. The amount of radioactivity in the extracts (directly) and/or the remaining residue (after combustion) was determined by LSC. Principle components (metabolites present in >5% AR)

in the extracts were identified by LC-MS after separation by reverse phase HPLC/UV with co-chromatography. Bile components were in addition extracted with ethyl acetate (pH 2 and 10) and radioactivity in the extracts and the aqueous residue was determined after further chromatographic separation by HPLC and two-dimensional TLC. Separate bile samples were subjected to enzymatic hydrolysis with β -glucuronidase/sulphatase (with and without β -glucuronidase inhibitor) for 24 hours at 37°C, extracted with methanol and analysed by HPLC with UV- and radio detection.

Results

No abnormalities were observed during the study with the exception of females in group 1 that showed (not further identified) reversible signs of acute toxicity. Radiochemical analysis of the test compound post dosing showed that radiochemical purity of ^{14}C -pyridaben was between 96.9 and 98.2%. Achieved doses were 95-103% of nominal.

Mass balance

Excretion study (group 1 and 2)

After a single oral dose of 30 mg/kg bw after 72 hours the majority of administered radioactivity was excreted in faeces (67-84% AR), whilst radioactivity in urine accounted for 4-6% AR after dosing [3,6-pyridazinone-ring ^{14}C]-pyridaben and 14-24% after dosing [benzyl ring ^{14}C]-pyridaben (Table 6.1.1-16). Radioactivity in the gastro-intestinal tract accounted for 4-7.2% of the applied dose. In skin and liver <1.5% AR was recovered. Total recovery for 3,6-pyridazinone-ring ^{14}C -pyridaben was 93-96% AR and for [benzyl ring ^{14}C]-pyridaben) 92-103% AR. From the results of this study, oral absorption based on recovered radioactivity in urine, liver, skin and carcass is estimated to be $\leq 27\%$. Bile-duct cannulation was not included in the study design.

Table 6.1.1-16 Recovery of radioactivity (% AR) in rats after single oral exposure to ^{14}C -pyridaben

sample		30 mg/kg bw [3,6-pyridazinone-ring ^{14}C]-pyridaben		30 mg/kg bw [benzyl ring ^{14}C]-pyridaben	
		M	F	M	F
Urine	0-24 h	2.74	1.44	9.40	4.34
	24-48 h	2.85	2.05	12.0	7.16
	48-72 h	0.47	0.60	5.09	2.00
	0-72 h	6.06	4.09	23.5	13.5
Faeces	0-24 h	17.1	23.2	16.2	23.8
	24-48 h	55.3	36.2	34.3	32.2
	48-72 h	11.6	25.2	16.5	13.8
	0-72 h	84.0	79.5	67.0	69.8
Cage wash	0-24 h	0.43	0.66	0.87	0.71
	24-48 h	0.27	0.37	0.78	0.73
	48-72 h	0.09	0.14	0.45	0.37
	0-72 h	0.79	1.7	2.11	1.81
Liver	72 h	0.12	0.12	1.52	0.76
GI-tract	72 h	4.00	7.22	5.31	5.14
Skin	72 h	0.16	0.08	0.96	0.33
Carcass	72 h	0.65	0.95	1.21	0.89
Recovery	72 h	95.8	93.1	102	92.2

Biliary excretion study (group 3 and 4)

The mass balance 24 hours after a single dose of 30 mg/kg bw ^{14}C -pyridaben to bile-duct cannulated rats was 92-98% AR (Table 6.1.1-17). Most radioactivity was recovered from the faeces and the gastro-intestinal tract (56-72% AR). In urine 1.6-4.0% AR was recovered. Radioactivity in bile accounted for 22-30% AR. Differences in percentage AR reached statistical significance between males and females for urine and bile (benzyl ring label) ($p < 0.01$ -0.05) and between both radiolabels for urine and faeces ($p < 0.01$).

Based on the results of this biliary excretion study it can be concluded that absorption via the gastro-intestinal tract is 26-39% AR.

Table 6.1.1-17 Recovery of radioactivity (% AR) in rats after single oral exposure to ^{14}C -pyridaben

sample		30 mg/kg bw [3,6-pyridazinone-ring ^{14}C]-pyridaben		30 mg/kg bw [benzyl ring ^{14}C]-pyridaben	
		M	F	M	F
Bile	0-24	25.6	27.7	22.4	29.9
Urine	0-24 h	2.05	1.56	1.89	4.01
Faeces	0-24 h	29.6	26.4	50.9	17.9
Cage wash	0-24 h	0.12	0.54	0.15	0.64
GI-tract	24 h	31.8	36.3	21.0	38.5
Skin	24 h	0.13	0.09	0.33	0.38
Carcass	24 h	2.76	4.95	1.65	5.06
Recovery	24h	92.1	97.5	98.3	96.4

Plasma, liver kidney study (group 5 and 6)

The amounts of applied radioactivity recovered from liver and kidney after a single dose of [3,6-pyridazinone-ring ^{14}C]-pyridaben were ≤ 0.70 % AR. After [benzyl ring ^{14}C]-pyridaben higher amounts were recovered from the liver (up to 3.7% AR) (Table 6.1.1-18). Plasma concentrations were lower after [3,6-pyridazinone-ring ^{14}C]-pyridaben than after [benzyl ring ^{14}C]-pyridaben. Differences between both radio-labels were significant for liver, kidney and plasma ($p < 0.01$). Differences between the sexes reached statistical significance for liver (benzyl ring label, $p < 0.01$), kidney (both labels, $p < 0.01$ -0.05) and plasma (benzyl ring label, $p < 0.05$)

Table 6.1.1-18 Concentration of radioactivity (ng pyridaben equivalents/g) in rats after single oral exposure to ^{14}C -pyridaben

sample		30 mg/kg bw [3,6-pyridazinone-ring ^{14}C]-pyridaben		30 mg/kg bw [benzyl ring ^{14}C]-pyridaben	
		M	F	M	F
Liver	24 h	3890 (0.70% AR)	4270 (0.69% AR)	19600 (3.67% AR)	10600 (1.62% AR)
Kidney	24 h	2100 (0.09% AR)	1450 (0.04% AR)	2620 (0.10% AR)	2060 (0.06% AR)
Plasma	24 h	386	363	1610	1020

Metabolite identification

Metabolites separated by HPLC were identified by co-chromatography of reference compounds. The following compounds were included as references: parent (PB-1), PB-5, PB-5/PB-7, PB-7, PB-8, PB-9, PB-11, PB-12, PB-13, PB-13/B-3, P-7, B-1, B-1/B-13, B-3, B-7 and B-1. Identification accuracy with this method

proved to be poor and the results can only be considered as indicative. Only some principal components were accurately identified by LC-MS.

After [3,6-pyridazinone-ring¹⁴C]-pyridaben extraction recovery in urine and faeces was 87-100% and 53-97%, respectively. P-7 was identified in urine (0-48 h) and parent (0-72 h), PB-8 (males 0-48 h, females 0-72 h) PB-9 (0-72 h), PB-11 (males 0-48 h) and PB-12 (males 0-72 h, females 0-48 h) in faeces. The principal component in urine of males (unknown 1.64% AR) was identified as P-9 by LC-MS. In faeces of females the parent (20.8% AR) was confirmed by LC-MS (see table 6.1.1-19). Metabolite profiles of males and females were very similar.

After [benzyl ring¹⁴C]-pyridaben extraction recovery in urine and faeces was 88-102% and 60-95%, respectively. B-1/B-13, B-7, B-11 and PB-8 were reported in urine in all samples (males and females 0-72 h). In faeces parent (0-72 h), PB-9 (0-72 h), PB-12 (0-72 h) and B-3 (males 0-72 h) were reported. The principle component in urine of males was confirmed to be B-11 by LC-MS (co-elution with B-15 confirmed by LC-MS). Metabolite profiles of females showed more polar metabolites than that of males. In general, especially faeces metabolites were poorly separated.

Table 6.1.1-19 Metabolite identification (% AR) in urine and faeces of male and female rats after single oral exposure to ¹⁴C-pyridaben

Fraction	% AR in urine				%AR in faeces				Identity
	[3,6-pyridazinone-ring ¹⁴ C]-pyridaben		[benzyl ring ¹⁴ C]-pyridaben		[3,6-pyridazinone-ring ¹⁴ C]-pyridaben		[benzyl ring ¹⁴ C]-pyridaben		
	M	F	M	F	M	F	M	F	
P-7	0.07	0.13							Not specified
B-7			0.37	0.07					2-(4-carboxyphenyl)-2-methylpropionic acid
PB-1					10.8	20.8*	18.8	24.4	parent
PB-8			2.52	1.25	3.33	1.34			5-[4-(1-carboxy-1-methylethyl)benzylthio]-4-chloropyridazin-3(2H)-one
PB-9					1.96	1.80	1.22	1.39	2-tert-butyl-4-chloro-5-[4-(1,1-dimethyl-2-hydroxyethyl)pyridazin-3(2H)-one
PB-11					0.86				5-(4-tert-butylbenzylthio)-4-chloro-2-(1,1-dimethyl-2-hydroxyethyl)pyridazin-3(2H)-one
PB-12					1.01	0.38	0.72	0.58	5-(4-tert-butylbenzylthio)-2-(1-carboxy-1-methylethyl)-4-chloropyridazin-3(2H)-one
B-1/B-13			0.35	0.11					4-tert-butylbenzoic acid/
B-11			6.03*	1.45					2-(4-carboxyphenyl)-2-methyl-1-propanol
B-3								3.64	4-tert-butylbenzylalcohol
no. unidentified ^(A)	15	20	20	17	22	19	27	20	
	(<1%)	(<1%)	(<4%)	(<3%)	(<4.5%)	(<4.3%)	(<3.5%)	(<3.2%)	

(A) values in brackets represent the individual percentages unidentified

* Identification confirmed by LC-MS

In bile of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben PB-8 (2.2-2.7% AR) and PB-9 (0.15% AR in males) was identified and one of the 24 unknowns was present in 6.5% AR (males) and 10.2% AR (females), but could not be identified by LC-MS. After treatment with [benzyl ring¹⁴C]-pyridaben PB-8 (2.3-2.4% AR) and B-13 (1.7-2.0% AR) were found (24-25 unknowns). The principle metabolite (unknown present at 5.5% in males and 4.8% in females) could not be further identified by LC-MS. Recovery extraction in bile was 85-

100% for both compounds. No major differences in metabolite pattern between sexes and different labels were found.

Treatment of bile with hydrolytic enzymes showed more effects in males than in females after 3,6-pyridazinone-ring¹⁴C-pyridaben and similar effects in bile of both sexes after [benzyl ring¹⁴C]-pyridaben. The results are indicative for presence of some hydrolysable metabolites, but no conclusions on their identity can be drawn. The proportion of radio-activity extracted under acidic conditions from was 35-56% of total radioactivity in bile and 1-4% under basic conditions. No further confirmation of identity of metabolites in bile was included

In liver extracts of female rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben very small amounts of the parent compound were identified (0.08% AR). The number of unknowns was 1-4 in both sexes (all <0.2%). After [benzyl ring¹⁴C]-pyridaben B-1 (2.3% males, 0.7% females) and PB-13/B-3 (0.03-0.07%) were found and 1-2 unknowns (<0.15%). No further confirmation of identification was included.

No information on metabolites recovered from kidney and plasma was available.

Conclusions

In 0-72 hour urine of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben (total of AR 4-6%) two metabolites were found: P-7 (not defined) and P-9 (identified by LC-MS). Radioactivity in faeces accounted for 80-84% AR. Metabolites in faeces included parent, PB-8, PB-9, PB-11 and PB-12. The parent was confirmed by LC-MS. Minor amounts of radio activity were found in cage wash, organs (liver, GI-tract (4-7%), skin) and carcass. Total radioactivity recovered was 92-98% AR. In bile-duct cannulated rats receiving [3,6-pyridazinone-ring¹⁴C]-pyridaben amounts of radio activity in urine, cage wash, skin and carcass were <5% AR. Most radio activity was recovered in faeces and GI-contents (61-63% AR). Bile accounted for 26-30% AR with PB-8 as sole identified metabolite. Total absorption from the gastro-intestinal tract was 31-34% AR. In the liver (0.7% AR), skin and plasma low amounts of radioactivity were present, with the parent identified in the liver.

For [benzyl ring¹⁴C]-pyridaben treated rats radio activity in urine accounted for 14-24% of the applied amount (B-1/B-13, B-7, B-11 and PB-8). B-11 (B-15) was confirmed by LC-MS. In faeces 67-70% AR was identified. Main metabolites were parent, PB-9, PB-12 and B-3 (total 18-24% AR). In females in general more polar metabolites were found than in males. After treatment of bile-duct cannulated rats with a single dose of [benzyl ring¹⁴C]-pyridaben faeces and GI-tract contents accounted for 72% AR and 56% AR in males and females, respectively. Uptake into the systemic circulation based on recovery in bile, urine, skin and carcass was 26% in males and 39% in females. In bile (22-30% AR) no metabolites could be identified. In liver B-1 and PB-13/B-3 were present.

In urinary, faecal and biliary fractions used for metabolite identification up to 27 unidentified metabolites (liver only 1-4 unknowns) were present that appeared difficult to separate. Metabolite patterns did not differ substantially between the sexes. Most fractions were < 5% AR. It is clear that extensive metabolism of ¹⁴C-pyridaben occurs, but no clear overview of the exact pathway can be derived from this study.

Acceptability

- (1) Metabolite identification was based on HPLC. For a limited number of compounds evidence of confirmation by LC-MS was provided.
- (2) Metabolites were not completely separated and differences between samples and reference compounds could not be explained based on the available information.
- (3) Overall evaluation by RMS. The mass balance study and the identification of the metabolites confirmed by LC-MS are considered acceptable. All other metabolites were insufficiently identified and thus the results can only be considered preliminary.

Additional information is requested.

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Characteristics

reference	: Haynes, 2005	exposure	: single by gavage
type of study	: Biliary excretion	doses	: 3 mg/kg bw
year of execution	: 2004-2005	vehicle	: 0.5% CMC with 1% Tween at 5 ml/kg
test substances	: Non-labelled pyridaben (NC-129), lot no. 129S020709, purity 99.9%. [3,6-pyridazinone-ring ¹⁴ C]-pyridaben: lot NPE/NAS691/9, chemical purity not reported, radiochemical purity >98%. [benzyl ring ¹⁴ C]-pyridaben: lot NPE/NAS691/12, chemical purity not reported, radiochemical purity >98%.	GLP statement	: yes
route	: oral	guideline	: Not indicated
species	: Han Wistar rat (183-250 g)	acceptability	: acceptable
group size	: 4/sex		

Study design

The absorption and excretion of ¹⁴C-pyridaben was investigated after a single oral dose of 3 mg/kg bw of 3,6-pyridazinone-ring¹⁴C-pyridaben or [benzyl ring¹⁴C]-pyridaben. All ¹⁴C-labelled pyridaben was isotopically diluted with unlabelled pyridaben to the required specific activity. Cannulation of the bile-duct was performed. All animals received food and water *ad libitum* throughout the study. From 4 males and 4 females per dose group urine and faeces were collected over 0-24 and 24-48 hours post-dosing. Bile was collected 0-3, 3-6, 6-9, 9-12, 12-24 and 24-48 hours after dosing. During the first 24 hours salts were replaced via the cannula. After 48 hours animals were sacrificed and the gastro-intestinal tract and liver were removed. Cage wash was performed after rinsing the cages with water (fraction added to urine samples).

Radioactivity in urine, bile and cage washings was quantified by LSC. Radioactivity in faeces was quantified by combustion/LSC (homogenised residue) and LSC (supernatant). Radioactivity in liver and GI-tract homogenates was quantified by combustion/LSC. Carcasses were solubilised and analysed by LSC.

Results

Radiochemical analysis of the test compound post dosing showed that radiochemical purity of [3,6-pyridazinone-ring¹⁴C]-pyridaben was between 98 and 100% and of [benzyl ring¹⁴C]-pyridaben 98.6-99.5%. Achieved doses were >96 % of nominal.

After a single oral dose of 3 mg/kg bw after 48 hours the majority of administered radioactivity was excreted in faeces (45-51% AR) and bile (47-56% AR), whilst radioactivity in urine accounted for 1-2.4% AR after dosing [3,6-pyridazinone-ring¹⁴C]-pyridaben and 4.5-5.7% after dosing [benzyl ring¹⁴C]-pyridaben. Excretion was essentially completed (99-101% AR after 48 hours. From the results of this study, oral absorption is calculated (sum of in urine, bile, liver and carcass) to be 49-57% [3,6-pyridazinone-ring¹⁴C]-pyridaben and 54% for [benzyl ring¹⁴C]-pyridaben.

Table 6.1.1-20 Excretion and retention of radioactivity (% AR) in rats after single oral exposure to ¹⁴C-pyridaben

sample		3 mg/kg bw [3,6-pyridazinone-ring ¹⁴ C]-pyridaben		3 mg/kg bw [benzyl ring ¹⁴ C]-pyridaben	
		M	F	M	F
bile	0-3 h	22.3	21.5	19.6	18.8
	3-6 h	17.0	13.0	13.1	14.8
	6-9 h	6.7	5.7	7.8	7.8
	9-12 h	3.4	2.9	2.7	3.0
	12-24 h	4.5	2.8	3.8	4.2
	24-48 h	1.6	0.93	0.87	0.83
Total bile	0-24h	55.5	46.7	47.9	49.4
urine	0-24 h	0.9	2.4	5.3	3.8
	24-48 h	0.07	0.05	0.42	0.64
Total urine	0-48 h	0.97	2.4	5.7	4.5
Cage wash	48 h	0.05	0.04	0.17	0.16
faeces	0-24 h	34.9	47.4	38.8	36.6
	24-48 h	10.0	3.1	6.2	8.5
Total faeces	0-48	44.9	50.5	44.9	45.1
liver	48 h	0.01	0.01	0.06	0.07
GI-tract (contents)	48 h	0.56	0.12	0.37	0.35
carcass	48h	0.27	0.13	0.24	0.23
Recovery	48 h	102.3	99.9	99.3	99.8

Conclusions

The absorption, distribution and excretion of ¹⁴C-pyridaben was investigated after a single oral dose of 3 mg/kg bw in bile-duct cannulated rats. Overall recoveries were 99-102% AR. Biliary and faecal excretion accounted for 47-56% and 45-51% of the applied radioactivity, respectively. Urinary excretion was 1-5.7% AR. Oral absorption was found to be ≤ 57% based on %AR in bile, urine, liver and carcass.

Acceptability

The study is acceptable.

In this study oral absorption was measured over a 48 hour period, while in other studies observations over a period of 168 hours.

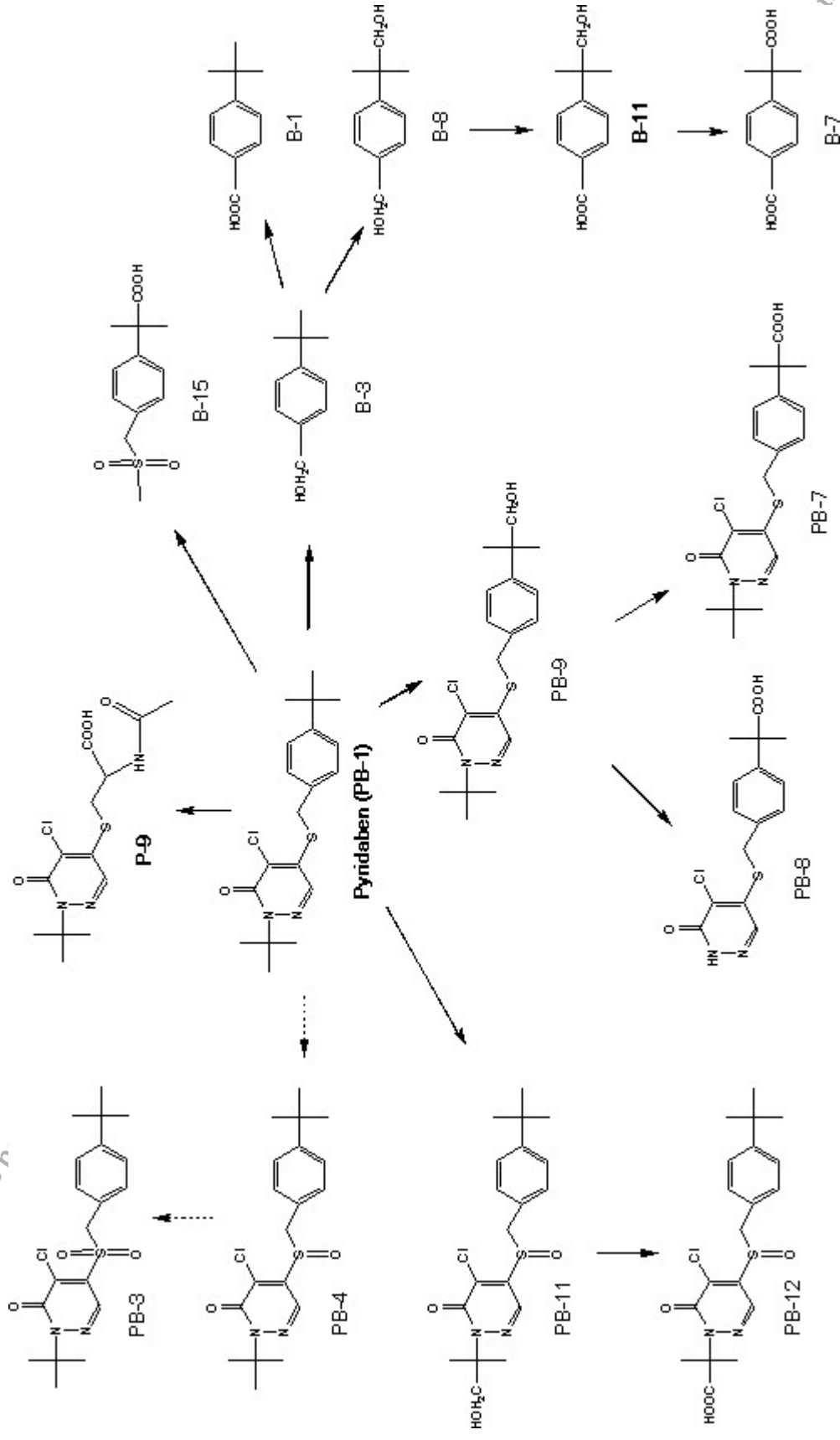
B.6.1.2 Proposed metabolic pathway

The proposed metabolic pathway of pyridaben in rat is shown in Fig. 6.1.2-01.

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Fig. 6.1.2-01 Proposed metabolic pathway of pyridaben in rat

The identification of the substances with their names in bold have been confirmed adequately.



WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.6.1.3 List of identified compounds during *in vivo* metabolism studies with pyridaben

Table 6.1.3-01 presents an overview of the metabolite identification achieved during *in vivo* metabolism studies in rat. Metabolites in bold were confirmed by LC-MS

Table 6.1.3-01 Identified compounds during *in vivo* metabolism studies with pyridaben in rat

Code	Compound	Description	Matrix
Pyridaben (PB-1)	2-tert-butyl-5-(4-tert-butylbenzylthio)-4-chloropyridazin-3(2H)-one	Parent	Urine, faeces, liver
PB-3	2-tert-butyl-5-(4-tert-butylbenzylsulfonyl)-4-chloropyridazin-3(2H)-one	Metabolite	Urine, faeces
PB-4	2-tert-butyl-5-(4-tert-butylbenzylsulfinyl)-4-chloropyridazin-3(2H)-one	Metabolite	Urine, faeces, liver
PB-7	2-tert-butyl-5-(4-(1-carboxy-1-methylethyl)-4-chloropyridazin-3(2H)-one	Metabolite	Urine, faeces
PB-8	5-[4-(1-carboxy-1-methylethyl)benzylthio]-4-chloropyridazin-3(2H)-one	Metabolite	Urine, faeces, bile
PB-9	2-tert-butyl-4-chloro-5-[4-(1,1-dimethyl-2-hydroxyethyl)pyridazin-3(2H)-one	Metabolite	Urine, faeces, liver
PB-11	5-(4-tert-butylbenzylthio)-4-chloro-2-(1,1-dimethyl-2-hydroxyethyl)pyridazin-3(2H)-one	Metabolite	Urine, faeces
PB-12	5-(4-tert-butylbenzylthio)-2-(1-carboxy-1-methylethyl)-4-chloropyridazin-3(2H)-one	Metabolite	Urine, faeces, liver
PB-13	4-chloro-2-(1,1-dimethyl-2-hydroxyethyl)-5-[4-(1,1-dimethyl-2-hydroxyethyl)benzylthio]-4-chloropyridazin-3(2H)-one	Metabolite	Liver
B-1	4-tert-butylbenzoic acid	Metabolite	Urine, faeces, liver
B-3	4-tert-butylbenzylalcohol	Metabolite	Urine, faeces, liver
B-7	2-(4-carboxyphenyl)-2-methylpropanoic acid	Metabolite	Urine, faeces, liver
B-8	2-(hydroxymethylphenyl)-2-methyl-1-propanol	Metabolite	Urine, faeces, liver
B-11	2-(4-carboxyphenyl)-2-methyl-1-propanol	Metabolite	Urine, faeces, liver
B-15	2-methyl-2-[4-(methylsulfonylmethyl)phenyl]propanoic acid	Metabolite	Urine, faeces
P-1	2-tert-butyl-4-chloro-5-mercaptopyridazin-3(2H)-one	Metabolite	Urine, faeces
P-3	2-tert-butyl-4-chloro-5-hydroxypyridazin-3(2H)-one	Metabolite	Urine, faeces
P-7	unknown	Metabolite	Urine
P-9	5-(2-acetamido-2-carboxyethylthio)-2-tert-butyl-4-chloropyridazin-3(2H)-one	Metabolite	Urine
D-1	di-[2-tert-butyl-4-(4-tert-butylbenzyl)pyridazin-3(2H)-one-5-yl]disulfide	Metabolite	Faeces

B.6.1.4 Summary and conclusions

In all studies available [3,6-pyridazinone-ring¹⁴C]-pyridaben and [benzyl ring¹⁴C]-pyridaben were studied in parallel. In case no differences between the findings for both compounds were present the text gives a single range and/or refers to ¹⁴C-pyridaben.

Absorption

In three studies with Sprague Dawley rats 168 hours after a single oral dose of 3 and 30 mg/kg bw, or 14-day repeated oral dosing at 3 mg/kg bw, 80-97% AR was excreted with faeces. In urine concentrations of applied radioactivity were 3-4% after [3,6-pyridazinone-ring¹⁴C]-pyridaben and 11-19% after low dose [benzyl ring¹⁴C]-pyridaben and slightly higher after 30 mg/kg bw [benzyl ring¹⁴C]-pyridaben (13-24%). In expired air very low amounts of radioactivity were recovered (<0.07% AR). In tissues 5-11% AR was retained after 2 hours and <1.4% AR 168 hours after a single dose ¹⁴C-pyridaben. Peak ¹⁴C-concentrations in blood were observed after 12 hours in both sexes after an oral single or repeated dose of 3 mg/kg bw, but only after 24 hours after a single oral dose of 30 mg/kg bw. Oral absorption was found to be 3-5% and 11-19% after low dose [3,6-pyridazinone-ring¹⁴C]-pyridaben and [benzyl ring¹⁴C]-pyridaben, respectively, and 3-7% and 13-24% after high dose [3,6-pyridazinone-ring¹⁴C]-pyridaben and [benzyl ring¹⁴C]-pyridaben, respectively. Actual absorption might have been higher because of the fact that excretion via the entero-hepatic circulation was not taken into account (no bile-duct cannulation and analysis of radioactivity in bile). No differences between the sexes were observed.

In another study with Sprague Dawley rats 67-84% AR was retained from faeces 72 hours after a single dose of 30 mg/kg bw ¹⁴C-pyridaben, whilst radioactivity in urine accounted for 4-6% AR after dosing [3,6-pyridazinone-ring¹⁴C]-pyridaben and 14-24% after dosing [benzyl ring¹⁴C]-pyridaben. Oral absorption based on recovered radioactivity in urine, liver, skin and carcass is estimated to be 5-7% and 15-27% for [3,6-pyridazinone-ring¹⁴C]-pyridaben and [benzyl ring¹⁴C]-pyridaben, respectively. Bile-duct cannulation was not included in the study design. After cannulation of the bile-duct of additional groups of rats in the same study (single dose 30 mg/kg bw), radioactivity in bile accounted for 22-30% AR. In faeces (and the gastro-intestinal tract) and in urine 56-72% AR and 1.6-4.0% AR, respectively, were recovered. A statistical significance difference between males and females for urine and bile (benzyl ring label) ($p < 0.01-0.05$) and between both radiolabels for urine and faeces ($p < 0.01$) was found. Based on the results of this study it can be concluded that absorption via the gastro-intestinal tract is 26-39% AR, based on %AR in bile, urine, skin and carcass.

A much higher absorption (49-57%) was found in another study with Wistar rats receiving a single dose of 3 mg/kg bw. In this study the bile-duct of the rats was cannulated. After 48 hours biliary and faecal excretion accounted for 47-56% and 45-51% of the applied radioactivity, respectively. Urinary excretion was 1-5.7% AR.

From the studies with bile-duct cannulation it can be concluded that oral absorption after a single low dose is 49-57% and 26-39% after a single high dose. However, it is not clear whether the difference in absorption rate was caused by the height of the dose or by the use of two different strains of rats with a strain dependent different absorption. In these studies no consistent differences between the sexes or both radiolabels were reported.

As the liver is not the main target organ, the %AR in bile should not be included in the final oral absorption rate. Therefore, based on %AR in urine, skin and carcass, oral absorption amounts to 11-19%. Although a direct effect on liver was not seen in the repeated dose toxicity studies with pyridaben, the working mechanism of pyridaben is very aspecific (inhibition of mitochondrial electron transport) and an effect on liver cannot completely be ruled out. Data on elimination through bile were available. Based on %AR in bile,

urine, skin and carcass oral absorption amounts to 26-39% for 30 mg/kg bw, and 49-57% for 3 mg/kg bw, the latter being measured over 48 hours, whereas the other studies had a duration of 168 hours. The majority of %AR passed the bile within the first 6 hours.

Considering the fact that the working mechanism is aspecific, it is considered too worst-case to take the lowest level for oral absorption. However, it is also concluded that the amount excreted in bile should not be included in the total oral absorption, as the liver is not the critical effect. Relevant absorption for risk assessment purposes will be between 10 and 30%. Therefore, the highest amount of oral absorption of the low dose based on urine, skin and carcass will be considered for risk assessment purposes, being 20%.

Elimination

In three studies with Sprague Dawley rats 168 hours after a single oral dose of 3 and 30 mg/kg bw, or repeated oral dosing at 3 mg/kg bw, the majority of administered radioactivity was excreted in faeces (80-97% AR), whilst radioactivity in urine accounted for 3-19% AR and in expired air for <0.07% AR. Pre-treatment for 14 days did not affect the pattern of excretion and retention.

In another study 72 hours after a single dose of 30 mg/kg bw ¹⁴C-pyridaben 67-84% AR was retained from faeces and 4-6% AR from urine after dosing [3,6-pyridazinone-ring ¹⁴C]-pyridaben and 14-24% from urine after dosing [benzyl ring ¹⁴C]-pyridaben.

The elimination half-life of [3,6-pyridazinone-ring ¹⁴C]-pyridaben was longer than that for [benzyl ring ¹⁴C]-pyridaben: 23 hours versus 9-10 hours after a single dose of 3 mg/kg bw, 60-70 hours versus 12 hours after repeated dosing and 49-56 hours versus 18-24 hours after a single dose of 30 mg/kg bw. No differences between the sexes became apparent.

Distribution

In one study, at 2 hours after a single oral dose of 3 mg/kg bw, low radioactivity concentrations were found in tissues (4-10% and 5-6% AR after [3,6-pyridazinone-ring ¹⁴C]-pyridaben and [benzyl ring ¹⁴C]-pyridaben, respectively). The highest amounts were recovered from liver (563 and 1150 ng eq./g (1.0-2.1 % AR), 1250-1330 ng eq./g (2.0-2.9% AR) after [3,6-pyridazinone-ring ¹⁴C]-pyridaben and [benzyl ring ¹⁴C]-pyridaben, respectively). In other organs radioactivity ranged from 256-392 ng eq./g (kidneys), 1110 and 2170 ng eq./g (uterus), 896-3290 ng eq./g (ovary) and 849-1390 ng eq./g (lymph nodes). Concentrations decreased rapidly thereafter.

In a second study 24 hours after a single dose of 30 mg/kg bw a comparable pattern of distribution to that after a single oral dose of 3 mg/kg bw was shown. The concentrations in high dose rats were on average a factor of 12 and 17 for [3,6-pyridazinone-ring ¹⁴C]-pyridaben and [benzyl ring ¹⁴C]-pyridaben, respectively, higher than in low dose rats (hence roughly proportional to the dose). In a separate study with 30 mg/kg bw (single dose) radioactivity recovered from liver and kidney was 6 and 11 times higher than that recovered after a single dose of 3 mg/kg bw, again reasonably proportional to dose.

After repeated oral administration of 3 mg/kg bw radioactivity in tissues 168 after the last dose was below 0.1% AR.

There were no remarkable differences between tissue levels and depletion rates of male and female rats.

Metabolism

Three studies are available using TLC analysis and co-elution of reference compounds as analytical method. The analyses were stated to be confirmed by two-dimensional TLC, but the results were not adequately included in the reports. The fourth study uses either HPLC or TLC to separate the metabolites and confirmed metabolites by co-elution of reference compounds or LC-MS.

All metabolites identified were <5% AR except for the parent in faeces, P-9 and B-11 in urine and two unknowns.

In 0-48 hour urine of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben (single oral dose or repeated dosing of 3 mg/kg bw), which contained 2-3% AR, no metabolites were identified and confirmed. Metabolites identified by TLC included parent, P-9, P-1, P-3 and PB-12. After a single dose of 30 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben P-9 was identified and confirmed by LC-MS in 0-72 hour urine (total 2-6% AR). In addition parent, P-1, P-3, P-7, PB-3, PB-9, PB-11 and PB-12 were identified by TLC or HPLC. The metabolite pattern was essentially the same for both sexes.

In urine of rats treated with a single dose of 3 or 30 mg/kg bw [benzyl ring¹⁴C]-pyridaben or treated repeatedly with 3 mg/kg bw (0-48 hour after low dose (6-14% AR) and 0-72 hour after high dose (12-24% AR)) B-11 was identified and confirmed by LC-MS. Several other metabolites were present and identified by TLC or HPLC including B-1 and B-8 and in addition parent, B-3, B-7, B-15, PB-4, PB-7, PB-8, PB-12. The metabolite pattern was essentially the same for both sexes and dosing regimes.

In faeces of rats treated with [3,6-pyridazinone-ring¹⁴C]-pyridaben (total radioactivity 45-54% AR low dose, 61-91% AR high dose) the parent and PB-12 were identified. At 30 mg/kg bw the parent (confirmed by LC-MS) was the main component accounting for >10% AR. In addition PB-3, PB-4, PB-7, PB-8, PB-9, PB-11, P-1 and P-3 were identified by TLC or HPLC, but not further confirmed. No major differences between sexes or dosing regimes could be found.

After treatment with [benzyl ring¹⁴C]-pyridaben TLC or HPLC analysis showed the parent to be the main component of faeces (13-32%). In addition B-1, B-3, B-7, B-8, B-11, B-15, PB-3, PB-4, PB-7, PB-9, PB-11, PB-12 and D-1 were found, but not confirmed adequately. Total radioactivity in faeces was 57-68% AR after a single dose and 35-42% AR after repeated dosing.

In liver the parent was recovered after HPLC analysis after a single dose at 30 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben (total radioactivity 0.7% AR). TLC analysis did not show any metabolites. After [benzyl ring¹⁴C]-pyridaben (single dose of 3 or 30 mg/kg bw) B-1 was identified after TLC and HPLC analysis. In addition parent, B-3, B-7, B-8, B-11, PB-4, PB-9, PB-12 and PB-13 were found. In bile PB-8 was found after a single dose of 30 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben. No metabolites were found after [benzyl ring¹⁴C]-pyridaben.

In three studies, metabolites were poorly separated on the TLC plates and not accurately confirmed. This renders the studies supplementary. However, all studies combined do provide information on the metabolic pattern of pyridaben. In the fourth study (at 30 mg/kg bw ¹⁴C-pyridaben) metabolites separated by HPLC could not be sufficiently identified by co-chromatography of referent compounds. In this study accurate confirmation of identity by LC-MS was available for P-9 and B-11 in urine and the parent compound in faeces.

B.6.2 Acute toxicity including irritancy and skin sensitisation (Annex IIA 5.2)

B.6.2.1 Acute toxicity

STUDY 1

Characteristics

reference	: Kawakami & Sunaga, 1990a	exposure	: Once by gavage
type of study	: Acute oral toxicity study	doses	: 0, 50, 100, 200, 400, 550, 800, 1100, and 1600 mg/kg bw (both sexes)
year of execution	: 1989-1990	vehicle	: Sodium carboxymethylcellulose
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Oral	guideline	: In accordance with OECD 401 (1987)
species	: Rat, Crj:CD (SD)	acceptability	: Acceptable
group size	: 5/sex/dose	LD ₅₀	: Female rats: 570 mg/kg bw

Study design

The study was performed in accordance with OECD 401 (1987). Doses were based on a dose-range finding study. In this study, males were found dead at 750 mg/kg bw and females at 1000 mg/kg bw. In a pharmacodynamic study, females were found dead at 300 mg/kg bw.

Results

Mortality: One male and one female given 200 mg/kg bw, and one female given 550 mg/kg bw were found dead on day 2. Within 10 days, 2/5 males and 4/5 females given 800 mg/kg were found dead. Within 7 days, 2/5 males and 5/5 females given 1100 mg/kg were found dead. In the 1600 mg/kg dose group, death occurred from 2 hours after treatment to 12 days in 3/5 males and 5/5 females.

Symptoms of toxicity: Most animals showed decreased activity, decreased faeces and diarrhoea. Other symptoms were arched back posture, ataxia, nasal discharge or reddish stain on facial area and/or forelimbs. These findings generally disappeared before the end of the experiment in surviving animals. In animals given 400 mg/kg or more, piloerection was observed which generally did not disappear before the end of the study.

Body weight: All rats found dead showed a loss of body weight during the time before death. Decreased body weight (gain) was observed in males (dose groups: ≥ 400 mg/kg) from day 2-11, and in females (dose groups: ≥ 100 mg/kg) from day 2-7. However, at the end of the experiment, surviving animals showed increased body weight.

Pathology: No significant observations were seen at necropsy for animals surviving to study termination from any of the dosage levels. Lung haemorrhage was regularly found, even in male control rats. In most animals that died during the study, haemorrhages in the thymus, the lung and the stomach were observed and whitish contents of the GI-tract.

Acceptability

This study is considered acceptable.

Conclusions

The acute oral LD₅₀ of NC-129 was found to be 1100 mg/kg bw in male rats and 570 mg/kg bw in female rats.

STUDY 2

Characteristics

reference	: Kawakami & Sunaga, 1990b	exposure	: Once by gavage
type of study	: Acute oral toxicity study	doses	: 0, 50, 100, 200, 400, 550, 800, 1100, and 1600 mg/kg bw (both sexes)
year of execution	: 1989-1990	vehicle	: Sodium carboxymethylcellulose
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Oral	guideline	: In accordance with OECD 401 (1987)
species	: Mouse, Crj:CD-1 (ICR)	acceptability	: Acceptable
group size	: 5/sex/dose	LD ₅₀	: Female mice: 383 mg/kg bw

Study design

The study was performed in accordance with OECD 401 (1987). Doses were based on a dose-range finding study. In this study, males were found dead at 500 mg/kg bw and above, and females at 100 mg/kg bw and above.

Results

Mortality: All animals given 550 mg/kg bw or more were found dead within 3 days after treatment, with the first deaths starting within 1 hour after treatment. In the 400 mg/kg dose group, 2/5 females died on day 2, and 1/5 males died on day 7.

Symptoms of toxicity: Most animals showed reduced activity, arched back posture and eye closing. Other symptoms were prone posture, diarrhoea, bradypnoea, piloerection and ataxia. These findings generally disappeared before the end of the experiment in surviving animals.

Body weight: In the surviving animals, no clear effects on body weight (gain) were observed. Only on day 2, in both males and females, body weight decreased with increasing dose.

Pathology: No significant observations were seen at necropsy for animals surviving to study termination from any of the dosage levels. Lung haemorrhage and yellowish/whitish contents of the gastro-intestinal tract was regularly found in mice that died after treatment. Sometimes gastric haemorrhagic erosion, pale-coloured liver or foamy substance in the lung was observed.

Acceptability

This study is considered acceptable.

Conclusions

The acute oral LD₅₀ of NC-129 was found to be 424 mg/kg bw in male mice and 383 mg/kg bw in female mice.

STUDY 3

Characteristics

reference	: Cummins, 1989	exposure	: Once by gavage
type of study	: Acute oral toxicity study	doses	: 182, 310, 528, 900, 1533 and 2691 mg/kg bw (both sexes)
year of execution	: 1989	vehicle	: carboxymethylcellulose
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Oral	guideline	: In accordance with OECD 401 (1987)
species	: Rat, CD strain	acceptability	: Acceptable
group size	: 5/sex/dose	LD ₅₀	: Female rats: 820 mg/kg bw

Study design

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

Results

Mortality: One female given 310 mg/kg bw was found dead on day 3. Three females given 528 mg/kg bw were found dead on day 2. In the 900 mg/kg dose group, all females died between day 2 and 5, and one male was found dead on day 7. In the 1533 mg/kg dose group, 3/5 males were found dead between 3 hours and 7 days after treatment, and 3/5 females died between day 2 and 6. All animals given 2691 mg/kg bw died between day 3 and 6.

Symptoms of toxicity: In surviving animals, no abnormalities were observed at doses up to 310 mg/kg. At 528 mg/kg and above, surviving animals showed decreased motor activity, piloerection, hunched posture, ataxia, thin body conformation, hair loss and ungroomed appearance. These observations generally disappeared before the end of the experiment. Animals that were found dead during the study showed the same symptoms before death, and in addition among others bradypnoea, hyperpnoea, and lethargy.

Body weight: Rats given 528 mg/kg or more showed a decrease in body weight during the first week and a regain in the second week. Body weight gain decreased at 528 mg/kg and above.

Pathology: No significant observations were seen at necropsy for animals surviving to study termination from any of the dosage levels. In most animals that died during the study, yellow or brown staining (external) was observed, and yellow viscous fluid in the small intestine and distended stomachs were found.

Acceptability

This study is considered acceptable.

Conclusions

The acute oral LD₅₀ of NC-129 was found to be 1350 mg/kg bw in male rats and 820 mg/kg bw in female rats.

STUDY 4

Characteristics

reference	: Rees, 1994	exposure	: Once by gavage
type of study	: Acute oral toxicity study	doses	: 81, 128 and 202 mg/kg bw (both sexes)
year of execution	: 1993	vehicle	: Maize oil
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Oral	guideline	: In accordance with OECD 401 (1987)
species	: Rat, CD strain	acceptability	: Acceptable
group size	: 5/sex/dose	LD ₅₀	: Male rats: 161 mg/kg bw

Study design

The study was performed in accordance with OECD 401 (1987). Doses were based on a dose-range finding study. In this study, males were found dead at 300 and 600 mg/kg bw, and females at 150, 300 and 600 mg/kg bw.

Results

Mortality: One female given 128 mg/kg bw was found dead on day 2. In the 202 mg/kg dose group, 3/5 females (1 was humanely killed) and 5/5 males died between day 2 and 5.

Symptoms of toxicity: In all dose groups, nearly all animals showed ungroomed appearance. Surviving animals in the 128 and 202 mg/kg dose groups, showed reduced activity, staggering gait, hair loss, piloerection, salivation, thin body conformation and hunched posture. These symptoms were also seen in rats before death. In surviving animals the symptoms disappeared before the end of the study in the low-dose group, in females of the mid-dose group, and in one female of the high-dose group. **Body weight:** Rats given 128 and 202 mg/kg showed a decrease in body weight during the first 4 days and a regain thereafter. Body weight gain decreased too.

Pathology: No significant observations were seen at necropsy for animals surviving to study termination from any of the dosage levels. Three animals that died during the study showed yellow staining (external), and one showed hair loss. No internal macroscopic findings were observed.

Acceptability

This study is considered acceptable.

Conclusions

The acute oral LD₅₀ of NC-129 was found to be 161 mg/kg bw in male rats and 181 mg/kg bw in female rats.

STUDY 5

Characteristics

reference	: Hayashi, 1989a	exposure	: Once by gavage
type of study	: Acute oral toxicity study	doses	: 0, 31, 50, 80, 128, 205, 328, 524, 839 and 1342 mg/kg bw
year of execution	: 1988-1989	vehicle	: Sodium carboxymethylcellulose
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Oral	guideline	: In accordance with OECD 401 (1987)
species	: Mouse, Crj:CD-1 (ICR), males	acceptability	: Acceptable
group size	: 10/dose	LD ₅₀	: Male mice: 253 mg/kg bw

Study design

The study was performed in accordance with OECD 401 (1987). Doses were based on a dose-range finding study. In this study, males were found dead at 50 mg/kg bw and above.

Results

Mortality: Deaths occurred from one hour until four days after treatment. Within 1 day, 1, 2, and 3 rats were found dead in the 50, 80 and 128 mg/kg dose group, respectively. Within 2 days, 4 animals were found dead in the 205 mg/kg group and 8 animals were found dead in the 328, 839 and 1342 mg/kg dose groups. In the 524 mg/kg dose group all animals died within 4 days.

Symptoms of toxicity: At dosages of 50 mg/kg bw and above, most animals showed decreased locomotor movement, prone and/or crouching posture and ataxia. Other symptoms were disappearance of the righting reflex, bradypnoea, decreased dermal temperature, piloerection and urinary incontinence. These findings generally appeared within 1 day after treatment and disappeared within 1 week in surviving animals.

Body weight: At 205 mg/kg and above, body weights decreased on day 1, but recovered within a week to their original value.

Pathology: No significant observations (only stomach thickening) were seen at necropsy for animals surviving to study termination from any of the dosage levels. Stomach haemorrhage and reddish contents of the small intestine was regularly found in mice that died after treatment. Sometimes discoloured organs, dilated small intestine and stomach congestion were observed.

Acceptability

This study is considered acceptable.

Conclusions

The acute oral LD₅₀ of NC-129 was found to be 253 mg/kg bw in male mice.

STUDY 6

Characteristics

reference	: Hayashi, 1989b	exposure	: Once by gavage
type of study	: Acute oral toxicity study	doses	: 0, 31, 50, 80, 128, 205, 328, 524, 839 and 1342 mg/kg bw
year of execution	: 1988-1989	vehicle	: Sodium carboxymethylcellulose
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Oral	guideline	: In accordance with OECD 401 (1987)
species	: Mouse, Crj:CD-1 (ICR), females	acceptability	: Acceptable
group size	: 10/dose	LD ₅₀	: Female mice: 205 mg/kg bw

Study design

The study was performed in accordance with OECD 401 (1987). Doses were based on a dose-range finding study. In this study, females were found dead at 100 mg/kg bw and above.

Results

Mortality: Deaths occurred from one hour until seven days after treatment. Within 1 hour, 1, 1, and 3 rats were found dead in the 50, 80 and 128 mg/kg dose group, respectively. Within 3 days, 6 animals were found dead in the 205 and 328 mg/kg groups and within 2 days 9 animals were found dead in the 524 and 839 mg/kg groups. After 7 days all animals had died in the 1342 mg/kg dose group.

Symptoms of toxicity: At dosages of 50 mg/kg bw and above, most animals showed decreased locomotor movement, prone and/or crouching posture and ataxia. Other symptoms were disappearance of the righting reflex, bradypnoea, decreased dermal temperature, piloerection and diarrhoea. These findings generally appeared within 1 day after treatment and disappeared within 1 week in surviving animals.

Body weight: At 205 mg/kg and above, body weights decreased on day 1, but recovered within a week to their original value.

Pathology: No significant observations (only stomach thickening) were seen at necropsy for animals surviving to study termination from any of the dosage levels. Stomach haemorrhage and reddish contents of the small intestine was found in mice that died after treatment.

Acceptability

This study is considered acceptable.

Conclusions

The acute oral LD₅₀ of NC-129 was found to be 205 mg/kg bw in female mice.

STUDY 7

Characteristics

reference	: Yamamoto, 1986	exposure	: 24 hours on a skin area of 20 cm ² (occlusive exposure).
type of study	: Acute dermal toxicity study	doses	: 2000 mg/kg bw (both sexes)
year of execution	: 1986	vehicle	: Moistened with water
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Dermal	guideline	: In accordance with OECD 402
species	: Rat, Crj: CD(SD)	acceptability	: Acceptable
group size	: 10/sex/dose	LD ₅₀	: > 2000 mg/kg bw

Study design

The study was performed in accordance with OECD 402.

Results

Mortality: No mortality was observed.

Symptoms of toxicity: No toxic signs were observed.

Body weight: Mean body weights decreased in males during the first 3 days after treatment, but increased thereafter. Mean body weights in females decreased 1 day after treatment, but increased thereafter.

Pathology: No abnormalities were observed.

Acceptability

The study is considered acceptable.

Conclusions

The acute dermal LD₅₀ of NC-129 in rats was found to be greater than 2000 mg/kg bw in both males and females.

STUDY 8

Characteristics

reference	: Cummins, 1987	exposure	: 24 hours on a skin area of 10% of the total body surface area (occlusive exposure).
type of study	: Acute dermal toxicity study	doses	: 2000 mg/kg bw (both sexes)
year of execution	: 1986	vehicle	: Moistened with water
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Dermal	guideline	: In accordance with OECD 402
species	: Rabbit, New Zealand White	acceptability	: Acceptable
group size	: 5/sex/dose	LD ₅₀	: > 2000 mg/kg bw

Study design

The study was performed in accordance with OECD 402.

Results

Mortality: No mortality was observed.

Symptoms of toxicity: Two males showed tachypnoea and hypopnoea during the first fifteen minutes after dosing. One female showed decreased motor activity, hunched posture, thin body conformation and limited faecal production from day 9-11 and at the end of the study. No dermal responses were observed in any of the rabbits.

Body weight: Mean body weights decreased in 8/10 rabbits during the first 8 days after treatment, but increased thereafter. One female showed a decreased body weight at the end of the study. Pathology: Four males and one female showed dark areas in the lung. Three males and one female showed dark-coloured musculature areas at the treatment sites.

Acceptability

The study is considered acceptable.

Conclusions

The acute dermal LD₅₀ of NC-129 in rabbits was found to be greater than 2000 mg/kg bw in both males and females.

STUDY 9

Characteristics

reference	: Iwasaki, 1987	exposure	: 4 hours; whole-body
type of study	: Acute inhalation toxicity study	doses	: 0, 0.41, 0.50, 0.57, 0.66, 0.73, 0.86, 1.02 and 5.48 mg/l (actual concentrations); MMAD 3.7-4.8 µm; GSD 1.7-1.8 µm
year of execution	: 1986-1987	vehicle	: White carbon
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Inhalation	guideline	: In accordance with OECD 403 (1981)
species	: Rat, Fischer (F344/Ducrj)	acceptability	: Acceptable
group size	: 10/sex/dose	LC ₅₀	: Female rats: 0.62 mg/l

Study design

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

Results

Mortality: During exposure or within 1 hour after exposure, 5/10, 6/10, 10/10, 8/10 and 10/10 males were found dead at 0.66, 0.73, 0.86, 1.02 and 5.48 mg/l NC-129, respectively. 1/10 female given 0.41 mg/l was found dead on day 1. Within 1 day after exposure, 4/10 females given 0.50 and 0.57 mg/l and 6/10 females given 0.66 and 0.73 mg/l were found dead. All females died within 5 hours after exposure to 0.86, 1.02 and 5.48 mg/l.

Symptoms of toxicity: All animals (including controls) showed eyelid closure and slow and deep respiration during exposure. Several exposed females in all dose groups showed lacrimation. At 0.66 mg/l and above, some animals gasped during exposure. After exposure several animals in all dose groups showed slow and deep respiration, a blotted fur of the perianal region and/or loose faeces around the anus, and reddening of the auricles. Nearly all animals (including controls) showed reddish brown staining around the nose after exposure. In surviving animals all symptoms disappeared before the end of the study.

Body weight: Mean body weights of male rats given 1.02 and 0.73 mg/l decreased after exposure, and increased after day 5. Male rats exposed to 0.66 mg/l or less showed decreased mean body weights after exposure, and increases after day 3. Mean body weights of female rats given 0.73 mg/l or less decreased after exposure, and increased after day 3. Control animals showed a decrease after exposure that recovered after day 1.

Pathology: Several animals that survived to the end of the study (including controls) showed dark red-coloured lungs and/or dark red spots in the lungs. Symptoms seen in dead animals were among others distended stomach, lung oedema, dark red (spots in the) lungs, white powder in tracheal lumen, hydrothorax, and dark-coloured liver. No histopathological changes were observed.

Acceptability

The study is considered acceptable.

Conclusions

The acute inhalation LC_{50} of NC-129 in rats was found to be 0.66 mg/l for male rats, and 0.62 mg/l for female rats.

B.6.2.2 Irritation and sensitisation**STUDY 1****Characteristics**

reference	: Cummins, 1986a	exposure	: 4 hours, semi-occlusive, application area 6 cm ²
type of study	: Skin irritation study	doses	: 0.5 g
year of execution	: 1986	vehicle	: None
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Dermal	guideline	: In accordance with OECD 404 (1981)
species	: Rabbit, New Zealand White	acceptability	: Acceptable
group size	: 6 females	Effect	: Not irritating

Study design

The study was performed in accordance with OECD 404 (1981).

Results

The results are summarised in tables 6.2.2-01 and 6.2.2-02.

Table 6.2.2-01 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 6.2.2-02 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 number of animals tested could have been lower (n=3).

Conclusions

The skin irritating potential of NC-129 in rabbits was found to be non-irritating.

STUDY 2

Characteristics

reference	: Cummins, 1986b	exposure	: Single instillation
type of study	: Acute eye irritation study	doses	: 0.1 g
year of execution	: 1986	vehicle	: None
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Ocular	guideline	: In accordance with OECD 405 (1981)
species	: Rabbit, New Zealand White	acceptability	: Acceptable
group size	: 6 females	Effect	: Not irritating

Study design

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

Results

The results are summarised in tables 6.2.2-03 and 6.2.2-04. At 1 hour after exposure, the cornea of one rabbit appeared stippled, which disappeared within 24 hours.

Table 6.2.2-03

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	2,1,1,1,1,1	1,2,1,1,2,1	0,1,1,1,1,1	0,0,0,0,0,0
Conjunctiva chemosis	0,1,1,1,1,1	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0
Conjunctiva discharge	0,1,1,1,0,0	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0

Table 6.2.2-04 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	1	0
3	0	0	0,7	0
4	0	0	0,7	0
5	0	0	1	0
6	0	0	0,7	0

Acceptability

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

Conclusions

NC-129 has been found to be not irritating to rabbit eyes.

STUDY 3

Characteristics

reference	: Myer, 1987	exposure	: Intradermal and topical induction; topical challenge (occlusive, 24h).
type of study	: Skin sensitisation study (GPMT)	doses	: 5% intradermal induction 25% topical induction 2.5% challenge
year of execution	: 1986	vehicle	: Peanut oil (intradermal induction), petrolatum (topical induction)
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Dermal	guideline	: In accordance with OECD 406 (1981)
species	: Guinea-pig, Hartley	acceptability	: Acceptable
group size	: 20 controls (females) 20 test animals (females)	Effect	: not sensitising

Study design

The study was performed in accordance with OECD 406 (1981) and conducted according to Magnusson and Kligman. 2,4-dinitrochlorobenzene was used as the positive control.

Dose levels were based on the results of a range-finding study using 2 animals for the 0, 1, 3 and 5% intradermal injections, two animals for the 5, 10, 15 and 25% topical applications, and an additional 3 animals for 2.5, 5, 10 and 15% topical application. All intradermal injection with 1, 3 and 5% mainly induced moderate erythema and slight oedema, independent of concentration. In the first two "topical" animals, topical application with 5-25% induced slight erythema at 24 hours, which had disappeared after 48-72 hours, and no oedema. In the second set of "topical" animals, topical application of 10-15% induced slight patchy to slight erythema after 24 hours, which had disappeared after 48-72 hours, and no oedema was observed. No skin reactions were observed at 2.5 or 5%. Intradermal induction was performed with 5% test substance in peanut oil. Topical induction was initiated after intradermal induction with treatment of 10% concentration of sodium lauryl sulphate in petrolatum. One day later 25% test substance in petrolatum was dermally applied for 48 hours. Fourteen days later, challenge was performed with dermal application of 2.5% test substance in petrolatum.

Results

After intradermal induction with 5% NC-129 and after topical induction with 25% NC-129, no irritation was recorded. After topical challenge with 2.5% NC-129, slightly patch erythema in 2 females was observed after 24 hours only. No oedema was observed. Control animals showed no erythema or oedema. One exposed guinea pig was sacrificed in extremis on day 4, after appearing moribund. The cause of moribundity was not test substance related, and the guinea pig was replaced with another one.

Acceptability

The study is considered acceptable.

Conclusions

NC-129 is not sensitising to the skin of guinea pigs in this Maximisation test.

STUDY 4

Characteristics

reference	: Smith, 1990	exposure	: Topical induction and topical challenge (occlusive, 6h).
type of study	: Skin sensitisation study (modified Buehler)	doses	: 50% topical induction (day 1, 8, 15) 10% and 50% challenge (day 28)
year of execution	: 1989	vehicle	: Paraffin oil
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Dermal	guideline	: In accordance with OECD 406 (1981)
species	: Guinea-pig, Dunkin-Hartley	acceptability	: Supplementary
group size	: 10 controls/sex 10 test animals/sex	Effect	: not sensitising

Study design

The study was performed in accordance with OECD 406 (1981) and conducted according to Buehler. See 'Acceptability' for remarks on the conduction of this study. 2,4-dinitrochlorobenzene was used as the positive control.

Dose levels were based on the results of a range-finding study using 5, 10, 30 and 50% occluded topical applications. None of the dose levels induced any dermal response.

Topical induction was performed with 50% test substance in paraffin oil on day 1, 8 and 15 for 6 hours. On day 28, challenge was performed with dermal application of 10 and 50% test substance in paraffin oil. About 24 and 48 hours later, dermal reactions were assessed.

Results

After the first induction phase, no dermal responses were observed. After the second induction phase, 13/20 animals showed areas of thickened skin and/or faint erythema. Faint to moderate confluent erythema was noted in 13/20 animals after the third induction phase. No dermal responses were observed 24 hours after the challenge with 10 and 50% test substance. In the 10% challenge group, 2/20 animals showed eschar formation of which one also showed blistering. In the control group, one animal showed eschar formation and blistering. In the 50% challenge group, 2/20 animals showed faint erythema of which one also showed blistering. In the control group, one animal had areas of thickened skin, blistering and eschar formation.

Acceptability

It should be noted that for a correct classification and labelling in accordance with EU regulations, a Maximisation or LLNA test are the performed tests with regard to the assessment of dermal sensitisation potential. The results of the study are of limited value for the overall evaluation of pyridaben.

Conclusions

The test substance has no evidence of skin sensitization to the skin of guinea pigs in a Buehler test.

B.6.2.3 Summary

The results of the acute toxicity, irritation and sensitisation studies are presented in table 6.2.3-01, 6.2.3-02, and 6.2.3-03, respectively.

Table 6.2.3-01 Acute toxicity studies, LD₅₀/LC₅₀ values

Test substance	LD ₅₀ /LC ₅₀	Species	Route	Vehicle	Reference
NC-129	570 mg/kg bw (females)	Rat	Oral	CMC	Kawakami & Sunaga, 1990a
NC-129	820 mg/kg bw (females)	Rat	Oral	CMC	Cummins, 1989
NC-129	161 mg/kg bw (males)	Rat	Oral	Maize oil	Rees, 1994
NC-129	383 mg/kg bw (females)	Mouse	Oral	CMC	Kawakami & Sunaga, 1990b
NC-129	253 mg/kg bw (males)	Mouse	Oral	CMC	Hayashi, 1989a
NC-129	205 mg/kg bw (females)	Mouse	Oral	CMC	Hayashi, 1989b
NC-129	>2000 mg/kg bw	Rat	Dermal	Water	Yamamoto, 1986
NC-129	>2000 mg/kg bw	Rabbit	Dermal	Water	Cummins, 1987
NC-129	0.62 mg/l (females)	Rat	Inhalation	White carbon	Iwasaki, 1987

Table 6.2.3-02 Skin and eye irritation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
NC-129	Not irritating to skin	Rabbit	Dermal	None	Cummins, 1986a
NC-129	Not irritating to eyes	Rabbit	Ocular	None	Cummins, 1986b

Table 6.2.3-03 Sensitisation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
NC-129	No skin sensitization (Maximisation test)	Guinea-pig	Dermal	Peanut oil and petrolatum	Myer, 1987
NC-129	No skin sensitization (modified Buehler) ¹	Guinea-pig	Dermal	Paraffin oil	Smith, 1990

¹ The results of the Buehler test are of limited value

Pyridaben needs to be classified as toxic after ingestion (T, R25) on the basis of its acute oral toxicity in rats according to Directive 2001/59/EC. It should be noted that the choice of the vehicle could be contributory to the degree of toxicity.

Pyridaben also needs to be classified as toxic after inhalation (T, R23) on the basis of its acute inhalatory toxicity in rats. Pyridaben needs no classification for acute dermal toxicity, nor for skin and eye irritation.

Pyridaben does not have sensitising properties in a Maximisation nor in a Buehler test.

B.6.3 Short-term toxicity (Annex IIA 5.3)

B.6.3.1 Subacute oral studies

No data were submitted by the notifier.

A subacute dose range study in dogs was submitted (Spicer, 1989a), which is further discussed at the semichronic data on dogs.

B.6.3.2 Subacute dermal studies**STUDY 1****Characteristics**

Reference	: Blaszcak, 1992	exposure	: 22 days, 6 hours/day, 10% body surface (5x5 cm), dorsal area, semi-occlusive
type of study	: subacute dermal toxicity	dose	: 0, 30, 100, 300 and 1000 mg/kg bw/day
year of execution	: 1991	vehicle	: None
test substance	: NC-129 (Pyridaben), Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
Route	: Dermal	guideline	: In accordance with OECD 410 (1981)
Species	: Rats, CD (CD-Crl:CD(SD)BR)	acceptability	: Acceptable
group size	: 5/sex/dose	NOAEL systemic	: 100 mg/kg bw/day

Study design

The study was performed in accordance with OECD 410 (1981). Doses were based on a dose-range finding study with 0, 10, 100 and 1000 mg/kg bw/day. In this study, no mortality and no skin irritation was observed. At 1000 mg/kg, animals lost weight or had smaller weight gains compared to controls.

Results

The results are summarised in table 6.3.2-01.

Table 6.3.2-01

Dose (mg/kg bw/d)	0		30		100		300		1000		Dr	
	m	f	m	f	m	f	m	f	m	f		
Mortality	No mortality occurred											
Clinical signs	No treatment-related findings											
Body weight							d		dc	d	f	
Body weight gain							d		dc	dc		
Food consumption									dc			
Ophthalmoscopy	No treatment-related findings											
Neurobehavioural examination	No treatment-related findings											
Functional observations	No treatment-related findings											
Motor activity	No treatment-related findings											
Haematology												
- activated partial thromboplastin time										ic		
Clinical chemistry												
- alanine aminotransferase							dc		dc			
- alkaline phosphatase										dc		
- albumin										dc		
- albumin/globulin ratio										dc		

Dose (mg/kg bw/d)	0		30		100		300		1000		Dr
	m	f	m	f	m	f	m	f	m	f	
Urinalysis	No treatment-related findings										Data not to be granted on the h
Organ weights											
- liver									ic ^r	ic ^r	
- brain									ic ^r	ic ^r	
- kidney							ic ^r			ic ^r	
Pathology											
Macroscopy	No treatment-related findings										
Microscopy											
treated skin:											
- squamous cell hyperplasia	0/5	0/5	0/5	0/5	2/5	1/5	2/5	2/5	2/5	3/5	
- desquamated epithelial cells	0/5	0/5	0/5	0/5	1/5	3/5	2/5	3/5	3/5	4/5	
- inflammatory cells/debris	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5	

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

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

r relative organ weight

Acceptability

The study is considered acceptable.

Conclusions

Dermal exposure to test substance concentrations of 0, 30, 100, 300 and 1000 mg/kg bw/day did not result in local skin effects. No treatment-related effects on mortality, clinical observations, ophthalmoscopy, neurobehavioural examination, functional observations and motor activity were noted. Body weight gain was decreased in males at 1000 mg/kg bw/d (49% of control) and in females at 300 and 1000 mg/kg bw/d (54% and 45% of control, respectively). Decreased body weights were noted in males at 1000 mg/kg bw/d (86% of control) and in females at 300 and 1000 mg/kg bw/d (92% and 91% of control, respectively). Compared to controls, food consumption was decreased in the 1000 mg/kg dose group in males after 1 and 3 weeks (80% and 86% of controls, respectively), and in females after 1 week (50% of controls). In both males and females of the 300 mg/kg dose group, food consumption was marginally decreased after 1 week (92% and 83% of controls, respectively), and was normal again thereafter.

Mean activated partial thromboplastin time was significantly increased at 1000 mg/kg bw in females (120% of control). In the absence of deviations in other haematological parameters and histopathological data this effect is considered to be not toxicologically relevant. In the 1000 mg/kg dose group, females showed decreased levels of alkaline phosphatase (71% of control) and albumin (84% of control) and consequently a decreased albumin/globulin ratio (75% of control). Males showed decreased levels of alanine aminotransferase in the 300 and 1000 mg/kg groups (both 50% of control). These effects are considered to be not toxicologically relevant.

Relative liver weights were significantly increased at 1000 mg/kg bw in both males and females (121% of control). In males relative brain weight was significantly increased at 1000 mg/kg (117% of control) and in females relative kidney weight was increased at 300 (113% of control) and 1000 mg/kg bw (115% of control). These weight increases were considered to be due to the decrease in terminal body weight.

Macroscopic pathology showed no toxicological effects. Histopathology showed subminimal to minimal squamous cell hyperplasia and/or desquamated epithelial cells in the treated skin of rats exposed to 100, 300 and 1000 mg/kg bw. These were considered to be a result of a very low grade mechanical effect of the powdered test material/physiological saline mixture. In the absence of any inflammatory reaction at the site of dermal application, these findings were not considered to be of any toxicological significance. Other findings occurred sporadically and were not considered to be related to treatment.

Based on decreased body weights gains in females at 300 and 1000 mg/kg bw/day the NOAEL for systemic effects is set at 100 mg/kg bw/day.

B.6.3.3 Subacute inhalation studies

STUDY 1

Characteristics

reference	: Hoffman, 1989	exposure	: 6 hours/day, 5 days/week, 4 weeks; whole body
type of study	: Subacute inhalation toxicity	concentrations	: Target conc.: 0, 1, 3 and 10 mg/m ³ , MMAD 2.4-4.8 µm, GSD 1.7-4.5 µm
year of execution	: 1989	vehicle	: White carbon
test substance	: NC-129 (Pyridaben), Lot no. 610704, purity 92.6% (and 7.4% white carbon)	GLP statement	: Yes
route	: Inhalation	guideline	: In accordance with OECD 412 (1981)
species	: Rat, Sprague-Dawley (CD)	acceptability	: Acceptable
group size	: 5/sex/dose (and 5/sex/dose in control and high dose as satellite group)	NOAEL	: 3.0 mg/m ³

Study design

The study was performed in accordance with OECD 412 (1981). The test substance consisted of 7.4% white carbon, which was also the vehicle used in this study. The target concentrations used in this study were 0, 1, 3 and 10 mg/m³. The vehicle control group (target concentration 0 mg/m³) are exposed to a white carbon level of 0.74 mg/m³. The measured gravimetric concentrations were 1.1, 0.96, 3.1 and 10 mg/m³ (respectively) and the analytically measured concentrations were 0, 0.87, 3.0 and 10 mg/m³ (respectively). The analytical concentrations are used for determining the NOAEL.

In the study, 5 rats/sex/dose were used. In the control group and the highest dose group, an additional 5 rats/sex were used in the study and during a 2-week recovery period.

Results

The results are summarised in Table 6.3.3-01.

Table 6.3.3-01

Dose (mg/m ³)	0		0.87		3.0		10		dr
	m	f	m	f	m	f	m	f	
Mortality	none								and should not be read in isolation. Registration must not be granted on this basis
Clinical signs									
- nasal discharge	7/10	6/10	4/5	5/5	5/5	5/5	10/10	10/10	
- dried material on facial area	4/10	9/10	2/5	3/5	3/5	4/5	10/10	10/10	
Body weight									
Food consumption									
Ophthalmoscopy	No treatment-related findings								
Functional observations	Not assessed								
Motor activity	Not assessed								
Haematology	No treatment-related findings								
Clinical chemistry									
- ALAT									
- albumin									
- cholesterol									
Organ weights									
- brain									
- heart									
- liver									
- spleen									
- lungs									
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 absolute organ weight
r relative organ weight
rec recovery group

Acceptability

The study is considered acceptable.

Conclusions

Rats inhaled 0, 0.87, 3.0 or 10 mg/m³ pyridaben 6 hours/day for 5 days/week for 4 weeks. Two satellite groups (0 and 10 mg/m³) were additionally observed for 14 days post-treatment.

No mortality occurred. Nasal discharge and dried material on the facial area were the most common clinical observations. In the highest dose group, sporadically excess lacrimation, ano-genital staining, chromodacryorrhea and alopecia were observed. Weight loss was observed on day 2 in males and females from the 10 mg/m³ dose group, and in females from the 3.0 mg/m³ dose group. Terminal body weights were reduced in males and females at 10 mg/m³ (93% of controls). The differences in body weights disappeared

during the recovery period. Food consumption was significantly decreased only in the first week after treatment with 10 mg/m³ in both males and females.

No treatment-related effects were observed on haematological parameters.

ALAT was significantly decreased in the 3.0 and 10 mg/m³ dose groups (77 and 62% of controls in males; 70 and 65% in females, respectively) and were not considered toxicologically significant. In females (3.0 and 10 mg/m³) albumin levels were significantly decreased (91 and 87% of controls, respectively), and at the highest dose females showed increased cholesterol levels (144% of controls). In the absence of effects on food consumption at the end of the exposure period and in the absence of evidence of liver dysfunction, the toxicological significance of the changes in cholesterol and albumin remains unclear. The clinical chemistry effects disappeared during the recovery period.

In the high dose males, relative weights of the brain (113% of control), the heart (112% of control), the liver (112% of control) and the spleen (126% of control) were significantly increased. In absence of biochemical or histopathological correlates, changes in relative organ weight were considered to be due to decreased body weight. In the recovery group of the high dose females, both absolute and relative weights of the lungs (113 and 116% of control, respectively) and the spleen (124 and 127% of control, respectively) were significantly increased. In the absence of histopathological correlates, the toxicological significance of these organ weight changes remains unclear.

Macroscopically no treatment-related effects were observed. Histopathologically, no treatment-related effects were observed either.

Based on decreased body weights at 10 mg/m³, the NOAEL is set at 3.0 mg/m³.

B.6.3.4 Semichronic oral studies

STUDY 1

Characteristics

Reference	: Holmes, 1988a	exposure	: 13 weeks, diet; 4 weeks recovery
Type of study	: 13-week oral toxicity study	dose	: 0, 30, 65, 155 and 350 mg/kg food (nominal) ¹
year of execution	: 1986/87	vehicle	: none
test substance	: NC-129 lot no. 129T8605, purity 97.8%	GLP statement	: yes
route	: oral	guideline	: predominantly in accordance with OECD 408 (1998)
species	: rat, CD	acceptability	: acceptable
group size	: 10/sex/dose (+10/sex/dose as recovery group in control and at 350 mg/kg food)	NOAEL	: 2.6 mg/kg bw/day for females

¹equal to 0, 2.3, 4.9, 11.6 and 25.7 mg/kg bw/d for males and 0, 2.6, 5.5, 12.8 and 27.7 mg/kg bw/d for females

Study design

The study was generally in compliance with OECD 408 (1998). Blood and urine were collected in week 13 and after 4 weeks recovery. Additional to normal clinical biochemical parameters plasma cholinesterase (using butyryl and acetyl substrates) and erythrocyte cholinesterase (using acetyl substrate) activities were measured.

Results

The results are summarised in table 6.3.4-01a and 6.3.4-01b

Table 6.3.4-01a Results after 13 weeks.

Dose (mg/kg food)	0		30		65		155		350		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	no treatment-related findings										m/f m/f
Clinical signs	no treatment-related findings										
Body weight gain ¹						dc	dc	dc	dc	dc	
Food consumption							d	d	d	d	
Water consumption							d	d	d	d	
Ophthalmoscopy	no treatment-related findings										
Haematology											
- platelet count									ic		
- prothrombin time									dc		
- haematocrit						i		ic		ic	
- haemoglobin						ic		ic		ic	
Clin. Chemistry											
- GGT									ic	ic	
- alkaline phosphatase										ic	
- urea									ic	ic	
- total protein				dc		dc		dc	dc	dc	
- albumin				dc		dc		dc	dc	dc	
- A/G ratio									ic		
- chloride			dc	dc		dc	dc	dc			
- potassium				ic					ic		
- phosphorus						ic		ic		ic	
- calcium							dc		dc	dc	
- plasma butyryl cholinesterase										dc	
- plasma acetyl cholinesterase											
Urine analysis	no treatment-related findings										
Organ weights											
- see text											
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

1 no statistical analysis performed on body weight

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Table 6.3.4-01b Results after 4-week recovery period.

Dose (mg/kg food)	0		350		dr
	m	f	m	f	
Mortality	no treatment-related findings				
Clinical signs	no treatment-related findings				
Body weight gain¹			ic	ic	
Food consumption			dc		
Water consumption	no treatment-related findings				
Ophthalmoscopy	no treatment-related findings				
Haematology	not performed				
Clin. Chemistry					
- alkaline phosphatase			ic		
- urea			ic	ic	
- total protein				dc	
- albumin				dc	
- α1-globulin			ic		
- α2-, β-globulin			dc		
- calcium				dc	
Urine analysis	no treatment-related findings				
Organ weights	see text				
Pathology					
<u>macroscopy</u>	no treatment-related findings				
<u>microscopy</u>	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

1 no statistical analysis performed on body weight

Acceptability

The study is considered acceptable.

Conclusions

Rats were administered 0, 30, 65, 155 and 350 mg/kg food of NC-129 for 13 weeks. Two females at 30 mg/kg food died during blood sampling. No treatment-related mortality or clinical signs were observed. Body weight gain was significantly decreased at 155 and 350 mg/kg food in males (77 and 50% of control, resp.) and at 65, 155 and 350 mg/kg food in females (82%, 68% and 47% of control, respectively). During the recovery phase significantly higher body weight gains were observed in both sexes that had previously received 350 mg/kg food. Food consumption was decreased at 155 and 350 mg/kg food in males and females (86% and 70-68% of control, respectively). Food consumption was restored in the female recovery group but in the male group food consumption increased only up to 90% of the control group. The efficiency of food conversion was decreased at 155 and 350 mg/kg food in males and females and at 65 mg/kg food in females. During the recovery period the food conversion ratio was increased in males and females that had

previously received 350 mg/kg food. Water consumption was decreased by approximately 15-20% at 155 and 350 mg/kg food in both sexes during the exposure period and was restored to control levels during recovery.

At the end of the exposure period platelet count was increased and prothrombin time was decreased in males at 350 mg/kg food (112 and 95% of control, resp.). At the end of the exposure period haemoglobin and the haematocrit were slightly, but significantly increased in females at 65 mg/kg food and above (haematocrit not significantly changed at 65 mg/kg food; 103-105% and 104-106% of control, resp.), which could be a result of dehydration. In the absence of similar effects in males the effects observed are considered to be not toxicologically relevant. Haematological parameters were not tested after recovery.

Gamma glutamyltransferase was significantly increased at 350 mg/kg food in males (300% of control) and females (200% of control), but no difference was observed after the recovery period. Alkaline phosphatase was significantly increased at 350 mg/kg food in females (169% of control) at the end of the exposure period and in males at the end of the recovery period (128% of control). Urea was significantly increased at 350 mg/kg food in males (147% of control) and females (133% of control), which may have been a result of dehydration and a reduced renal blood flow. Total protein was significantly decreased at 155 and 350 mg/kg food in males (94-92% of control) and at all treatment levels in females (96-87% of control). This was clearly due to a significant decrease in albumin in females at all treatment levels (93-82% of control) according to electrophoretic analysis. The decrease in protein and albumin at 30 mg/kg food were only slight and not dose-related, and they are therefore, not considered to be toxicologically significant. Albumin blood concentration was also determined by chemical analysis with bromocresol green indicating significantly decreased albumin concentration at 155 and 350 mg/kg food in females only (92-89% of control). The data suggest that albumin synthesis may have been negatively affected by the decreased food consumption at 155 and 350 mg/kg food. The albumin/globulin ratio was slightly but significantly increased in males at 350 mg/kg food.

Some of these effects were still present at 350 mg/kg food after the recovery period (other groups were not investigated). α 1-Globulin concentration increased and α 2- and β -globulin concentrations decreased in males at the end of the recovery period. Phosphorus was significantly increased at 65 and 155 mg/kg food in males, which is considered to be not treatment-related in view of the fact that no increase at 350 mg/kg food in males was observed. In females phosphorus was significantly increased at 350 mg/kg food, which could be related to a reduced renal blood flow that also caused increased urea. No increased phosphorus concentrations were observed at the end of the recovery period. The significantly decreased calcium concentration at 350 mg/kg food in both sexes (94-96% of control) was probably an artefact caused by reduced albumin concentrations. The effect was still present in females at the end of the recovery period. Plasma butyryl- and acetyl cholinesterase activity were decreased at 350 mg/kg food in females (51 and 55% of control, resp.). The effect had disappeared at the end of the recovery period. No changes in erythrocyte cholinesterase activity were noted. Other deviations observed (chloride, potassium) were considered to be incidental or not dose-related, and were not observed at the highest dose level(s), and therefore, not considered to be toxicologically relevant.

Urinalysis showed no treatment-related effects.

Almost all absolute organ weights determined were significantly decreased at 350 mg/kg food in both sexes, while relative weights were significantly increased at the end of the exposure. Absolute kidney and liver weight were also (significantly) decreased at 155 mg/kg food. The decreased organ weights were all considered to be an effect of reduced body weight gain and, therefore, not considered to be toxicologically relevant. Effects were still present at the end of the recovery period.

No treatment-related effects were observed at necropsy nor at microscopy.

The NOAEL is set at 30 mg/kg food (equal to 2.6 mg/kg bw/d for females) based on decreased body weight gain and changes in clinical biochemistry (protein and albumin) in females at 65 mg/kg food and above.

STUDY 2

Characteristics

Reference	: Holmes, 1988b	exposure	: 13 weeks, diet
Type of study	: 13-week oral toxicity study	dose	: 0, 30, 90, 270 and 810 mg/kg food/day (nominal) ¹
year of execution	: 1986/87	vehicle	: none
test substance	: NC-129 lot no. 129T8605, purity 97.8%	GLP statement	: yes
route	: oral	guideline	: predominantly in accordance with OECD 408 (1998)
species	: mouse; CD-1	acceptability	: acceptable
group size	: 12/sex/dose	NOAEL	: 4.1 mg/kg bw/day in males

¹equal to 0, 4.1, 13.0, 40.1 and 119.2 mg/kg bw/d for males and 0, 4.9, 14.6, 43.1 and 124.6 mg/kg bw/d for females

Study design

The study was generally in compliance with OECD 408 (1998). Blood was collected in week 13 to measure haematological parameters and prior to necropsy (week 14) to measure clinical biochemical values. Urine was collected overnight in week 12.

Results

The results are summarised in table 6.3.4-02.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. No conclusion must be drawn on the basis of this document.

Table 6.3.4-02

Dose (mg/kg food)	0		30		90		270		810		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	no treatment-related findings										m/f
Clinical signs	no treatment-related findings										
Body weight gain ¹					dc	d	dc	dc	dc	dc	
Food consumption								d	d	d	
Water consumption						d	d	d	d	d	
Ophthalmoscopy	no treatment-related findings										m/f
Haematology											
- haematocrit							dc		dc	dc	
- haemoglobin									dc	dc	
- MCHC									ic		
- MCV						dc		dc	dc	dc	
- MCH									dc	dc	
- platelet count										dc	
Clin. Chemistry											
- alkaline phosphatase									ic		
- ASAT									ic		
- OCT									i	ic	
- urea							ic	ic	ic	ic	
- glucose			ic		ic		ic		ic		
- total bilirubin							dc		dc		
- total protein									dc		
- chloride							ic		ic		
- phosphorus					ic				ic		
Urine analysis	no treatment-related findings										
Organ weights											
- brain								dc ^a	ic ^r	dc ^a	
- epididymides									dc ^a		
- heart							ic ^r		ic ^r	ic ^r	
- kidneys							ic ^r		ic ^r	ic ^r	
- liver							ic ^r	ic ^r	ic ^r	ic ^r	
- lungs									dc ^a , ic ^r		
- spleen									ic ^r		
- subman. sal. gland								dc ^a	dc ^a	dc ^a ic ^r	
- testes									ic ^r		
- uterus + cervix										dc ^a	
Pathology											
macroscopy	no treatment-related findings										
microscopy											
- liver: acute inflam. and necrosis	6/12	6/11	5/12	9/12	6/12	9/12	5/12	10/11	4/11	7/11	

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

1 no statistical analysis performed on body weight

Acceptability

Acute inflammation and necrosis of the liver may be caused by a parasitic infection. This may have masked effects that otherwise would have been observed. However, since the liver was not the target organ and

comparable effects on body weight, food consumption and clinical biochemistry were noted in other repeated dose studies with rats and dogs, the study is considered acceptable for derivation of a NOAEL.

Conclusions

Mice were administered 0, 30, 90, 270 and 810 mg/kg food of NC-129 for 13 weeks. One male at 810 mg/kg food and one female in the control group and at 270 and 810 mg/kg food each died during blood sampling. No treatment-related mortality or clinical signs were observed. Body weight loss was observed in the first week of exposure at 810 mg/kg food. Body weight gain was significantly and slightly to markedly decreased at 90, 270 and 810 mg/kg food in males (82%, 58% and 49% of control, respectively) and females (88%, 61% and 20% of control, respectively; at 90 mg/kg food not significantly) with a dose-relationship. Food consumption was decreased at 810 mg/kg food in males (86% of control) and at 270 and 810 mg/kg food in females (91% and 76% of control, respectively). The food conversion efficiency was decreased at 90, 270 and 810 mg/kg food in males and at 270 and 810 mg/kg food in females, with a dose-related trend. Water consumption was decreased at 270 and 810 mg/kg food in males (85% and 81% of control, respectively) and at 90-810 mg/kg food in females (77%, 70% and 57% of control, respectively) in a dose-related manner.

The haematocrit was significantly decreased at 270 mg/kg food in males and at 810 mg/kg food in both sexes (95% of control). Haemoglobin was significantly decreased at 810 mg/kg food in both sexes (95-97% of control). The mean cell haemoglobin concentration was slightly, but significantly increased in males at 810 mg/kg food (103% of control) and is not considered to be a biologically relevant effect. Mean cell volume was slightly, but significantly decreased at 810 mg/kg food in both sexes (94% of control) and at 90 and 270 mg/kg food in females (96% of control). The mean cell haemoglobin content was significantly decreased at 810 mg/kg food in both sexes (94% of control). Platelet count was slightly, but significantly decreased at 810 mg/kg food in females (89% of control).

Alkaline phosphatase and aspartate aminotransferase were significantly increased at 810 mg/kg food in males (158 and 157% of control, resp.). Ornithine carbamyltransferase was increased in males (163% of control) and significantly increased in females (224% of control) at 810 mg/kg food. Urea was significantly increased at 270 and 810 mg/kg food in both sexes (122-143% of control), which was probably due to dehydration. Glucose was significantly increased in all treated males (143-152% of control). For glucose a mean value and range were given for mice (n=33) as background data (origin unknown). The glucose mean control value was rather low compared to this background data and no dose-response relationship was present. Therefore, the increase in glucose in males is considered to be not treatment-related. Total bilirubin was significantly decreased in males at 270 and 810 mg/kg food (67-83% of control; not dose-related), but this effect is not considered to be toxicologically relevant. Total protein was slightly, but significantly decreased at 810 mg/kg food in males (94% of control), possibly due to a reduced food consumption. Chloride was significantly increased in males at 270 and 810 mg/kg food (104-105% of control). Phosphorus was significantly increased at 90 and 810 mg/kg food in males (109 and 112% of control, respectively). Other deviations observed were considered to be incidental and not to be toxicologically relevant.

Urinalysis showed no treatment-related findings.

The decreased absolute organ weights and increased relative organ weights are all considered to be related to the decreased gain in body weight.

No treatment-related effects were observed at necropsy. Microscopy revealed acute inflammation and necrosis in the liver of the control group and all treated groups with similar incidences. Therefore, the effect is not considered to be treatment-related.

The NOAEL is set at 30 mg/kg food (equal to 4.1 mg/kg bw/day for males and 4.9 mg/kg bw/day for females) based on marked decrease in body weight gain.

STUDY 3

Characteristics

Reference	: Spicer, 1989b	exposure	: 13 weeks, gelatin capsule
Type of study	: 13-week oral toxicity study	dose	: 0, 0.5, 1.0, 4.0 and 16.0 mg/kg bw/day
year of execution	: 1988	vehicle	: none
test substance	: NC-129 tech., Lot no. 129T8605, purity 98.0%	GLP statement	: yes
route	: oral	guideline	: predominantly in accordance with OECD 409 (1998)
species	: dog, Beagle	acceptability	: acceptable
group size	: 4/sex/dose	NOAEL	: 1.0 mg/kg bw/day

Study design

The study was generally in compliance with OECD 409 (1998). Also the weight of epididymides, thymus and uterus was not determined. Haematological and biochemical parameters and urinalysis were measured pre-test and after 1, 2 and 3 months of exposure. Dosing was based on a 28-day study (Spicer, 1989a), in which dogs were administered 0, 10, 30, 100 and 300 mg/kg bw/day. Emesis was observed in all treated animals and one control male. Diarrhoea or soft stool was observed in all treated groups. Ptyalism was seen at 100 and 300 mg/kg bw. Body weight loss was observed for one male at 300 mg/kg bw and food consumption was decreased at 300 mg/kg bw in males.

Results

The results are summarised in table 6.3.4-03.

Table 6.3.4-03 Results after 13 weeks.

Dose (mg/kg bw/day)	0		0.5		1.0		4.0		16.0		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	None										
Clinical signs											
- food-like emesis	1/4	0/4	1/4	0/4	1/4	1/4	1/4	2/4	3/4	4/4	m/f
- soft stool	0/4	0/4	2/4	3/4	0/4	2/4	1/4	1/4	3/4	4/4	m/f
- ptyalism	1/4	0/4	1/4	0/4	0/4	1/4	4/4	2/4	4/4	4/4	m/f
Body weight gain							d		d		
Food consumption			d		d		d		d		
Ophthalmoscopy	No treatment-related findings										
Haematology							dc ¹		dc ¹		
- lymphocyte count										dc ²	
- prothrombin time											
Clin. Chemistry											
- BUN			dc		dc		dc		dc		
Urinalysis	No treatment-related findings										
Organ weights	No treatment-related findings										
Pathology											
<u>macroscopy</u>	No treatment-related findings										
<u>microscopy</u>											
- mediastinal lymph node: lymphoid hyperplasia	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4	0/4	

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

¹ after 3 months² after 2 and 3 months**Acceptability**

The study is considered acceptable.

Conclusions

Dogs were administered 0, 0.5, 1.0, 4.0 or 16.0 mg/kg bw/day of NC-129 technical for 13 weeks. No mortality was observed. A dose-related increase of animals with food-like emesis and ptyalism was observed at 4.0 and 16.0 mg/kg bw/day. Soft stool was seen in all treated groups. Body weight gain was decreased at 4.0 and 16.0 mg/kg bw/day in males (46 and 31% of control resp.); at both levels body weight loss was observed in one animal. Food consumption was variable in all groups but was lower than control, overall, for males in treated groups, although the groups receiving 0.5 and 1 mg/kg bw/d were essentially unaffected.

Lymphocyte count was decreased after 3 months only, at 4.0 and 16.0 mg/kg bw/day in males compared to pre-test values and control value. Prothrombin time was decreased at 16.0 mg/kg bw at 2 and 3 months in

females. However, the pre-test value was already lower than the control group. Therefore, both these effects are not considered to be treatment-related.

In males decreased blood urea nitrogen was seen in all treated groups after 3 months compared to the control group. Since the mean value of the control group was higher than pre-test value after 3 months, the effect is not considered to be treatment-related. Other deviations were incidental and not treatment-related.

Urinalysis showed no treatment-related effects.

No effect was observed on organ weights or macroscopic examination. Histological examination of the mediastinal lymph node revealed mild lymphoid hyperplasia in 2 males at 16.0 mg/kg bw/day.

The NOAEL is set at 1.0 mg/kg bw/day based on decreased body weight gain, food-like emesis and ptialism.

STUDY 4

Characteristics

Reference	: Spicer, 1990 including additional study Spicer, 1991	exposure	: 1-year, gelatin capsule
Type of study	: 1-year oral toxicity study	dose	: 0, 0.5, 1.0, 4.0, 16.0 and 32.0 mg/kg bw/day
year of execution	: 1988/89	vehicle	: none
test substance	: NC-129 techn., Lot no. 129T8605, purity 98.0%	GLP statement	: yes
route	: oral	guideline	: in accordance with OECD 452 (1981)
species	: dog, Beagle	acceptability	: acceptable
group size	: 4/sex/dose	NOAEL	: 1.0 mg/kg bw/day

Study design

The study was generally in compliance with OECD 452 (1981). Haematological parameters and urinalysis were not determined after 3 months of exposure. The lowest dose level of the original study was 1.0 mg/kg bw/d. However, to reconfirm body weight reductions and emesis, five months after the start of exposure, a level of 0.5 mg/kg bw/d was added and a concurrent control group, and these groups were also treated for one year. Since the period between start of the main study and the additional study is only five months, the studies will be treated combined below.

Results

The results are summarised in table 6.3.4-04.

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Table 6.3.4-04 Results.

Dose (mg/kg bw/day)	0		0.5*		1.0		4.0		16.0		32.0		dr
	m	f	m	f	m	f	m	f	m	f	m	f	
Mortality	None												
Clinical signs see text													
Body weight gain			ic	d	d	d	d	d	d	d	d	d	
Food consumption	No treatment-related findings												
Ophthalmoscopy	No treatment-related findings												
Haematology	No treatment-related findings												
Clin. Chemistry - calcium									dc ¹		dc ²		
Urinalysis	No treatment-related findings												
Organ weights	No treatment-related findings												
Pathology													
macroscopy													
- emaciation	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	
- pale tissues	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	
microscopy													
- bone marrow: hypercellularity	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	
- liver: hypertrophy	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	
- skeletal muscle: atrophy	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	
- thymus: atrophy	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	

* The 0.5 mg/kg bw/d was started as an additional study and compared to its own concurrent control group.

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

¹ after 12 months

² after 6 and 12 months

Acceptability

The study is considered acceptable. Although the variations in body weights and body weight gains are high in this study, the effect of a decreased body weight gain is clear from the height of the decrease.

Conclusions

Dogs were administered 0, 0.5, 1.0, 4.0, 16.0 or 32.0 mg/kg bw/d of NC-129 technical for 12 months. No mortality was observed. Diarrhoea was observed incidentally in more animals with increasing dose. Soft stool was seen in a higher number of animals at 4.0 mg/kg bw/d with a higher incidence at 32.0 mg/kg bw/d. Food-like emesis was observed at all dose levels and in all animals at 4.0 mg/kg bw/d incidentally with an increased incidence and higher severity from 16.0 mg/kg bw/d onwards. Ptyalism was observed at all dose levels and in an increased number of animals with an increased incidence and higher severity at higher doses. Body weight gain was significantly increased in males at 0.5 mg/kg bw/d (163% of control), and

decreased in females at 0.5 mg/kg bw/d (64% of control) and at all other dose levels (77, 60, 50 and 53% of control for males and 68, 40, 44 and 20% of control for females at 1.0, 4.0, 16.0 and 32.0 mg/kg bw/d. The significant reduction in body weight in 32.0 mg/kg bw/d females was mainly due to substantial weight loss by one female). A high variation in body weight and body weight gain is seen within groups and between groups, also between the two control groups. Since deviations in males at 0.5 compared to 1.0 mg/kg bw/d and higher were contradictory, the effect at 0.5 mg/kg bw/d is not considered to be treatment-related. One female at 32 mg/kg/day appeared to be thin throughout the study and, in addition to having ptialism, soft stool/diarrhoea and emesis, also appeared to be weak during some study weeks with decreased activity, a dry nose, pale gums and felt cold to the touch. A large variation in food consumption with no significant treatment-related effect was observed.

Calcium concentration was significantly decreased at 16.0 and 32.0 mg/kg bw/d in females. Since also the pre-test value was lower than the control value, the decrease is not considered to be treatment-related. Other deviations were incidental and not considered to be treatment-related.

Haematology, clinical biochemistry and urinalysis showed no treatment-related effects.

No effect on organ weights was observed.

One female at 32.0 mg/kg bw/d that was thin throughout the study, was emaciated and all tissues were pale at macroscopy. Histopathological examination of this female revealed overall hypocellularity of the bone marrow, mild hypertrophy of hepatocytes, and mild atrophy of skeletal muscle and thymus.

Only body weight gain was decreased probably due to emesis and no other effects were seen, e.g. organ weights. In view of the large variation in body weights at the start of the experiment, the decrease in body weight gain at 0.5 and 1.0 mg/kg bw/d (64 and 68% of control) is considered to be no clear effect. However, the decrease at 4.0 mg/kg bw/d and higher (40, 44 and 20% of control at 4.0, 16.0 and 32.0 mg/kg bw/d are considered adverse. The NOAEL is set at 1.0 mg/kg bw/d.

B.6.3.5 Summary

The results of the subacute and semichronic toxicity studies are summarised in tables 6.3.5-01 and 6.3.5-02, respectively.

Table 6.3.5-01 Subacute studies with pyridaben

Duration	Species	Route	NOAEL mg a.i./kg bw/day	LOAEL mg a.i./kg bw/day	Critical effects	Reference
3 weeks	rat	dermal	systemic: 100	systemic: 300	- Reduced body weight gain.	Blaszczak, 1992
4 weeks	rat	inhalation	3.0 mg/m ³	10 mg/m ³	- Reduced body weight.	Hoffman, 1989

Table 6.3.5-02 Semichronic studies with pyridaben

Duration	Species	Route	NOAEL mg a.i./kg bw/day	LOAEL mg a.i./kg bw/day	Critical effects	Reference
13 weeks	rat	oral	2.7	5.5	Decreased body weight gain in females.	Holmes, 1988a
13 weeks	mouse	oral	4.1	13.0	Decreased body weight gain and water consumption.	Holmes, 1988b
3 months	dog	oral	1.0	4.0	Decreased body weight gain, lymphocyte count.	Spicer, 1989b
12 months	dog	oral	1.0	4.0	Decreased body weight gain.	Spicer, 1990,1991 incl. additional study

Three weeks of dermal exposure of rats to 0, 30, 100, 300 and 1000 mg/kg bw/day resulted in decreased body weight gain at 300 and 1000 mg/kg bw/day. The NOAEL for systemic effects was set at 100 mg/kg bw/day.

After subacute inhalation exposure of rats to 0, 1, 3 and 10 mg/m³, reduced body weight was observed at 10 mg/m³. Based on these effects a NOAEL of 3.0 mg/m³ was established for systemic effects.

Dietary exposure of rats to 0, 30, 65, 155 and 350 mg/kg food/day of pyridaben (equal to 0, 2.3, 4.9, 11.6 and 25.7 mg/kg bw/d for males and 0, 2.6, 5.5, 12.8 and 27.7 mg/kg bw/day for females) for 13 weeks caused decreased body weight gain at 65 mg/kg food in females and at 155 and 350 mg/kg food in both sexes. Food and water consumption were decreased at 155 and 350 mg/kg food. Food conversion efficiency was also decreased at 155 and 350 mg/kg food in both sexes and at 65 mg/kg food in females. No toxicological significant changes were noted in haematology. At clinical biochemistry, gamma glutamyltransferase was increased at 350 mg/kg food in both sexes. Alkaline phosphatase was increased at 350 mg/kg food in females at the end of the exposure period and in males at the end of the recovery period. Total protein was decreased at 65 mg/kg food in females and at 155 and 350 mg/kg food in both sexes. Albumin was decreased in females at 65, 155 and 350 mg/kg food. Albumin/globulin ratio was slightly increased in males at 350 mg/kg food. α 1-Globulin concentration was increased and α 2- and β -globulin concentrations were decreased in males at 350 mg/kg food at the end of the recovery period only. The NOAEL is established at 30 mg/kg food (equal to 2.7 mg/kg bw/d for females) based on decreased body weight gain and changes at clinical biochemistry (decreased protein and albumin).

Dietary exposure of mice to 0, 30, 90, 270 and 810 mg/kg food (equal to 0, 4.1, 13.0, 40.1 and 119.2 mg/kg bw/d for males and 0, 4.9, 14.6, 43.1 and 124.6 mg/kg bw/d for females) for 13 weeks, caused reduced body weight gain and water consumption at 90 mg/kg food and above. Food consumption and food conversion efficiency were decreased from 180 and 90 mg/kg food onwards, respectively. Changes in haematology (a.o. decreased haematocrit and haemoglobin) were noted at 810 mg/kg food. Changes in clinical biochemistry were noted at 270 mg/kg food and above and included changes in ALP, ASAT, OCT, urea, total protein, chloride and phosphorus. Changes in relative organ weights were noted at 270 and 810 mg/kg food, but were considered to be due to the decreased body weight gain. Histopathology revealed acute inflammation

and necrosis of the liver in all groups including control. A NOAEL of 30 mg/kg food is established (equal to 4.1 mg/kg bw/day in males and 4.9 mg/kg bw/day in females) based on decreased body weight gain.

Dogs exposed to 0, 0.5, 1.0, 4.0 and 16.0 mg/kg bw/d of pyridaben in a gelatin capsule for 3 months showed a dose-related increased incidence of food-like emesis and ptyalism at 4.0 and 16.0 mg/kg bw/d. Body weight gain was reduced at 4.0 and 16.0 mg/kg bw/d in males. Food consumption was reduced in all treated males but this was not dose-related. Mild lymphoid hyperplasia was noted in 2/4 males at 16.0 mg/kg bw/d. The NOAEL is set at 1.0 mg/kg bw/d based on decreased body weight gain, food-like emesis and ptyalism.

Dogs exposed to 0, 0.5, 1.0, 4.0, 16.0 and 32.0 mg/kg bw/d of pyridaben in a gelatin capsule for 12 months showed a reduced body weight gain at 1.0-32.0 mg/kg bw/d in both sexes and at 0.5 mg/kg bw/d in females. A high variation in body weight and body weight gain was observed within groups and between groups, also between the two control groups. The variation in food consumption was also high. An increased incidence of soft stool was noted at 4.0 mg/kg bw/d and above. Food-like emesis and ptyalism were observed at all dose levels, including control group, with an increased incidence and higher severity at higher dose levels.

In view of the large variation in body weights at the start of the experiment, the decrease in body weight gain at 0.5 and 1.0 mg/kg bw/d is considered to be no adverse effect. The NOAEL is set at 1.0 mg/kg bw/d based on decreased body weight gain.

In conclusion, in all rodent and dog studies with oral, dermal and inhalation exposure decreased body weight (gain) was the adverse effect.

Considering the identity of the observed effects at the LOAELs after oral, dermal and inhalation repeated exposure, classification with R48 is not considered necessary.

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B.6.4.1 *In vitro*

Study design and results

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
B: <i>S. typh.</i> TA 98 TA 100 TA 1535 TA 1537	point mut. point mut. point mut. point mut.	- - - -	- - - -	rat liver	Arochlor 1254	Mutation tests ¹⁾ : 50, 158, 500, 1580 and 5000 µl/plate Solvent: DMSO Positive and negative control: Included	Bootman and May, 1986a
B : <i>E.coli</i> WP2uvrA	point mut.	-	-				

Test substance: NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3(2H)-pyridazinone), batch nr. 129 T 8605, white crystalline solid, purity 98.0%.
Precipitation was not observed.
GLP statement: yes
According to OECD 471: yes

The study fulfils the requirements of the recent OECD 471 guideline of 1997.

Pyridaben did not induce point mutations in *S. typhimurium* and *E.coli*.

Type of study: Lethal DNA damage test with *Escherichia coli* strains

¹⁾ Dose levels were based on a dose-range finding study with dose levels of 31.6-10000 µg/ml.

Test substance: NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3(2H)-pyridazinone),
batch nr. 129 T 8605, white crystalline solid, purity 98.0%.
Precipitation was not indicated.
Toxicity was not observed.
GLP statement: yes
No OECD guideline available.

Acceptability

There is no OECD or EC guideline available for this type of study. However, the results of the present study are considered suitable of the overall evaluation of the genotoxic properties of pyridaben.

Conclusion

Pyridaben did not produce lethal DNA damage in *E.coli* WP2, WP67 and CM871.

STUDY 3

Study design and results

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) cells	chromosome aberration			rat liver	Phenobarbital and 5,6-benzoflavone	Dose range finding: 0.001 to 1000 µg/ml Main test 1: (-S9 mix: 24 h treatment) 1.1, 3.4, 10.4, 32.3 and 100 µg/ml (-S9 mix: 48 h treatment) 0.1, 0.3, 1.0, 3.2 and 10 µg/ml Main test 2: (-S9 and +S9 mix: 6 h treatment) 3.1, 6.3, 12.5, 25 and 50 µg/ml Vehicle: DMSO Negative and positive controls included	Kaneko, 1988a
Test substance: NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3-(2H)-pyridazinone), batch nr. 129 T 8605, white crystalline solid, purity 98.0%. Cytotoxicity observed at dose levels: Main test 1: (-S9 mix: 24 h treatment) 100 µg/ml, (-S9 mix: 48 h treatment) 0.1 µg/ml, Main test 2: (-S9 mix: 6 h treatment) 3.1 µg/ml, (+S9 mix: 6 h treatment) 50 µg/ml No precipitation observed. GLP statement: yes According to OECD 473: no, see acceptability							

Acceptability

The chromosome aberration study does not fulfil the requirements of the recent OECD 473 guideline of 1997. No duplicate cultures were used (the guideline states that at least 200 metaphases should be scored per concentration and control equally divided amongst the duplicates). At least three analysable concentrations should be used. In case of cytotoxicity these concentrations should cover a range from the maximum to little or no toxicity. The highest concentration should show a mitotic index greater than 50%. Although 5 concentrations were used for analysis in this study, at the 24 h treatment and 6 h treatment (+S9-mix) four concentrations showed little or no cytotoxicity and the highest concentration was very toxic. At the 6 h and 48 h treatment all concentrations were toxic to very toxic and no concentration was used for analysis with little or no cytotoxicity.

Conclusion

No conclusion could be drawn, because the study was not acceptable.

STUDY 4

Study design and results

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 (V79) cells, 4-1 clone 9 3/12	gene mutations (HGPRT)	-	-	rat liver	Arochlor 1254	Dose range finding (-S9 and + S9): 1.84 to 1150 µg/ml ¹⁾ Main experiment 1 and 2: (-S9 and +S9) 3, 125, 6.25, 12.5, 25 and 50 µg/ml Additional experiment 3 (+S9) 3, 125, 6.25, 12.5, 25 and 50 µg/ml Solvent: DMSO Negative and positive controls included	Hodson-Walker, 1989a
Test substance: NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3-(2H)-pyridazinone), batch nr. 129 T 8605, fine white powder (agate ground), purity 98.0%. 1) Visible precipitation was observed at 50 µg/ml GLP statement: yes According to OECD 476: yes							

Acceptability

The study was performed in accordance with OECD 476 of 1984. The study does not fulfil the requirements of the more recent OECD 476 guideline of 1997, such as cytotoxicity criteria and number of dose levels tested. However, the study is considered acceptable.

Conclusion

Under the conditions of the study, pyridaben did not induce gene mutations in mammalian cells.

STUDY 5**Study design and results**

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 ovary (CHO) cells	chromosome aberration	-	-	rat liver	Phenobarbital and 5,6-benzoflavone	Dose range finding: 0.001 to 1000 µg/ml Main test 1: (- S9 mix: 4 h treatment) 0.1, 1 and 10 µg/ml (- S9 mix: 24 and 48 h treatment) 0.001, 0.01 and 0.1 µg/ml (+ S9 mix: 4h treatment) 0.1, 1, 10 and 20 µg/ml Additional test 2: (-S9 mix: 4 h treatment) 0.1, 1 and 10 µg/ml (-S9 mix: 24 h treatment) 0.001, 0.01 and 0.1 µg/ml Vehicle: DMSO Negative and positive controls included	Hodson-Walker, 1989b
Test substance: NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3-(2H)-pyridazinone), batch nr. 129 T 8605, white crystalline solid, purity 98.0%. Cytotoxicity observed at dose levels: Main test 1: (-S9 mix: 4 h treatment) 1 and 10 µg/ml, (+S9 mix: 4 h treatment): no 50% reduction was observed, (-S9 mix: 24 h treatment) 0.1 µg/ml, (-S9 mix: 48 h treatment) 0.01 and 0.1 µg/ml, Main test 2: (-S9 mix: 4 h treatment) 1 and 10 µg/ml, (-S9 mix: 24 h treatment) 0.1 µg/ml No precipitation observed. GLP statement: yes According to OECD 473: yes							

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Results CA

Main1 See below. Values printed bold represented a statistically significant difference from the control.

dose µg/ml	-S9		+S9	
	4 hours treatment/20-h harvest			
	Mean mitotic index %	Mean cells with aberrations	Mean mitotic index %	Mean cells with aberrations
Solvent c.	3.8	5.0	7.4	3.5
0.1	3.8	10.5 ¹⁾	5.0	5.5
1	1.5	7.0	4.1	6.0
10	0.7	10.5 ¹⁾	4.4	3.5
pos. c.	4.8	19.0 ²⁾	5.6	57.0 ²⁾

Main1 continued

dose µg/ml	-S9			
	24 hours treatment		48 hours treatment	
	Mean mitotic index %	Mean cells with aberrations	Mean mitotic index %	Mean cells with aberrations
Solvent c.	2.5	7.0	5.7	6.0
0.001	3.6	7.0	4.2	3.5
0.01	3.6	12.5 ¹⁾	2.3	5.0
0.1	0.9	6.5	1.0	3.5
pos. c.	1.4	60.0 ²⁾	1.6	94.5 ²⁾

1) significant 0.05 > p > 0.01

2) very highly significant p < 0.001

Main2

dose µg/ml	-S9			
	4 hours treatment/20-h harvest		24 hours treatment	
	Mean mitotic index %	Mean cells with aberrations	Mean mitotic index %	Mean cells with aberrations
Solvent c.	4.9	9.0	4.3	6.0
0.001			4.7	8.5
0.01			3.0	13.0 ¹⁾
0.1	3.5	6.5	1.1	5.6
1	0.7	3.5		
10	0.8	6.0		
pos. c.	4.8	15.0 ²⁾	1.9	74.5 ²⁾

1) significant 0.05 > p > 0.01

2) very highly significant p < 0.001

Acceptability

The study was performed in accordance with OECD 473 of 1984. The study does not fulfil the requirements of the more recent OECD 473 guideline of 1997, such as duplicate cultures. However, the study is considered acceptable.

Conclusion

Pyridaben did not induce chromosome aberrations in Chinese hamster ovary (CHO) cells.

STUDY 6**Study design and results**

Type of study: in vitro unscheduled DNA synthesis, with independent repeat

Indicator cells	Endpoint	Result	Dose range	Reference
male rat hepatocytes, Wistar	DNA repair	-	Pre test: 0.01 to 80 µg/ml ¹⁾ Experiment 1: 0.015, 0.045, 0.15, 0.45 and 1.5 µg/ml Experiment 2: 0.002, 0.004, 0.015, 0.045 and 0.15 µg/ml Exposure period: 18 hr Positive control included. Solvent DMSO	Fautz, 1992
Test substance: NC-129, batch nr. 129 T 8605, colourless solid, purity 98.0%. ¹⁾ Precipitation was observed at dose level: ≥ 80 µg/ml directly and at dose levels higher than 5.33 µg/ml during the treatment period. Toxicity was observed in the preliminary test at dose level of 0.8 µg/ml and above. Toxicity was observed in first experiment at dose level of 1.5 µg/ml and above and in second experiment at dose level of 0.15 µg/ml and above GLP statement: yes According to OECD 482: yes				

Acceptability

The study was performed in accordance with OECD 482 of 1986. The study is considered acceptable.

Conclusion

Pyridaben did not induce DNA damage in rat hepatocytes.

B.6.4.2 In vivo**STUDY 7****Study design and results**

Type of study: mouse micronucleus test

Indicator cells	Endpoint	Result	Dose range	Reference
ICR mouse, (Crj: CD-1) 6/sex/dose (low, intermediate and positive control) 18/sex/dose (solvent control) 24/sex/dose (high dose)	micronuclei (bone marrow)	-	Dose range finding test: 50 to 800 mg/kg bw, once by gavage, sacrifice at 72 hours after dosing. Part 2: 0, 30, 65, 140 mg/kg bw, once by gavage, sacrifice: low, intermediate dose and positive control at 24 hours after dosing. High dose and solvent control at 24, 48 and 72 hours after dosing. Positive control included. Vehicle: 1% CMC	Kaneko, 1988b
Test substance: NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3(2H)-pyridazinone), batch nr. 129 T 8605, white crystalline solid, purity 98.0%. DRF: Toxicity observed at dose level of 200 and 400 mg/kg (1 male and 2 female animals died); 800 mg/kg (3 male and 2 female animals died). Main test: Toxicity observed at dose level 140 mg/kg (2 male and 3 female animals died). GLP statement: yes According to OECD 474: yes				

Acceptability

The study was performed in accordance with OECD 474 of 1983. The study does not fulfil the requirements of the recent OECD 474 guideline of 1997, since only 1000 immature erythrocytes per animal were scored for the incidence of micro nucleated immature erythrocytes instead of at least 2000. However, the study is considered acceptable.

Conclusion

Pyridaben did not induce micronuclei in mouse bone marrow cells.

B.6.4.3 Summary

The results from the *in vitro* and *in vivo* genotoxicity studies are summarised in table 6.4.3-01 and 6.4.3-02, respectively.

Table 6.4.3-01 *In vitro* genotoxicity studies

Test substance	Type of study		Result		Reference
	Indicator cells	Endpoint	without activation	with activation	
NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3(2H)-pyridazinone), batch nr. 129 T 8605, white crystalline solid, purity 98.0%.	B: <i>S. typh.</i> TA 98 TA 100 TA 1535 TA 1537	point mut. point mut. point mut. point mut.	- - - -	- - - -	Bootman and May, 1986a
	B: <i>E. coli</i> WP2uvrA	point mut.	-	-	
NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3(2H)-pyridazinone), batch nr. 129 T 8605, white crystalline solid, purity 98.0%	B: <i>E. coli</i> WP2 WP67 CM871	DNA damage (repair deficient).	- - -	- - -	Bootman and May, 1986b
NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3(2H)-pyridazinone), batch nr. 129 T 8605, fine white powder (agate ground), purity 98.0%.	Chinese hamster (V79) cells, 4-1 clone 9 3/12	gene mutations (HGPRT)	-	-	Hodson-Walker, 1989a
NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3(2H)-pyridazinone), batch nr. 129 T 8605, white crystalline solid, purity 98.0%.	Chinese hamster ovary (CHO) cells	chromosome aberration	-	-	Hodson-Walker, 1989b
NC-129, batch nr. 129 T 8605, colourless solid, purity 98.0%.	male rat hepatocytes, Wistar	DNA repair	-	-	Fautz, 1992

Table 6.4.3-02 *In vivo* genotoxicity studies

Test substance	Type of study		Result	Reference
	Species	Endpoint		
NC-129 (4-chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3(2H)-pyridazinone), batch nr. 129 T 8605, white crystalline solid, purity 98.0%.	ICR mouse, (Crj: CD-1)	Micronuclei (bone marrow)	-	Kaneko, 1988b

Pyridaben did not induce point mutations in *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and in the *Escherichia coli* tester strain WP₂uvrA, both with and without metabolic activation. Pyridaben was

negative in an *in vitro* chromosome aberration test with a Chinese hamster ovary cell line CHO. Pyridaben was negative in a gene mutation test using Chinese hamster cells. In addition, pyridaben was negative in an *in vitro* DNA repair study using male rat hepatocytes, pyridaben was also negative in an *in vivo* mouse micronucleus test. In conclusion, pyridaben is considered non-genotoxic.

B.6.5 Long-term toxicity and carcinogenicity studies (Annex IIA 5.5)

B.6.5.1 Chronic toxicity and carcinogenicity

STUDY 1

Characteristics

reference	: Holmes, 1990a	exposure	: 24 months, diet
type of study	: 104-week combined toxicity/carcinogenicity study	doses	: 0, 4, 10, 28 and 80/120 mg/kg food ¹
year of execution	: 1987-1989	vehicle	: None
test substance	: NC-129 techn., Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Oral	guideline	: OECD guideline 453
species	: rat, CD strain	acceptability	: acceptable
group size	: 85/sex/dose	NOAEL	: 1.1 mg/kg bw/day for males

¹ equal to 0, 0.16, 0.40, 1.13 and 5.00 mg/kg bw/day for males and 0, 0.20, 0.54, 1.46 and 6.52 mg/kg bw/day for females in the toxicity phase; equal to 0, 0.16, 0.39, 1.09 and 3.18 mg/kg bw/day for males and 0, 0.20, 0.51, 1.47 and 4.23 mg/kg bw/day for females in the oncogenicity phase.

Study design

In general, the study was performed in accordance with OECD guideline 453. Epididymides were not weighed.

Dose levels were based on a 13-week oral toxicity study in rats (Holmes, 1988).

In total 85 animals/sex/dose were used in the present study. 50 animals/sex/dose were used for the evaluation of the carcinogenic potential. 35 animals/sex/dose were used for the toxicity phase (10-12 animals/sex/dose for interim sacrifice, 23-25 animals/sex/dose for terminal sacrifice). For the animals used to assess chronic toxicity (toxicity phase) and carcinogenic potential (oncogenicity phase) the highest dose was set at 120 and 80 mg/kg food, respectively. Haematological investigations and urinalysis were performed in weeks 12, 23, 50, 76, 104 on toxicity phase animals (20 animals/sex/dose from the terminal sacrifice group with an additional 10/sex/dose from the interim sacrifice on week 12, 23, and 50). Clinical biochemistry investigations were performed on 20 animals/sex/dose from the toxicity phase (including 10/sex/dose from the interim group) in week 23 and 50, and on 10 animals/sex/dose in weeks 12, 76 and 104. Mean values at 76 and 104 weeks were often based on reduced group sizes due to mortality. Where possible, blood samples were taken from animals killed (*in extremis* or for humane reasons) before the designated termination. After 104 weeks treatment, blood samples were taken from some oncogenicity phase animals to supplement numbers. Blood smears were taken in weeks 55 and 77 from all surviving oncogenicity phase animals, and in week 104 from those not sampled for detailed haematology.

Results

The results are summarised in table 6.5.1-01a and -01b.

Table 6.5.1-01a Animals toxicity phase

Dose (mg/kg food)	0		4		10		28		120		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	18/24	10/24	18/24	14/25	15/24	13/23	23/24	17/24	19/24	15/24	m
Clinical signs	No treatment-related findings										
Body weight gain			d		d		i		dc ¹	d	
Food consumption									d	d	
Ophthalmoscopy	No treatment-related findings										
Haematology											
- WBC					d		d			dc	
- neutrophils			d		dc	d	dc	d	dc	dc	
- lymphocytes										dc	
Urinalysis											
- volume							dc		dc		
- specific gravity									ic		
- protein										ic	
Clinical chemistry	No treatment-related findings										
Organ weights											
week 53											
- liver										dc ^a	
- testes									ic ^{a,r}	ic ^r	
- adrenals											
- heart									ic ^r		
- kidneys									ic ^r	i ^r	
- lungs+bronchi									ic ^r	i ^r	
Pathology											
Macroscopy (n=35)	No treatment-related findings										
microscopy (n=35)	No treatment-related findings										
non-neoplastic lesions											
microscopy	No treatment-related findings										
neoplastic lesions											

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

1 Body weight gain was decreased until week 76 of treatment relative to control; a higher body weight gain was observed from week 78-104 at 120 mg/kg food so that no difference in body weight gain was observed after 104 weeks.

WARNING: This document forms part of an Evaluation data package and should not be read in isolation. Registration must not be based on the basis of this document.

Table 6.5.1-01b Animals oncogenicity phase

Dose (mg/kg food)	0		4		10		28		80		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality (n=50)	43	31	39	33	37	22	41	26	36	24	
Clinical signs					No treatment-related findings						
Body weight gain									d	d	
Food consumption									d	d	
Ophthalmoscopy					Not performed for this group						
Haematology					Not performed for this group						
Urinalysis					Not performed for this group						
Clinical chemistry					Not performed for this group						
Organ weights - ovaries										ic ^{a,r}	
Pathology											
<u>Macroscopy</u> (n=50)					No treatment-related findings						
<u>microscopy</u> (n=50) <i>non-neoplastic lesions</i>					No treatment-related findings						
<u>microscopy</u> (n=50) <i>neoplastic lesions</i>					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

Acceptability

Mortality should be less than 50% after 2 years according to OECD 453. However, mortality is higher in most groups. For females median survival time is acceptable, but for males median survival time is about 4 months lower than normal. The notifier submitted the following explanation:

In the CD rat of this period it was typical to find high incidences of chronic myocarditis (and senile nephropathy), predominantly in males, and considered to be geriatric change exacerbated by high bodyweight and fat accumulation in this strain. It was also typical for mortality to be high in the last months of the study. Reducing survival in this strain was a known phenomenon linked to genetic selection and exacerbated by high protein diet (LAD-2, as used for the pyridaben study, was a breeding diet containing approximately 21% protein). It is clear from the study that no oncogenic effect is to be expected from the test substance and comparable effects on body weight and food consumption with other studies in rats and dogs were found. Therefore, the study is considered acceptable and no additional data are requested.

Conclusions

Animals were given 0, 4, 10, 28, and 80 (oncogenicity phase) or 120 (toxicity phase) mg/kg food for up to 104 weeks. Mortality was high in all groups including the control group, and generally higher in males than in

females. Animals died or were killed predominantly in the second half of the study. The median survival time was 87, 86, 85, 83, 92 weeks and 99, 97, >106, 102, >106 weeks for oncogenicity males and females at 0, 4, 10, 28 and 80 mg/kg food, respectively. No treatment-related clinical signs were observed, except a delayed onset time of the first palpable swelling for treated females in the toxicity as well as the oncogenicity phase group.

Body weight gain was decreased by approximately 25% in males in the toxicity group given 120 mg/kg food during the first 76 weeks of treatment, but total weight gains (over 104 weeks) were similar to those of male controls (body weight gain was observed at 120 mg/kg food during weeks 78-104, while the other groups showed body weight loss (predominantly control animals) or no body weight gain for the same week during that period). Females in the toxicity group showed a decreased body weight gain at 120 mg/kg food at the end of the study (approximately 18% relative to female controls). The decreases at 4 and 10 mg/kg food in females are not considered to be treatment-related, because body weight gain of females at 28 mg/kg food showed an increase relative to the control group (7%). In the oncogenicity groups, total body weight gain was decreased by 12-13% in both sexes at 80 mg/kg food.

Overall food consumption was decreased in the toxicity group at 120 mg/kg food in both sexes (approximately 10%) and in the oncogenicity group at 80 mg/kg food (approximately 5%). Food conversion efficiency was determined for the first 14 weeks and was decreased at 120 mg/kg food for the toxicity group and at 80 mg/kg food for the oncogenicity group in both sexes indicating that the effects on body weights were not due only to reduced food consumption. At ophthalmoscopy no treatment-related findings were noted.

Leucocytes were decreased at 10 and 28 mg/kg food in week 76 (87-88% of control) and significantly decreased at 120 mg/kg food in females in week 76 and 104 (67-80% of control). Neutrophils were decreased at 4, 10, 28 and 120 mg/kg food in females in week 76 (91-74% of control) and significantly decreased in week 104 at 10, 28 and 120 mg/kg food in males (75-58% of control) and 120 mg/kg food in females (55% of control). Observed changes in leucocytes and neutrophils were considered to be due to bacterial infection and not test substance related. All other statistically significant changes in haematology parameters were not considered toxicologically significant, since findings were only slight, not dose-related, or did not occur consistently throughout the study period.

All statistically significant changes in clinical biochemistry were not considered toxicologically significant, since findings were only slight, not dose-related, or did not occur consistently throughout the study period.

Urinalysis showed a significantly decreased urine volume in males at 120 mg/kg food in week 12-76 (71-58% of control) and decreased volume in week 104 (56% of control). Urine volume was also significantly decreased in males at 28 mg/kg food in week 76 (65% of control). Decreased volume at 28 mg/kg food is not accompanied by changes in clinical biochemistry and is therefore considered to be not toxicologically relevant. Specific gravity was significantly increased at 120 mg/kg food in males in week 12-76 (101% of control). Protein content was significantly increased at 120 mg/kg food in females in week 104 (155% of control). Protein content of urine increased during the study in males and females.

At interim sacrifice, absolute testes weight was slightly, but significantly increased at 120 mg/kg food (109% of control). Decreased absolute liver weight, increased relative testes weight, increased relative weights of

adrenals, heart, kidney, and lungs and bronchi in males and/or females were considered to be related to reduced body weight.

At terminal sacrifice, no treatment-related changes in organ weights were observed in the toxicity group.

In the oncogenicity group absolute and relative weight of the ovaries was significantly increased at 80 mg/kg food (139 and 156% of control). Further changes in organ weights were not dose-related and not considered toxicologically relevant.

There were no macroscopic or microscopic findings at interim necropsy or terminal necropsy that distinguished treated animals from controls.

Based on reduced body weight, food consumption and reduced food conversion efficiency, the NOAEL is established at 28 mg/kg food (equal to 1.1 mg/kg bw/day in males and 1.5 mg/kg bw/day in females). No oncogenic potential of the test substance in rats was observed.

STUDY 2

Characteristics

reference	: Holmes, 1990b	exposure	: 53 (interim) or 78 weeks, diet
type of study	: 78-week carcinogenicity study	doses	: 0, 2.5, 8.0, 25 or 80 mg/kg food ¹
year of execution	: 1987-1989	vehicle	: None
test substance	: NC-129 techn., Lot no. 129T8605, purity 98.0%	GLP statement	: Yes
route	: Oral	guideline	: OECD guideline 451
species	: mouse, CD-1 strain	acceptability	: acceptable
group size	: 12/sex/dose (interim sacrifice) 52/sex/dose (terminal sacrifice)	NOAEL	: 0.81 mg/kg bw/day in males not oncogenic (see acceptability)

¹ equal to 0, 0.27, 0.81, 2.78 and 8.88 mg/kg bw/day for males and 0, 0.29, 0.91, 2.78 and 9.74 mg/kg bw/day for 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 (Holmes, 1988).

This carcinogenicity study was extended with haematology. Blood samples were collected from 10 animals/sex/dose in week 52 and 78.

Results

The results are summarised in table 6.5.1-02.

Table 6.5.1-02 Results

Dose (mg/kg food)	0		2.5		8		25		80		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality (n=52)	22	15	24	15	21	11	25	18	28	10	m
Clinical signs					No treatment-related findings						
Body weight gain							dc		dc	dc	
Food consumption					No treatment-related findings						
Haematology (n=10)									dc ¹		
- MCV									dc ¹		
- MCH									ic ¹		
- platelet count											
- PCV											
- Hb										i ²	
- RBC										i ²	
Organ weights					No treatment-related findings						
Pathology											
<u>Macroscopy</u> (n=52)					No treatment-related findings						
<u>microscopy</u> (n=52)					No treatment-related findings						
<i>non-neoplastic lesions</i>					No treatment-related findings						
<u>microscopy</u> <i>neoplastic lesions</i>					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

1 week 52

2 week 78

Acceptability

The study is considered acceptable. The present carcinogenicity study was extended with haematology. Although no clinical biochemistry and urinalysis was performed, the study is considered suitable for establishment of a NOAEL for chronic toxicity based on the critical effect. From other studies the critical effect was shown to be reduced body weight (gain).

Conclusions

A slightly increased mortality was observed in males at 80 mg/kg food relative to the control group. No treatment-related clinical signs were noted.

Body weight gain was significantly decreased at 80 mg/kg food in both sexes (77% and 86% of control for males and females, respectively) and at 25 mg/kg food in males (86% of control). No effect on food consumption was observed. Food conversion efficiency during the first 14 weeks of the study was lower at 80 mg/kg food in both sexes

Mean cell volume and mean cell haemoglobin were decreased at 80 mg/kg food in males after 52 weeks only (94% of control). Platelet count was increased at 80 mg/kg food in males after 52 weeks only. Packed cell volume, haemoglobin and erythrocyte count were increased at 80 mg/kg food in females after 78 weeks.

Since the effects were noted only once, no effects in the other sex were noted, and the values are within normal biological variation the effects are not considered to be treatment-related.

Relative organ weights at 80 mg/kg food that were increased were considered to be due to reduced body weight. No treatment-related effect on organ weights was observed.

No macroscopic or microscopic findings were attributed to treatment.

Based on reduced body weight gain a NOAEL of 8.0 mg/kg food (equal to 0.81 and 0.91 mg/kg bw/d for males and females, resp.) can be derived.

No oncogenic potential of the test substance in mice was observed.

B.6.5.2 Summary

The results of the chronic toxicity and carcinogenicity studies are summarised in table 6.5.2-01.

Table 6.5.2-01 Chronic toxicity and carcinogenicity studies with pyridaben

Duration	Species	Route	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Critical effects	Reference
104-weeks	rat	Oral	1.1 (M)	5.0 (M)	Reduced body weight, food consumption and food conversion efficiency.	Holmes, 1990a
78-weeks	mouse	Oral	0.81 (M)	2.78	Reduced body weight gain.	Holmes, 1990b

In a 2-year combined toxicity/carcinogenicity study in rats, administration of pyridaben at 0, 4, 10, 28 and 80 (oncogenicity group)/120 (toxicity group) mg/kg food (equal to 0, 0.2, 0.4, 1.1 and 3.2-5.0 mg/kg bw/day in males and 0, 0.2, 0.5, 1.5 and 4.2-6.5 mg/kg bw/day in females) resulted in reduced body weight and food consumption at 80 and 120 mg/kg food. Leukocyte count was decreased at 10-120 mg/kg food in females. Neutrophils were decreased at 4 mg/kg food in females and at 10-120 mg/kg food in both sexes. Observed changes in leucocytes and neutrophils were considered to be due to bacterial infection and not test substance related. Urine volume was decreased and specific gravity was increased in males at 120 mg/kg food. Protein content was increased at 120 mg/kg food in females and increased in both sexes in the course of the study. Absolute testes weight was increased at 120 mg/kg food in the toxicity group and ovary weight was increased at 80 mg/kg food in the oncogenicity group.

Based on reduced body weight, food consumption and food conversion, the NOAEL is set at 28 mg/kg food (equal to 1.1 mg/kg bw/day in males and 1.5 mg/kg bw/day in females). No oncogenic potential of the test substance in rats was observed.

In a 78-week carcinogenicity study in mice, pyridaben was administered via the diet at 0, 2.5, 8.0, 25 or 80 mg/kg food (equal to 0, 0.27, 0.81, 2.78 and 8.88 mg/kg bw/day for males and 0, 0.29, 0.91, 2.78 and 9.74 mg/kg bw/day for females). Treatment at 80 mg/kg food resulted in slightly reduced survival in males. Body weight gain was decreased at 80 mg/kg food in both sexes and at 25 mg/kg food in males. Food conversion efficiency during the first 14 days of the study was lower at 80 mg/kg food in both sexes.

No oncogenic potential was observed in mice. Based on reduced body weight gain a NOAEL of 8.0 mg/kg food (= 0.81 and 0.91 mg/kg bw/d for males and females, resp.) is established.

B.6.6 Reproductive toxicity (Annex IIA 5.6)

B.6.6.1 Reproductive toxicity

STUDY 1

Characteristics

reference	: Willoughby, 1990	exposure	: Diet starting 14 weeks before mating (F0) or at weaning (F1), throughout mating, gestation and lactation periods until scheduled necropsy
type of study	: 2-generation reproduction study	doses	: 0, 10, 28 and 80 mg/kg food ¹
year of execution	: 1988/89	vehicle	: None
test substance	: NC-129 techn. (pyridaben), Lot no. 129T8605, purity 98%	GLP statement	: Yes
route	: Oral	guideline	: In accordance with OECD 416 (1983 and 2001)
species	: Rat, CD-strain	acceptability	: Acceptable
group size	: 25 /sex/dose	NOAEL _{par}	: 1.87 mg/kg bw/day
		NOAEL _{dev}	: 5 mg/kg bw/day
		reproductive effects	: 5.33 mg/kg bw/day

¹ equivalent to 0, 0.67, 1.87 and 5.33 mg/kg bw/day (default conversion factor 15). The NOAEL_{dev} of 28 mg/kg food is equivalent to 5 mg/kg bw/d based on actual food intake (LOAEL 15 mg/kg bw/d), see conclusions.

Study design

The study was generally performed in accordance with OECD guideline 416 (1983 and 2001). Weight of brain, liver, kidneys, spleen, pituitary, thyroid and adrenals were not determined in adult animals. Sperm was not evaluated. Sexual maturation of pups was not evaluated. Organ weights of pups were not determined.

Results

The results of the study are summarized in table 6.6.1-01.

Table 6.6.1-01

Dose (mg/kg food)	0		10		28		80		dr
	m	f	m	f	m	f	m	f	
<u>F₀ animals</u>									
Mortality			no treatment-related findings						
Clinical signs			no treatment-related findings ¹						
Body weight gain							dc	d	
Food consumption							d		
Mating/fertility/gestation			no treatment-related findings						
Oestrus cycle			no treatment-related findings						

Dose (mg/kg food)	0		10		28		80		dr
	m	f	m	f	m	f	m	f	
Sperm evaluation			not performed						
Organ weight - ovaries - epididymides - seminal vesicles - testes						ic ^a	ic ^r ic ^r ic ^r	ic ^{a,r}	
Pathology									
<u>Macroscopy</u>			no treatment-related findings						
<u>Microscopy</u>			no treatment-related findings						
<u>F₁ pups</u>									
Litter size			no treatment-related findings						
Post implantation loss (%)			no treatment-related findings						
Live birth index			no treatment-related findings						
Viability index			no treatment-related findings						
Lactation index			no treatment-related findings						
Sex ratio			no treatment-related findings						
Clinical signs			no treatment-related findings ¹						
Body weight day 25 post-partum								dc	
Sexual maturation			not performed						
Pup development			no treatment-related findings						
Auditory and visual response			no treatment-related findings						
Organ weights			not performed						
Pathology									
<u>Macroscopy</u>			no treatment-related findings						
<u>F₁ animals</u>									
Mortality			no treatment-related findings						
Clinical signs			no treatment-related findings						
Body weight gain - males 22 weeks - females pre-mating period							dc	d	
Food consumption - pre-mating period							dc	d	
Mating/fertility/gestation			no treatment-related findings						
Oestrus cycle			no treatment-related findings						
Sperm evaluation			not performed						
Organ weight - epididymides - seminal vesicles - uterus							dc ^a dc ^a	ic ^r	

Dose (mg/kg food)	0		10		28		80		dr
	m	f	m	f	m	f	m	f	
Pathology									
Macroscopy			no treatment-related findings						
Microscopy pituitary: degenerate cells	0/25	0/25					5/25	0/25	
F₂ pups									
Litter size			no treatment-related findings						
Post implantation loss			no treatment-related findings						
Live birth index			no treatment-related findings						
Viability index			no treatment-related findings						
Lactation index			no treatment-related findings						
Sex ratio			no treatment-related findings						
Clinical signs			no treatment-related findings ¹						
Body weight day 25 post-partum								dc	
Pup development			no treatment-related findings						
Auditory and visual response			no treatment-related findings						
Organ weights			not performed						
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 when compared to controls (p =< 0.05)

1 clinical signs were not reported, only in the result section it was stated that no treatment-related clinical signs were seen

Acceptability

The study is considered acceptable.

Conclusions

In the 2-generation reproduction study, F₀ animals showed no treatment-related mortality or clinical signs. One male at 80 mg/kg food was found dead during week 6 of treatment. At necropsy a dark mass in the urinary bladder and chronic inflammation of the urinary bladder and prostate was noted. Since no masses or chronic inflammation were observed in the combined carcinogenicity study (B.6.5.1) the effects are considered to be incidental. Body weight gain was decreased at 80 mg/kg food in males (88% of control) and in females (91-93% of control) during the pre-mating and gestation period. During lactation body weight gain was significantly increased relative to control resulting in a body weight comparable to the control group at the end of the study. Food consumption was slightly reduced in males at 80 mg/kg food during the pre-mating period.

There were no changes observed between parental animals of the treated and control groups in mating indices, pregnancy rates, male fertility and oestrus cycle. Sperm was not evaluated. No changes in post implantation loss were observed.

The increased relative weight of epididymides, seminal vesicles and testes is considered to be due to lower body weight. Absolute ovaries weight was increased in females at 28 and 80 mg/kg food (115% of control) and increased relative ovaries weight was observed in females at 80 mg/kg food (118% of control). In the absence of a dose-response relationship in absolute ovary weight and histopathological changes in the ovaries, the toxicological significance of increased ovary weight remains unclear.

At necropsy no treatment-related abnormalities were observed, including histopathological examinations.

No treatment-related changes were detected in litter size, live birth index, viability index, lactation index, sex ratio or clinical signs of the F_0 offspring (F_1 pups). Sexual maturation was not determined. Pup development, and auditory and visual responses were unaffected by treatment.

F_1 pup weights were initially similar to the control group, but significantly decreased on day 25 post partum at 80 mg/kg food (87% of control) due to the fact that pups are joining in on eating the test-diet. Unselected offspring killed after culling of the F_1 generation showed no treatment-related macroscopic findings.

F_1 animals showed no treatment-related mortality or clinical signs. One control female was humanely killed during parturition because a dead foetus became lodged in the vagina. One female at 28 mg/kg food was killed after her litter had been weaned, following rupture of a subcutaneous mass (benign fibroma). Body weight gain was significantly decreased in males at 80 mg/kg food after 22 weeks of treatment (85% of control). Body weight gain in females was decreased at 80 mg/kg food during the pre-mating period (93% of control). During lactation body weight gain was significantly increased relative to control resulting in a body weight comparable to the control group at the end of the study. Food consumption was significantly reduced in males at 80 mg/kg food and slightly reduced in females during the pre-mating period.

No changes were noted between parental animals of the treated and control groups in mating indices, pregnancy rates, male fertility and oestrus cycle. Sperm was not evaluated. There were no changes in post implantation loss for F_1 females.

Decreased absolute weight of epididymides and seminal vesicles and increased relative weight of the uterus was probably due to a lower body weight.

At necropsy no treatment-related abnormalities were observed. Eosinophilic degenerate cells were observed in the pituitary in 5/25 males at 80 mg/kg, however, the degree of change was generally only slight to minimal. In the absence of similar findings in other toxicity studies, it is considered unlikely to be treatment related.

No treatment-related changes were observed in litter size, live birth index, viability index, lactation index, sex ratio or clinical signs of the F_1 offspring (F_2 pups). Pup weights were decreased at 80 mg/kg food at day 25 post-partum due to the fact that pups are joining in on eating the test-diet. Pup development, and auditory and visual responses were unaffected by treatment. At necropsy no treatment-related findings were observed.

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 80 mg/kg food (equivalent to 5.33 mg/kg bw/d).

Based on decreased body weight gain and food consumption, the NOAEL for parental effects is set at 28 mg/kg food (equivalent to 1.87 mg/kg bw/day).

The NOAEL for developmental toxicity is set at 28 mg/kg food, based on decreased body weight seen in weeks 3 and 4 post partum for both F1 and F2 litters, appearing as soon as they start eating diet. Therefore, as the key effect is on offspring eating diet up to and around weaning, achieved intake associated with this effect is used as a refinement of the default factor 15. The nearest interval of measurement of food consumption is the first week of the selected F1 generation (week 5 of age). Achieved intake for F1 animals eating 28 mg/kg food in week 5 of age was calculated to be 5.17 and 5.00 mg/kg bw/day for males and females respectively (the achieved intakes at the LOAEL of 80 mg/kg food were 15.07 and 14.88 mg/kg bw/d for males and females respectively). The NOAEL for developmental toxicity of 28 mg/kg food is therefore considered equivalent to 5 mg/kg bw/day.

B.6.6.2 Teratogenicity studies

STUDY 1

Characteristics

reference	: Willoughby, 1988	exposure	: days 6-15 of gestation, gavage (10 ml/kg)
type of study	: teratogenicity study	doses	: 0, 2.5, 5.7, 13.0 or 30.0 mg/kg bw/day ¹
year of execution	: 1987	vehicle	: 1% (w/v) aqueous carboxymethylcellulose
test substance	: NC-129 techn. (pyridaben), Lot 129T8605, purity 98.0%	GLP statement	: Yes
route	: oral	guideline	: OECD guideline 414 (1981 and 2001)
species	: Rat, Charles River CD	acceptability	: acceptable
group size	: 22 females/dose	NOAEL _{mat}	: 4.7 mg/kg bw/day
		NOAEL _{dev}	: 12.5 mg/kg bw/day
		teratogenic effects	: 26.7 mg/kg bw/day

¹ equal to 1.8, 4.7, 12.5 and 26.7 mg/kg bw/d after correction for measured concentration of the test substance (72, 82, 96 and 89% of nominal concentration, respectively, see also study design)

Study design

The study was generally performed in accordance with OECD guideline 414 (1981 and 2001), except that uterus weight was not determined. Dose levels were based on a preliminary study (LSR Report No. 87/NAS012/005; not available to reviewer).

The achieved concentrations of test substance in the dosing preparations was analysed on day 1, 5, 10 and 13. The two lowest dose preparations were considered below acceptable ranges by the RMS, hence the nominal (intended) doses were corrected for mean measured analytical concentrations.

Formulation analysis					
Intended dose (mg/kg bw/d)	Actual dose (% of intended dose)				
	1 st day	5th day	10th day	13 th day	Mean
2.5	64	57	82	82	72
5.7	58	100	73	92	81
13.0	103	100	89	94	96
30.0	75	94	91	94	89

Results

The results are summarised in table 6.6.2-01.

Table 6.6.2-01

Nominal dose (mg/kg bw/day)	0	2.5	5.7	13.0	30.0	dr
<u>Maternal effects</u>						
Mortality	None					
Clinical signs	No treatment-related findings					
Pregnant animals	22	22	22	22	22	
Body weight (gain)						
Day 6-16				dc	dc	
Day 6-20					dc	
Food consumption						
Day 6-15				dc	dc	
Uterus weight	Not determined					
Pathology						
<u>macroscopy</u>	No treatment-related findings					
<u>Litter response</u>						
Number of dams examined	22	22	22	22	22	
Corpora lutea/dam	No treatment-related findings					
Dams with live fetuses	22	22	22	22	22	
Live fetuses/dam	No treatment-related findings					
Foetal weight					dc	
Placental weight					dc	
Post implantation loss	No treatment-related findings					
Sex ratio	No treatment-related findings					
<u>Examination of the fetuses</u>						
External observations¹						
- small foetus	2.2	1.5	2.3	2.7	9.2*	
Skeletal findings¹						
Variations:						
- supra-occipital bone:						
incomplete ossification	20.8	17.4	15.3	22.6	38.8*	
- 2 sternbrae:						
incomplete ossification	69.4	72.2	74.8	70.1	52.7*	
- thoracic vertebral						
centra: incomplete	33.3	30.8	38.7	39.8	55.6**	
ossification						
Visceral findings	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

*

p<0.05

**

p<0.01

1

% foetal incidence

Acceptability

The study was considered acceptable.

Conclusions

No effect on mortality or clinical signs was observed. Mean body weight was slightly decreased during the first three days of exposure at 30.0 mg/kg bw and subsequently, their weight gain was slightly, but significantly reduced (83% of control). Body weight gain at 13.0 mg/kg bw was delayed one day relative to the control after starting treatment and was therefore, significantly reduced until day 16 of gestation (86% of control). During treatment (day 6-15 of gestation) food consumption was decreased at 13.0 and 30.0 mg/kg bw (88 and 73% of control, respectively).

There were no significant differences in the number of corpora lutea or implantations, the number of live foetuses or sex ratio. Mean foetal and placental weight were significantly decreased at 30.0 mg/kg bw (93% and 90% of control, respectively), but values were still within historical ranges. Foetus examination revealed an increased incidence of small foetuses at 30.0 mg/kg bw, but within historical controls. Incomplete ossification of the supra-occipital bone and thoracic vertebral centra were significantly increased at 30.0 mg/kg bw and values were outside historical control ranges. Incomplete ossification of 2 sternbrae was significantly decreased at 30.0 mg/kg bw, but within historical control range. No significant deviations in the incidence of soft tissue anomalies were noted.

Based on the decrease in body weight gain and food consumption in maternal females, the NOAEL for maternal toxicity was set at 5.7 mg/kg bw/day (equal to 4.7 mg/kg bw/d after correction for measured concentration of the test substance). Based on retarded ossification of various bones and reduced foetal weight the NOAEL for developmental toxicity was set at 13.0 mg/kg bw/day (equal to 12.5 mg/kg bw/d after correction for measured concentration of the test substance). Since no irreversible structural effects were reported, the NOAEL for teratogenic effects was set at 30.0 mg/kg bw/day (equal to 26.7 mg/kg bw/d after correction for measured concentration of the test substance).

STUDY 2

Characteristics

reference	: Ross, 1988	exposure	: days 6-19 of gestation, gavage (5 ml/kg)
type of study	: teratogenicity study	doses	: 0, 1.5, 5 or 15 mg/kg bw/day
year of execution	: 1987	vehicle	: 1% (w/v) aqueous carboxymethylcellulose
test substance	: NC-129 techn. (pyridaben), Lot 129T8605, purity 98.0%	GLP statement	: Yes
route	: oral	guideline	: OECD guideline 414 (1981 and 2001)
species	: Rabbit, New Zealand White	acceptability	: acceptable
group size	: 19-20 females/dose	NOAEL _{mat}	: 5 mg/kg bw/day
		NOAEL _{dev}	: 15 mg/kg bw/day
		teratogenic effects	: 15 mg/kg bw/day

Study design

The study was generally performed in accordance with OECD guideline 414 (1981 and 2001), except that uterus weight was not determined. Dose levels were based on a preliminary study (LSR Report No. 87/NAS019/390; not available to reviewer).

Results

The results are summarised in table 6.6.2-02 and 02a.

Table 6.6.2-02

Dose (mg/kg bw/day)	0	1.5	5	15	dr
<u>Maternal effects</u>					
Mortality		None			
Pregnant animals	14/19	13/19	12/19	15/20	
Clinical signs - reduced faeces				+ ¹	
Body weight (gain)			d	dc	
Food consumption				d	
Uterus weight		Not determined			
Pathology <u>macroscopy</u>		No treatment-related findings			
<u>Litter response</u>					
Corpora lutea/dam		No treatment-related findings			
Dams with resorptions only	1	1	0	2 aborted	
Resorptions (total)	0.5	0.6	0.9	1.2	
Dams with live foetuses	13	12	12	13	
Live foetuses/dam	6.8	9.3	7.3	7.8	
Foetal weight	45.0	39.4	42.3	39.5	
Post implantation loss	7.3	5.9	11.1	12.9	
Sex ratio		No treatment-related findings			
<u>Examination of the foetuses</u>					
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

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

1 21.4% of females affected

Table 6.6.2-02a Body weight (kg) and body weight gain (kg)

Dose (mg/kg bw/day)	0	1.5	5	15
day 6	4.20	4.24	4.17	4.07
day 8	4.25	4.25	4.16	3.93*
day 10	4.28	4.27	4.17	3.93*
day 12	4.30	4.28	4.20	3.94**
day 18	4.44	4.40	4.30	4.11**
Body weight gain (kg)				
day 6-8	0.04	0.02	-0.01*	-0.14***
day 6-10	0.08	0.03	0.01*	-0.13***
day 6-12	0.09	0.05	0.03	-0.13***
day 6-18	0.23	0.16	0.13	0.04**

* significantly different from control, $P < 0.05$ ** significantly different from control, $P < 0.01$ *** significantly different from control, $P < 0.001$ **Acceptability**

The study was considered acceptable.

Conclusions

No mortality was observed. An increased incidence of reduced faeces was noted at 15 mg/kg bw. Concomitantly, food consumption was decreased during the first three weeks of the study and was similar to control in the last week of the study at 15 mg/kg bw/day. Rabbits lost weight during day 6-8 of gestation at 15 mg/kg bw/day followed by no body weight gain until day 12. At 5 mg/kg bw/day only a slight, transient effect on body weight was observed: no body weight gain was noted during day 6-10. Thereafter body weight gain was comparable to controls, although body weights were still slightly below control values. There were no significant differences in the number of corpora lutea or implantations, the number of live foetuses, sex ratio, foetal or placental weight. Two females at 15 mg/kg bw aborted. Total resorptions were increased at 15 mg/kg bw, but still within the historical range.

No effect was observed on external foetal examination and examination of viscerae and skeleton.

Based on the body weight loss and decrease in body weight gain, slightly reduced food consumption and reduced faeces in maternal females, the NOAEL for maternal toxicity was set at 5 mg/kg bw/day. Since no effects on foetal development and no irreversible structural effects were noted, the NOAEL for developmental and teratogenic effects was set at 15 mg/kg bw/day.

STUDY 3

Characteristics

Reference	: Hellwig, 1995	exposure	: days 6-19 of gestation, 6 h/d (3 ml/kg bw); semi-occlusive (10% of body surface, 18x11.5 cm)
type of study	: teratogenicity study	doses	: 0, 70, 170 or 450 mg/kg bw/day
year of execution	: 1994	vehicle	: 0.5% carboxymethylcellulose
test substance	: NC-129 techn. (pyridaben), Batch 900304, purity 99.7%	GLP statement	: Yes
Route	: dermal	guideline	: OECD guideline 414 (1981 and 2001)
Species	: Rabbit, Himalayan (Chbb:HM)	acceptability	: acceptable
group size	: 15 females/dose	NOAEL mat (local)	: <70 mg/kg bw/day
		NOAEL mat (systemic)	: 70 mg/kg bw/day
		NOAEL dev	: 450 mg/kg bw/day
		teratogenic effects	: 450 mg/kg bw/day

Study design

The study was generally performed in accordance with OECD guideline 414 (1981 and 2001). Dose levels were based on a rang-finding study, in which pregnant rabbits were treated with 100, 400 and 1000 mg/kg bw in distilled water containing 5 mg Cremophor EL/100 ml on day 6-19 of gestation. Body weight and food consumption was decreased at 400 and 1000 mg/kg bw. At all dose levels eschar formation at the application site was noted.

Results

The results are summarised in table 6.6.2-03.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration may not be granted on the basis of this document.

Table 6.6.2-03

Dose (mg/kg bw/day)	0	70	170	450	dr
<u>Maternal effects</u>					
Mortality		None			
Pregnant animals	15/15	14/15	15/15	14/15	
Body weight (gain)			d	dc	
Food consumption			dc	dc	
Dermal observations		see text			
Uterus weight		No treatment-related findings			
Pathology					
<u>macroscopy</u>		No treatment-related findings			
<u>Litter response</u>					
Corpora lutea/dam		No treatment-related findings			
Resorptions		No treatment-related findings			
Dams with live foetuses		No treatment-related findings			
Live foetuses/dam		No treatment-related findings			
Foetal weight		No treatment-related findings			
Post implantation loss		No treatment-related findings			
Sex ratio		No treatment-related findings			
<u>Examination of the foetuses</u>					
External observations (number of foetuses) - hernia umbilicalis	0	0	0	1	
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

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

Acceptability

The study was considered acceptable.

Conclusions

No mortality was observed. Food consumption was significantly decreased at 170 and 450 mg/kg bw during treatment and on day 19-21 after treatment at 450 mg/kg bw. Body weight loss was noted at the beginning of the treatment period at 170 and 450 mg/kg bw. Body weight was lower than that of the control group at the end of the study at 170 and 450 mg/kg bw. No effect was seen on body weight corrected for uterine weight. Severe erythema to slight eschar formation and/or slight oedema was noted in animals at 450 mg/kg bw. Moderate to severe erythema and/or slight eschar formation and/or slight oedema was observed in animals at

170 mg/kg bw. Moderate to severe erythema and/or slight eschar formation was seen in animals at 70 mg/kg bw. In all groups skin effects were observed during the second half of the treatment period and/or during the post-treatment period. No effect on uterus weight or macroscopy was noted. There were no significant differences in the number of corpora lutea, implantations or resorptions, the number of live foetuses, sex ratio, foetal or placental weight. External foetal examination revealed hernia umbilicalis in one foetus at 450 mg/kg bw. The incidence is so low, that this can be considered to be incidental for this species.

No treatment-related effect was observed on foetal examination of viscerae and skeleton.

Based on the presence of moderate to severe erythema and/or slight eschar formation at 70 mg/kg the NOAEL for local maternal toxicity is set at <70 mg/kg bw/day. Based on the decrease in body weight gain and food consumption in maternal females, the NOAEL for systemic maternal toxicity was set at 70 mg/kg bw/day. Since no treatment-related effects on foetal development and no treatment-related irreversible structural effects were noted, the NOAEL for developmental and teratogenic effects was set at 450 mg/kg bw/day.

B.6.6.3 Summary

The results of reproduction toxicity and teratogenicity studies are summarised in table 6.6.3-01.

Table 6.6.3-01 Summary of reproduction toxicity and teratogenicity studies with pyridaben

Species		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Critical effects	Reference
2-Generation reproduction toxicity					
rat	parental	1.87	5.33	Decreased body weight gain and food consumption. Decreased pup body weight.	Willoughby, 1990
	developmental reproduction	5 ≥5.33	15 -	No effects.	
Teratogenicity					
rat	maternal	4.7	12.5	Decreased body weight gain and food consumption.	Willoughby, 1988
	developmental	12.5	26.7	Decreased foetal weight, retarded ossification of various bones.	
	teratogenicity	≥ 26.7	-	No effects.	
rabbit (oral)	maternal	5	15	Decreased body weight gain and food consumption, reduced faeces.	Ross, 1988
	developmental	15	-	No effects.	
	teratogenicity	15	-	No effects.	
rabbit (dermal)	maternal, local	<70	70	Moderate to severe erythema and/or slight eschar formation.	Hellwig, 1995
	maternal, systemic	70	170	Decreased body weight gain and food consumption.	
	developmental	450	-	No effects.	
	teratogenicity	450	-	No effects.	

In an oral 2-generation reproduction study in rats (0, 10, 28 and 80 mg/kg food), a decrease in body weight gains and food consumption was noted among males and females from the F₀- and F₁-generation at 80 mg/kg food. An increase in absolute ovary weight was observed at 28 and 80 mg/kg food and accompanied by an increase in relative ovary weight at 80 mg/kg food in the F₀-generation. There were no changes observed between parental animals of the treated and control groups in mating indices, pregnancy rates, fertility, oestrus cycle, post-implantation loss and macroscopic findings.

Examination of the F₀ and F₁-offspring revealed decreased body weights of pups at 80 mg/kg food in weeks 3 and 4 post partum. No treatment-related changes were detected in clinical signs, litter size, birth weight, sex ratio, litter survival, pup development, auditory and visual responses or macroscopic observations of the F₀ and F₁-offspring. Based on the data presented in this study, the NOAEL for parental toxicity was 28 mg/kg food (equivalent to 1.87 mg/kg bw/day). The NOAEL for developmental toxicity was 28 mg/kg food (equivalent to 5 mg/kg bw/day). The NOAEL for reproductive toxicity was considered to exceed 80 mg/kg food (equivalent to 5.33 mg/kg bw/day).

In a teratogenicity study in rats (0, 2.5, 5.7, 13.0 and 30.0 mg/kg bw/day) the NOAEL for maternal effects was 5.7 mg/kg bw/day (equal to 4.7 mg/kg bw/day after correction for measured concentration of the test substance), based on decreased body weight gain and food consumption. The NOAEL for developmental effects was set at 13.0 mg/kg bw/day (equal to 12.5 mg/kg bw/day after correction for measured concentration of the test substance), based on decreased mean foetal weight, and retarded ossification of various bones. No treatment-related effects were observed on the number of corpora lutea or implantations, the number of live foetuses, and the sex ratio. There were no morphological changes observed in foetuses that could be attributed to treatment. Therefore, the NOAEL for teratogenicity was considered to exceed 30.0 mg/kg bw/day (equal to 26.7 mg/kg bw/day after correction for measured concentration of the test substance).

In an oral teratogenicity study in rabbits (0, 1.5, 5 and 15 mg/kg bw/day), a NOAEL for maternal effects of 5 mg/kg bw/day was derived, based on body weight loss and decrease in body weight gain, slightly reduced food consumption and reduced faeces. Since no effects on foetal development and no irreversible structural effects were noted, the NOAEL for developmental and teratogenic effects was set at 15 mg/kg bw/day.

In a dermal teratogenicity study in rabbits (0, 70, 170 and 450 mg/kg bw/day), the NOAEL for local maternal effects was <70 mg/kg bw/day, based on moderate to severe erythema and/or slight eschar formation at 70 mg/kg bw/day. The NOAEL for systemic maternal effects was set at 70 mg/kg bw/day, based on decreased body weight and food consumption. Since no effects on foetal development and no irreversible structural effects were noted, the NOAEL for developmental and teratogenic effects was set at 450 mg/kg bw/day.

B.6.7 Neurotoxicity / delayed neurotoxicity (Annex IIA 5.7)

B.6.7.1 Acute oral neurotoxicity and delayed neurotoxicity

STUDY 1

Characteristics

Reference	: Higgs, 1995a	exposure	: Once by gavage (10 ml/kg bw)
Type of study	: Acute oral neurotoxicity study	dose	: 0, 50, 100 and 200 mg/kg bw
year of execution	: 1994	vehicle	: 1% aqueous CMC
test substance	: NC-129, lot 129T8605, purity 98%	GLP statement	: Yes
route	: Oral	guideline	: OECD guideline 424
species	: Rats, CD strain	acceptability	: Acceptable
group size	: 10/sex/dose	NOAEL	: 50 mg/kg bw

Study design

The study was performed in accordance with OECD guideline 424. Dose levels were based on the results of a preceding study in which 2 animals/sex were treated with 200, 300 or 500 mg/kg bw, followed by a 7-day observation period. One male and one female given 500 mg/kg bw and one female given 300 mg/kg bw were found dead within 1 day after dosing. Clinical symptoms of animals given 300 and 500 mg/kg bw included tremors, hypoactivity, abnormal gait, pallor, irregular respiration, piloerection, partially closed eye and hunched posture. Animals given 200 mg/kg showed hypoactivity, piloerection and tremors.

In the main study, the FOB was performed on day 1, approximately 5 hours after dosing, the estimated time of peak effect, and on day 7 and 14.

Results

The results are summarised in table 6.7.1-01.

Table 6.7.1-01

Dose (mg/kg bw)	0		50		100		200		dr
	m	f	m	f	m	f	m	f	
Mortality	No mortality								
Clinical signs¹									
- piloerection	0/10	0/10	0/10	0/10	10/10	1/10	10/10	5/10	
- hypoactivity	0/10	0/10	0/10	0/10	7/10	0/10	9/10	8/10	
- tremors	0/10	0/10	0/10	0/10	1/10	0/10	6/10	3/10	
- partially closed eyes	0/10	0/10	0/10	0/10	1/10	0/10	4/10	5/10	
Body weight gain					d	dc	dc	dc	
Food consumption (day 1)²					d	d	d	d	
Functional observation battery									
<i>Home cage examination</i>									
- no locomotion (day 1)	1/10	2/10	3/10	0/10	7*/10	1/10	5*/10	0/10	
<i>Open field examination</i>									
- no locomotion (day 1)	0/10	1/10	0/10	1/10	1/10	0/10	2*/10	0/10	
<i>Manipulation</i>									
- absent or weak startle reflex (day 1)	0/10	0/10	0/10	0/10	1/10	0/10	2/10	0/10	
- absent or weak flexor responses (day 1)	0/10	0/10	0/10	0/10	1/10	0/10	2/10	1/10	
- slow or poorly coordinated righting reflex (day 1)	0/10	2/10	3/10	2/10	2/10	1/10	5*/10	2/10	
- body temperature						dc	dc	dc	
<i>Landing foot splay measurement</i>									
<i>Grip strength</i>									
<i>Motor activity</i>									
Brain and pituitary weights									
Pathology									
<u>macroscopy</u>									
<u>microscopy</u>									

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
/ maximum number of animals showing clinical signs at any of observation times.

- 2 No statistical analysis was performed.
 * statistically significant

Acceptability

The study is considered acceptable.

Conclusions

After single administration of 0, 50, 100 and 200 mg/kg bw by gavage, no mortality was observed. Animals given 100 and 200 mg/kg bw showed piloerection, hypoactivity, tremors and partially closed eyes, mainly on the day of administration. Thereafter occasional piloerection was observed in males given 100 and 200 mg/kg bw and in one female given 200 mg/kg bw, and tremors were observed in one male given 200 mg/kg bw. No clinical signs were observed from day 4 onwards. Body weight gains were reduced in males given 100 and 200 mg/kg bw (83 and 25% of controls) during the first 5 days and in females given 100 mg/kg bw (64% of controls) during the first 4 days. Females given 200 mg/kg bw lost weight during the first 4 days. Body weights recovered during the remaining study period. Food consumption was reduced on the day of dosing in all treated males and females (59, 64 and 27% of controls for males, 70, 55 and 50% of controls for females).

In the functional observation battery the following findings were noted at day 1:

- lower activity in males at 100 and 200 mg/kg bw in the home cage examination
- lower activity in single males at 100 and 200 mg/kg bw in the open field examination
- absent or weak startle reflex in one male at 100 mg/kg bw and in two males at 200 mg/kg bw
- absent or weak flexor responses in one male at 100 mg/kg bw and in two males and one females at 200 mg/kg bw
- increased incidence of slow or poorly coordinated righting reflex in males at 200 mg/kg bw
- slightly reduced body temperature in females at 100 mg/kg bw and both sexes at 200 mg/kg bw

No changes in landing foot splay, grip strength and motor activity were observed.

Furthermore, no changes in brain and pituitary weight were noted.

Macroscopic post mortem examination and histopathological examination of the central and peripheral nervous system did not reveal any abnormalities.

Based on the clinical observations and changes in FOB at 100 and 200 mg/kg bw, the NOAEL for acute toxicity is set at 50 mg/kg bw. Observed changes are likely to result from overt toxicity, rather than from specific neurotoxicity.

B.6.7.2 Semichronic oral neurotoxicity

STUDY 1

Characteristics

Reference	: Higgs, 1995b	exposure	: 13-week, diet
Type of study	: Semichronic oral neurotoxicity study	dose	: 0, 30, 100 and 350 mg/kg food ¹
year of execution	: 1994	vehicle	: None
test substance	: NC-129, lot 129T8605, purity 98%	GLP statement	: Yes
route	: Oral	guideline	: OECD guideline 424
species	: Rats, CD strain	acceptability	: Acceptable
group size	: 10/sex/dose	NOAEL	: 8.5 mg/kg bw/day

¹ equal to 0, 2.5, 8.5 and 28.8 mg/kg bw/day in males and 0, 2.8, 9.3 and 31.1 mg/kg bw/day in females

Study design

The study was performed in accordance with OECD guideline 424. Dose levels were based on previous performed studies. In the main study, the FOB was performed pre-test and in week 4, 8 and 13.

Results

The results are summarised in table 6.7.2-01.

Table 6.7.2-01

Dose (mg/kg food)	0		30		100		350		dr
	m	f	m	f	m	f	m	f	
Mortality	No mortality								
Clinical signs	No treatment-related findings								
Body weight gain							dc	dc	
Food consumption¹							d	d	
Functional observation battery	No treatment-related findings								
Organ weights							dc ^a , ic ^r	ic ^r	
- brain							dc ^a	i ^r	
- pituitary									
Pathology									
<u>macroscopy</u>	No treatment-related findings								
<u>microscopy</u>	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

1 No statistical analysis was performed

Acceptability

The study is considered acceptable.

Conclusions

No mortality and treatment-related clinical signs were observed. Body weight gains were reduced in males and females given 350 mg/kg food (59 and 62% of controls) and food consumption was reduced in males and females given 350 mg/kg food (76 and 79% of controls). In males given 100 mg/kg food, food consumption was reduced during the first 4 weeks only. No treatment-related findings were noted in the functional observation battery, grip strength and motor activity tests.

Decrease absolute brain and pituitary weights were noted in high dose males (93 and 80% of controls). In males and females at 350 mg/kg food, both relative brain weights (127 and 117% of controls) and relative pituitary weights (107 and 109% of controls) were increased. Organ weights changes were considered to be related to reduced body weight.

Macroscopic post mortem examination and histopathological examination of the central and peripheral nervous system did not reveal any abnormalities.

Based on the changes in body weight at 350 mg/kg food, the NOAEL is set at 100 mg/kg food (equal to 8.5 mg/kg bw/day in males and 9.3 mg/kg bw/day in females). No signs of neurotoxicity were observed, hence to the NOAEL for neurotoxicity is 350 mg/kg food (28.8 mg/kg bw/d).

B.6.7.3 Summary

In an acute neurotoxicity study in rats (0, 50, 100 and 200 mg/kg bw by gavage), no mortality was observed. Animals given 100 and 200 mg/kg bw showed piloerection, hypoactivity, tremors and partially closed eyes, mainly on the day of administration. Thereafter occasional piloerection was observed in males given 100 and 200 mg/kg bw and in one female given 200 mg/kg bw, and tremors were observed in one male given 200 mg/kg bw. No clinical signs were observed from day 4 onwards. Body weight gains were reduced in males and females given 100 and 200 mg/kg bw. Food consumption was reduced on the day of dosing in all treated males and females. In the functional observation battery a lower activity, weak startle reflex, weak flexor responses were noted at day 1 in males at 100 and 200 mg/kg bw. Furthermore, an increased incidence of slow or poorly coordinated righting reflex was noted in males at 200 mg/kg bw and reduced body temperature in females at 100 mg/kg bw and both sexes at 200 mg/kg bw. No changes in landing foot splay, grip strength and motor activity were observed. Macroscopic post mortem examination and histopathological examination of the central and peripheral nervous system did not reveal any abnormalities.

Based on the clinical observations and changes in FOB at 100 and 200 mg/kg bw, the NOAEL for acute toxicity is set at 50 mg/kg bw. Observed changes are likely to result from overt toxicity, rather than from specific neurotoxicity.

Dietary exposure of rats to 0, 30, 100 or 350 mg/kg food (equal to 0, 2.5, 8.5 and 28.8 mg/kg bw/day in males, and 0, 2.8, 9.3 and 31.1 mg/kg bw/day in females) for 13 weeks, caused reduced body weight gains in males and females given 350 mg/kg food. Food consumption was reduced in males and females given 350 mg/kg food. No treatment-related findings were noted in the functional observation battery, grip strength and motor activity tests. Macroscopic post mortem examination and histopathological examination of the central and peripheral nervous system did not reveal any abnormalities.

Based on the changes in body weight at 350 mg/kg food, the NOAEL is set at 100 mg/kg food (equal to 8.5 mg/kg bw/day in males and 9.3 mg/kg bw/day in females). No signs of neurotoxicity were observed, hence to the NOAEL for neurotoxicity is 350 mg/kg food (28.8 mg/kg bw/d).

B.6.8 Further toxicological studies (Annex IIA 5.8)

B.6.8.1 Toxicological data on metabolites

B.6.8.1.1 Toxicity studies on metabolites

Studies with metabolite PB-7 were submitted. The studies had been conducted to support a registration in Japan.

STUDY 1

Characteristics

reference	: Cummins, 1990	exposure	: Once by gavage
type of study	: Acute oral toxicity study	doses	: 400, 800, 2000, 3162 and 5000 mg/kg bw (both sexes)
year of execution	: 1989	vehicle	: Carboxymethylcellulose
test substance	: PB-7 (Pyridaben metabolite), Lot no. 890510, purity >98%	GLP statement	: Yes
route	: Oral	guideline	: In accordance with OECD 401 (1987)
species	: Rat, CD (SD)	acceptability	: Acceptable
group size	: 5/sex/dose	LD ₅₀	: Male rats: 2728 mg/kg bw

Study design

The study was performed in accordance with OECD 401 (1987). Doses were based on a dose-range finding study with 200, 400, 800 and 1600 mg/kg bw. In this study, male and female rats were found dead at 800 and 1600 mg/kg bw.

Results

Mortality: No mortality was observed in the 400 and 800 mg/kg dose groups. One male given 2000 mg/kg was found dead on day 3. In the 3162 mg/kg dose group, 3/5 males and 4/5 females were found dead within 5 hours after treatment. In the 5000 mg/kg dose group, all males died within 4 days, and 4/5 females were found dead within 5 hours.

Symptoms of toxicity: Most surviving animals showed decreased motor activity, ungroomed appearance, and at 3162 and 5000 mg/kg also ataxia, breathing difficulties, hunched posture and thin body conformation. These findings disappeared before the end of the experiment. Before death, non-surviving animals showed all before mentioned signs, and in addition lethargy, piloerection, diarrhoea and pupil dilation.

Body weight: Out of the surviving animals, one female given 3162 mg/kg and one female given 5000 mg/kg showed decreased body weights on day 8, which increased thereafter. No other remarkable findings on body weight were observed.

Pathology: No significant observations were noted at necropsy for animals surviving to study termination from any of the dosage levels. In several animals that died during the study, staining of the coat, and altered contents of the stomach and the small intestine was observed.

Acceptability

This study is considered acceptable.

Conclusions

The acute oral LD₅₀ of PB-7 was found to be 2728 mg/kg bw in male rats and 3086 mg/kg bw in female rats.

STUDY 2

Study design and results

Type of study: Ames test, plate incorporation method, with independent repeat

Indicator cells	Endpoint	Res. - act.	Res. + act.	Activation		Dose range	Reference
				Tissue	Inducer		
B: <i>S. typh.</i> TA 98 TA 100 TA 1535 TA 1537	point mut. point mut. point mut. point mut.	- - - -	- - - -	rat liver	Arochlor 1254	Pre test: TA98 and WP2uvrA (-S9): of 5 to 5000 µg/plate Mutation tests ¹⁾ : TA98, TA100, TA1535 and TA1537 (- and + S9): 25, 79, 250, 790 and 2500 µg/plate WP2uvrA (- and + S9): 50, 158,500, 1580 and 5000 µg/plate Solvent: DMSO Positive and negative control: Included	May, 1990a
B : <i>E.coli</i> WP2uvrA	point mut.	-	-				
Test substance: PB-7 (2-tert-butyl-5-[4-(1-carboxy-1-methylethyl)benzylthio]-4-chloropyridazin-3(2H)-one), batch nr.890510, off-white powder, purity >98.0%. Precipitation was not observed. Pre test: Toxicity was observed in strain TA98 at 2500 µg/plate and 5000 µg/plate in strain WP2uvrA. Mutation test: Toxicity was observed in strain TA1535, TA1537, TA98 and TA100 at 2500 µg/plate and in strain WP2uvrA at 5000 µg/plate GLP statement: yes According to OECD 471: yes							

Acceptability

The study fulfils the requirements of the recent OECD 471 guideline of 1997.

Conclusions

PB-7 did not induce point mutations in *S. typhimurium* and *E.coli*.

STUDY 3

Study design and results

Type of study: Lethal DNA damage test with *Escherichia coli* strains

Indicator cells	Endpoint	Res. - act.	Res. + act.	Activation		Dose range	Reference
				Tissue	Inducer		
B : <i>E.coli</i> WP2 WP67 CM871	DNA damage (repair deficient).	- - -	- - -	rat liver	Arochlor 1254	Main tests ¹⁾ : 100, 316, 1000, 3160, 10000 µg/ml Solvent: DMSO Positive and negative control: Included	May, 1990b
¹⁾ Dose levels were based on a dose-range finding study with dose levels of 31.6-10000 µg/ml. Test substance: PB-7 (2-tert-butyl-5-[4-(1-carboxy-1-methylethyl)benzylthio]-4-chloropyridazin-3(2H)-one), batch nr.890510, off-white powder, purity >98.0%. Precipitation was not indicated. Little toxicity was observed after 2 hours of exposure. Toxicity was observed in all three strains after 18 hours of exposure. GLP statement: yes No OECD guideline available.							

Acceptability

There is no OECD or EC guideline available for this type of study. However, the results of the present study are considered suitable for the overall evaluation of the genotoxic properties of metabolite PB-7.

Conclusions

PB-7 did not produce lethal DNA damage in *E.coli* WP2, WP67 and CM871.

B.6.8.1.2 Summary

An acute oral toxicity study and genotoxicity studies were performed with the metabolite PB-7.

The acute oral LD₅₀ of PB-7 was found to be 2728 mg/kg bw in male rats and 3086 mg/kg bw in female rats.

The results from the *in vitro* genotoxicity studies with PB-7 are summarised in Table 6.8.1-02.

Table 6.8.1-02 *In vitro* genotoxicity studies

Test substance	Type of study		RESULT		Reference
	Indicator cells	Endpoint	without activation	with activation	
PB-7 (2-tert-butyl-5-[4-(1-carboxy-1-methylethyl)benzylthio]-4-chloropyridazin-3(2H)-one), batch nr.890510, off-white powder, purity >98.0%	B: <i>S. typh.</i> TA 98 TA 100 TA 1535 TA 1537 B : <i>E.coli</i> WP2uvrA	point mut. point mut. point mut. point mut. point mut.	- - - - -	- - - - -	May, 1990a
PB-7 (2-tert-butyl-5-[4-(1-carboxy-1-methylethyl)benzylthio]-4-chloropyridazin-3(2H)-one), batch nr.890510, off-white powder, purity >98.0%.	B : <i>E.coli</i> WP2 WP67 CM871	DNA damage (repair deficient).	- - -	- - -	May, 1990b

PB-7 did not induce point mutations in *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and in the *Escherichia coli* tester strain WP2uvrA, both with and without metabolic activation. PB-7 was negative in a lethal DNA damage test in strains of *Escherichia coli* (WP2, WP67 and CM87).

The available genotoxicity package on PB-7 is considered insufficient to conclude on the genotoxic properties of PB-7.

Conclusion

Metabolite PB-7 showed to be less toxic than pyridaben for acute toxicity. No conclusion can be drawn on the genotoxicity of PB-7.

B.6.8.2 Mechanistic data

B.6.8.2.1 Mechanistic studies

STUDY 1

Characteristics

Reference	Mellert, 1992	exposure	: 3-month, gelatin capsule
Type of study	3-month oral toxicity study	dose	: 0 and 300 mg/kg bw/day
year of execution	1991	vehicle	: none
test substance	NC-129 (pyridaben), Lot no. 129T8605, purity 98.6%	GLP statement	: yes
route	: oral	guideline	: no guideline applicable
species	: dog, Beagle	acceptability	: acceptable
group size	: 4 females/dose	NOAEL	: -

Study design

The study was designed to investigate whether the salivation observed in former studies was due to systemic effects or local irritation or palatability.

Dosing was based on the 4-week (Spicer, 1989), 13-week (Spicer, 1991) and 1-year (Spicer, 1991 including study at additional dose level) studies in dogs and 2 studies not available to the reviewer that are described below from the summary given in the current study (4-week dog Project no. IRDC 563-003, 1989 and 13-week dog Project no. IRDC 563-005, 1989).

Palatability and toxicity were tested in 2 dogs per sex per group in a 4-week diet study (Project no. IRDC 563-003, 1989). Dose levels used were 0, 200, 1000, 4000 and 16000 mg/kg food. The test substance was less palatable at higher doses with a dose-related reduction in food consumption and body weight gain and organ weight changes. No mortality, no haematological and biochemical effects or findings at urinalysis or at ophthalmological examination were observed.

In the second 13-week study that was not available to the reviewer (Project no. IRDC 563-005, 1989) 4 dogs per sex per group were given 0, 2.4, 12, 60 and 300 mg/kg bw/d via gelatin capsule. Another 4 dogs per sex were used in the control group and at 300 mg/kg bw/d and sacrificed at week 10 (at 300 mg/kg bw/d all surviving dogs were sacrificed). Two males and 6 females died at 300 mg/kg bw/d and 1 male at 60 mg/kg bw/d. These animals showed soft stool/diarrhoea, emesis, inappetence, thinness and dehydration. Prostration, decreased physical activity, dry nose and ptyalism were also observed in some of these dogs. Surviving animals at 60 and 300 mg/kg bw/d had similar clinical signs. Reduced body weight gain or weight loss was observed in all treated females and at 12 mg/kg bw/d and higher in males. Food consumption and food conversion were also decreased. At necropsy depletion of body fat was noted. No histopathology was done.

In the current study dogs were given an empty stomach-soluble capsule (control group), a stomach-soluble capsule with 300 mg/kg bw/d pyridaben or a stomach-resistant, but small intestine-soluble capsule with 300 mg/kg bw/d pyridaben. Body weight was recorded weekly and food consumption daily. Mortality was checked at least once every day. Clinical signs were examined at least once every working day. Salivation was examined on working days prior to test substance administration and about 2, 4 and 6 hours thereafter. At the end of the study the gastro-intestinal tract and gross lesions of the dogs were macroscopically examined.

Results

The results are summarised in table 6.8.2.1-01.

Table 6.8.2.1-01 Results.

Dose (mg/kg bw/d)	0	300 (stomach- soluble)	300 (intestine- soluble)
	f	f	f
Mortality		none	
Clinical signs³			
vomiting		+	+
diarrhoea		+	+
Body weight gain		dc	
Food consumption⁴		d	
Pathology			
<u>Macroscopy</u>	No treatment-related findings		

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

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

3 + = present

4 no statistical analysis performed

Acceptability

The study is considered acceptable. However, the effects observed regarding salivation do not coincide with the 90-day and 1-year dog studies (paragraph 6.3.4). Moreover, mortality was high at 300 mg/kg bw in an earlier 13-week study (Project no. IRDC 563-005, 1989), while no mortality was observed at this level in the current study. Therefore, no conclusion can be drawn on salivation.

Conclusions

Dogs were administered 0 and 300 mg/kg bw pyridaben in a stomach-soluble capsule or small intestine-soluble capsule for 13 weeks. No mortality was observed. Vomiting and diarrhoea was observed in all animals treated with test substance and not in the control group. In none of the animals salivation was observed. Reduced body weight was seen in the stomach-soluble capsule group (82% of control). This body weight reduction was due to significant body weight loss during the first treatment week and reduced body weight gain during the remaining treatment period. Body weight gain was observed in the other two groups. Mean food consumption was decreased in the group receiving the test substance in the stomach-soluble capsule throughout the study, while mean food consumption in the other two groups was not.

No treatment-related effect was observed on macroscopic examination.

Pyridaben causes decreased food consumption and body weight loss when given in a stomach-soluble capsule, but not in a small intestine-soluble capsule.

STUDY 2

Characteristics

Reference	: Shimada, 1987	exposure	: single
Type of study	: body functions studies; <i>in vivo</i> and <i>in vitro</i>	dose	: <i>in vivo</i> : 1-1000 mg/kg bw
year of execution	: 1987	vehicle	: <i>in vivo</i> : 1% CMC <i>in vitro</i> : ethanol
test substance	: NC-129 (pyridaben), Lot no. 129T8605, purity 98 %	GLP statement	: no
route	: intragastrical	guideline	: no guideline applicable
species	: rat, Jcl-SD mouse, Jcl-ICR guinea pig, Hartley rabbit, KBL-JW dog, Beagle	acceptability	: acceptable
group size	: see study design	NOAEL	: -

Study design

Experiment 1a/b

Mice and rats (5/sex/group) were given a single dose of pyridaben in a 1% CMC solution intragastrically. Doses applied in mice were 0, 3, 10, 30, 100 and 300 mg/kg bw for males and 0, 1, 3, 10, 30 and 100 mg/kg bw for females. Doses applied in rats were 0, 10, 30, 100, 300 and 1000 mg/kg bw for males and 0, 3, 10, 30, 100 and 300 mg/kg bw for females. Gross behaviour was observed for 1 hour continuously after administration and once an hour afterwards for 6 hours and after 24 hours.

Experiment 2

Male rats were immobilised by i.v. injection with d-tubocurarine chloride and artificially respired at a tidal volume of 1 ml/breath and a rate of 90 breaths/min. Monopolar electrodes were placed on the dura mater of the sensory and motor cortices with a reference electrode at the frontal sinus. A bipolar electrode was inserted into the right hippocampus. After stable EEG activities were recorded, pyridaben was intragastrically administered at 0, 3, 30 and 300 mg/kg bw in a 1% CMC solution (5 rats/group). Neocortical and hippocampal EEG activities were recorded for 60 minutes after administration. Rats were injected intramuscularly every 30 minutes to remain immobilised.

Experiment 3

Male mice (8/group) were given 0, 3, 30 and 300 mg/kg bw pyridaben or 3 mg/kg bw diazepam in 1% CMC solution intragastrically. One hour after administration mice were put to sleep by injection with sodium pentobarbital and the sleeping time was recorded.

Experiment 4

Male rats (5/group) were given 0, 3, 30 and 300 mg/kg bw pyridaben in 1% CMC solution intragastrically. Body temperature was measured once an hour starting before administration until 6 hours after administration.

Experiment 5

Male rats (5/group) were anaesthetised and artificially respired. The sciatic nerve was electrically stimulated and contraction of the gastrocnemius muscle was recorded. When contractions were stable, 0, 3, 30 and 300 mg/kg bw pyridaben in 1% CMC solution was administered intragastrically.

Experiment 6

Male rats (5/group) were anaesthetised and respiration, blood pressure, electrocardiogram, and heart rate were recorded. After stabilisation, 0, 3, 30 and 300 mg/kg bw pyridaben in 1% CMC solution was administered intragastrically and blood pressure, heart rate, and electrocardiogram were recorded for 120 minutes.

Experiment 7

Male dogs (4/group) were anaesthetised and artificially respired. The pre- and post-ganglionic nerves of the superior cervical ganglion were electrically stimulated and the contraction of the nictating membrane was recorded. After stabilisation of blood pressure, blood flow, electrocardiogram, heart rate and contraction of the nictating membrane, 0, 3, 30 and 300 mg/kg bw pyridaben in 1% CMC solution was administered intragastrically. A positive control was included. Blood pressure, blood flow, electrocardiogram and heart rate were recorded till 1 hour after administration. One hour after administration the nerves were stimulated again and contraction of the nictating membrane was recorded.

Experiment 8

Part (2 cm) of the ileum of male guinea pigs (5/group) was isolated and incubated for 15 minutes with 1, 3, 10, 30 or 100 μ M pyridaben in ethanol, or 0.3 or 1% ethanol (vehicle control). Positive controls were included. The influence on contractions induced by acetylcholine and on histamine was determined.

Experiment 9

The vas deferens with the hypogastric nerves of male guinea pigs was isolated and incubated for 15 minutes with 10, 30 or 100 μ M pyridaben in ethanol and ethanol at 0.1, 0.3 and 1% (vehicle control). Positive controls were included. The influence on contractions induced by electric stimulation of the hypogastric nerve or noradrenalin was determined.

Experiment 10

Prothrombin time and activated partial thromboplastin time were determined in blood from 5 rabbits after addition of 10, 30 and 100 μ g/ml pyridaben and ethanol (vehicle control). Also the starting, maximal, and ending points of haemolysis, and the haemolysis width were determined of blood incubated for 30 minutes at 37 °C with 10, 30, 100 and 300 μ g/ml pyridaben and ethanol (vehicle control).

Acceptability

The study is considered acceptable.

Results and conclusions

Experiment 1a

One female mouse given 100 mg/kg bw was found death within 2 hours after administration. Male mice administered 300 mg/kg bw and female mice administered 100 mg/kg bw pyridaben showed diarrhoea (females only) abnormal body posture, decreased response to touch, loss of startle response, decreased motor activity, abnormal gait, decreased muscle tone and muscle strength (males only), loss of righting reflex and abnormal respiration within 1 hour after administration. All these effects, except for decreased muscle strength were still present at 24 hours in males. Males at 100 mg/kg bw showed abnormal body posture, loss

of startle reflex, decreased motor activity and abnormal respiration within 2 hours after administration and the effects disappeared within 24 hours. Diarrhoea, decreased motor activity and abnormal respiration each were observed in one male after 3-5 hours and effects disappeared within 6 hours. Females at 30 mg/kg bw showed diarrhoea, abnormal body posture, decreased response to touch, decreased motor activity, abnormal gait, decreased muscle tone and abnormal respiration within 1 hour after administration and the effects disappeared within 24 hours. Females at 3 and 10 mg/kg bw showed diarrhoea within 1-2 hours and the effect disappeared within 4 hours after administration.

Experiment 1b

In rats at 10 and 30 mg/kg bw diarrhoea was observed in one male each. Diarrhoea, abnormal body posture, decreased motor activity and abnormal respiration was observed at 100 and 300 mg/kg bw within 1 hour and effects disappeared within 24 hours after administration, except for diarrhoea at 300 mg/kg bw. At 1000 mg/kg bw all animals died within 7.5 hours after showing similar effects as seen at lower doses. Additionally decreased response to touch, loss of startle response, abnormal gait, decreased muscle tone and strength, loss of righting reflex and whitened skin colour were noted.

In female rats diarrhoea was observed at all treated levels. At 100 mg/kg bw also abnormal body posture, decreased response to touch and decreased motor activity were observed. Abnormal respiration was additionally seen at 300 mg/kg bw and one female died within 24 hours.

Experiment 2

After administration of 3 and 30 mg/kg bw no changes in the EEG were observed. At 300 mg/kg bw suppression of brain activity was noted. At this dose level the low voltage-fast wave component decreased from 45 minutes onwards, and had almost disappeared 60 minutes after administration in both neocortical sensory and motor cortices. The synchronised waves in the hippocampus became desynchronised slower waves. The frequencies of α - and β -waves in the sensory and motor cortex were significantly decreased from 30 minutes onwards after administration (sensory cortex not significantly at 30 minutes).

Experiment 3

Sleeping time was slightly increased at 100 mg/kg bw pyridaben (125% of control), while diazepam showed a marked increase (230% of control). Two mice died at 100 mg/kg bw after 50 minutes.

Experiment 4

Body temperature was lowered at 300 mg/kg bw starting 1 hour after administration.

Experiment 5

Pyridaben did not influence contraction of the gastrocnemius muscle.

Experiment 6

Respiration rate increased less during the observation period at 30 and 300 mg/kg bw pyridaben compared to that of the control group. Heart rate decreased from 20 minutes onwards at 300 mg/kg bw while the heart rate of the control group remained constant. Blood pressure decreased at 30 and 300 mg/kg bw from 50 and 40 minutes onwards, respectively, while blood pressure in the control group slightly increased.

Experiment 7

No change in blood pressure, blood flow, electrocardiogram and heart rate were observed after administration of pyridaben. Stimulated contraction of the nictating membrane was not influenced by pyridaben, but was influenced by hexamethonium bromide.

Experiment 8

NC-129 did not directly influence contraction. The contraction induced by acetylcholine was decreased at 30 and 100 μM pyridaben (at 100 μM the effect was partly caused by ethanol). The contraction induced by histamine was decreased at 3 μM and above (at 100 μM the effect was partly caused by ethanol).

Experiment 9

NC-129 did not directly influence contraction. Inhibition of contraction induced by electric stimulation was completely due to the ethanol present. Contraction induced by noradrenalin was inhibited at 30 and 100 μM pyridaben.

Experiment 10

Pyridaben did not influence prothrombin time and activated partial thromboplastin time. Haemolysis starting point and ending point were significantly increased at 300 $\mu\text{g/ml}$.

In conclusion, pyridaben induced behavioural changes in rats and mice, reduced brain activity in rats, increased sleeping time in mice, reduced body temperature in rats, decreased respiration rate, heart rate and blood pressure in rats at acute (lethal) dose levels. In dogs no changes in blood pressure, blood flow, electrocardiogram and heart rate were observed at doses up to 300 mg/kg bw. Pyridaben did not influence contraction of the gastrocnemius muscle after electric stimulation of the sciatic nerve in rats at doses up to 300 mg/kg bw. Pyridaben did influence acetyl- and histamine-induced contractions of isolated guinea pig ileum. Pyridaben did inhibit noradrenalin-induced contraction of the isolated vas deferens from guinea pigs. Pyridaben did not influence coagulation of rabbit blood, but it did influence haemolysis.

STUDY 3**Characteristics**

Reference	: Shimada, 1988	exposure	: single
Type of study	: body functions studies; <i>in vivo</i> and <i>in vitro</i>	dose	: <i>in vivo</i> : 3-300 mg/kg bw
year of execution	: 1987/88	vehicle	: <i>in vivo</i> : 1% CMC <i>in vitro</i> : ethanol
test substance	: NC-129 (pyridaben), Lot no. 129T8605, purity 98.0 %	GLP statement	: no
route	: intragastrical, ocular or intradermal	guideline	: no guideline applicable
species	: rat, Jcl-SD mouse, Jcl-ICR guinea pig, Hartley rabbit, KBL-JW	acceptability	: acceptable
group size	: see study design	NOAEL	: -

Study design

Experiment 1

Male rats (5/group) were given a single dose of 0, 3, 30 and 300 mg/kg bw pyridaben and chlorphentermine (positive control) in a 1% CMC solution intragastrically. Food consumption was determined at 6 and 24 hours after administration.

Experiment 2

Male rats (5/ group) were given a single dose of 0, 3, 30 and 300 mg/kg bw pyridaben and chlorpromazine (positive control) in 1% CMC solution intragastrically. The average motor activity per hour was measured over 6 hours.

Experiment 3

Male rats (5/group) were given 0, 3, 30 and 300 mg/kg bw pyridaben or diazepam in 1% CMC solution intragastrically. After 1 hour traction force was determined. Immediately after that the coordinative motion of the rats was tested by means of the Rotarod test.

Experiment 4

In the eyes of male guinea pigs (5/group) 0.1% and 1% pyridaben or 1% tetracaine in 1% CMC solution was applied at 2 drops/eye. Corneal reflex was observed for 60 minutes and compared to pre-test values.

Experiment 5

To male guinea pigs (5/group) 0.05 ml of 0, 0.1 and 1% pyridaben or 1% tetracaine in 1% CMC solution was applied intradermally. Skin twitch reflex was observed for 60 minutes and compared to pre-test values.

Experiment 6

Male mice (5/group) were given 0, 3, 30 and 100 mg/kg bw pyridaben or morphine intragastrically. An hour after administration responses within 2 seconds of pinching the tail were recorded as nociceptive responses and compared to pre-test values.

Experiment 7

Male mice (5/group) were given 0, 3, 30 and 100 mg/kg bw pyridaben or morphine intragastrically. After fifty-five minutes the mice were injected intraperitoneally with 10 ml/kg 0.6% acetic acid. Five minutes after acetic acid injection the number of writhing responses was counted for 5 minutes.

Experiment 8

Five isolated ears from rabbits were perfused with Lock-Ringer solution containing 1% of serum of the rabbit before and for 15 minutes after addition of 100 or 300 µg/ear pyridaben or isoproterenol or ethanol (vehicle control) and the flow rate was observed for 15 minutes after administration.

Experiment 9

Right and left atria preparations were isolated from male guinea pigs (5/group) and suspended in an organ bath. The left atrium was electrically stimulated and contractions of the left atrium and spontaneous contractions of the right atrium were recorded. After stable contractions had been observed, isoproterenol was added to the bath and responses recorded. Preparations were washed and treated with 10 and 100 µM pyridaben or 1 and 10 µM propranolol or 0.1% ethanol (vehicle control) for 15 minutes. Then responses on isoproterenol application were observed again.

Experiment 10

The pylorus of 5 male rats was ligated 1 hour after intragastrical administration of 0, 3, 30 and 300 mg/kg bw pyridaben or cimetidine in 1% CMC solution. Four hours after the ligation, the stomach was isolated and gastric juice was collected. After centrifugation, the volume and pH of the supernatant were determined.

Experiment 11

Male mice (5/group) were intragastrically administered with 5% activated charcoal suspended in 1% CMC 60 minutes after intragastrical administration of 0, 3, 30 and 300 mg/kg bw pyridaben or atropine in 1% CMC solution. The small intestine was isolated 30 minutes after administration of charcoal and the rate of mobility of the charcoal was determined.

Experiment 12

Male rats (5/group) were intragastrically administered with 0, 30 and 300 mg/kg bw pyridaben in 1% CMC and bile volume was determined every hour for 6 hours.

Experiment 13

The fundus of the stomach of male rats (5/group) were isolated and as a strip suspended in an organ bath. After stable contractions were recorded by serotonin addition, the preparation was washed and treated with 0.1, 1 and 10 μ M pyridaben, cyprohepatidine or 0.01% ethanol (vehicle control) for 15 minutes. Then contractions induced by serotonin were observed again.

Experiment 14

Male rats (5/group) were intragastrically administered with 0, 3, 30 and 300 mg/kg bw pyridaben or acetazolamide in 1% CMC solution. Urine was collected for 6 hours. The cumulative volume was determined each hour and sodium, potassium, chloride, pH and osmotic pressure were determined in the 6-hour accumulated volume.

Experiment 15

The uterus from non-pregnant rats (5/group) was isolated and suspended as a strip in an organ bath. After stable contractions of the preparation induced by oxytocin had been observed, the preparation was washed and treated with 0.1, 1 and 10 μ M of pyridaben and 0.01% ethanol (vehicle control) for 15 minutes. Then contractions induced by oxytocin were observed again.

Experiment 16

The uterus from pregnant rats (5/group) was isolated and suspended as a strip in an organ bath. Spontaneous contractions were recorded before and after the addition of 0.1, 1 and 10 μ M pyridaben and 0.01% ethanol.

Acceptability

The study is considered acceptable.

Results and conclusions

Experiment 1

Pyridaben did not influence food consumption after 6 hours and resulted in a decreased food consumption after 24 hours at 30 and 300 mg/kg bw (75 and 31% of control, resp.). However, chlorphentermine resulted in decreased food consumption after 6 and 24 hours (31% and 59% of control).

Experiment 2

Due to the high variation in activity from hour to hour no conclusion can be drawn.

Experiment 3

Traction force was reduced at 300 mg/kg bw pyridaben (78% of control) and in rats given diazepam (53% of control). The staying time on the rotating bar was not influenced by pyridaben or diazepam.

Experiment 4

No reduction in corneal reflex was observed with pyridaben, while a reduction was seen during 1-30 minutes after tetracaine.

Experiment 5

A reduced skin twitch reflex was seen in 1 animal treated with 1% pyridaben during the first 30 minutes and in all animals treated with tetracaine during 60 minutes.

Experiment 6

Two mice at 100 mg/kg bw pyridaben showed a reduced reflex and 4 mice given morphine.

Experiment 7

The number of responses was almost nullified at 100 mg/kg bw pyridaben (3% of control) and completely nullified with morphine.

Experiment 8

The flow rate after application of 100 or 300 µg/ear pyridaben showed a slight increase (not statistically significant), while isoproterenol showed a clear increase during the first 5 minutes.

Experiment 9

The strength of contraction was reduced by 0.1% ethanol (concentration similar to 10 µM pyridaben) and 10 and 100 µM pyridaben with 50%. The frequency of contractions was also reduced by all three treatments (98, 96 and 98% of value before treatment). Also the influence on the positive inotropic effects of isoproterenol was caused by ethanol in all treatments. The influence of chronotropic effects of isoproterenol was negatively influenced by ethanol (79% of control) and the negative effect was larger for 100 µM pyridaben (60% of control) and 10 µM propranolol (16% of control).

Experiment 10

The volume of gastric juice was reduced by cimetidine, while the pH was increased by 300 mg/kg bw pyridaben (pH 3.0) and cimetidine (pH 3.4).

Experiment 11

Pyridaben did not delay passage of the charcoal through the small intestine, while atropine did.

Experiment 12

Pyridaben showed no influence on the production of bile volume.

Experiment 13

The contractile force induced by serotonin was reduced significantly by 1 and 10 μ M pyridaben and cyproheptadine.

Experiment 14

Urine volume was significantly reduced during the first 3 hours after administration of 30 and 300 mg/kg bw pyridaben, while urine volume was significantly increased during 6 hours after administration of acetazolamide. Sodium and chloride were decreased at 30 mg/kg pyridaben and potassium was increased at 300 mg/kg pyridaben. Osmotic pressure was increased at 30 and 300 mg/kg bw pyridaben. After administration of acetazolamide sodium and potassium, and the pH were increased, while osmotic pressure was decreased. The effects on electrolytes, pH and osmotic pressure in the urine may be influenced by the collection of urine admixed with diarrhoea.

Experiment 15

Contractions induced by oxytocin were reduced after treatment with 1 and 10 μ M pyridaben.

Experiment 16

Contraction strength and frequency were not influenced by pyridaben.

In conclusion, pyridaben induced reduced food consumption in rats and reduced traction force in rats, a reduced skin twitch reflex in guinea pigs and a reduced reflex upon tail pinching and intraperitoneal injection of acetic acid in mice at acute toxic (lethal) dose levels. Pyridaben induced an increased pH of gastric juice, but not the volume, and a reduced urine volume, deviations in urine ion concentrations. Pyridaben did not influence coordinative motion in rats, corneal reflex after ocular administration in guinea pigs, mobility through the small intestine in mice or bile volume in rats. Pyridaben did influence chronotropic effects of isoproterenol on contractions of atria preparations from guinea pigs negatively. Pyridaben induced reduced contractile force of the isolated rat fundus, reduced oxytocin-induced contraction of the uterus, while spontaneous contraction of the uterus was not influenced. Pyridaben did not influence the flow rate in isolated rabbit ear vein.

B.6.8.2.2 Overall evaluation of the mechanistic studies

To further evaluate the effect of pyridaben on several body functions additional tests were conducted.

The first study was conducted to clarify why salivation was seen in the dog studies performed. From this 13-week study, in which 300 mg/kg bw was administered by stomach-soluble or small intestine-soluble capsule. Pyridaben causes decreased food consumption and body weight loss when given in a stomach-soluble capsule, but not in a small intestine-soluble capsule. In none of the animals salivation was observed. However, the effects observed regarding salivation do not coincide with the 90-day and 1-year dog studies (paragraph 6.3.4). Moreover, mortality was high at 300 mg/kg bw in an earlier 13-week study (Project no. IRDC 563-005, 1989), while no mortality was observed at this level in the current study. Therefore, no conclusion can be drawn on salivation.

In the other two studies a number of body functions were studied in several animal species after administration of a single dose intragastrically, ocular or intradermal, or in *in vitro* experiments.

Pyridaben induced behavioural changes in rats at 100 mg/kg bw and higher and in mice at 30 mg/kg bw and higher. Pyridaben resulted in reduced brain activity, reduced body temperature, in rats at 300 mg/kg bw, increased sleeping time at 100 mg/kg bw in mice. A decreased respiration rate and heart rate and decreased blood pressure compared to the control group was found at 30 and 300 mg/kg bw in rats. No change in blood pressure, blood flow, electrocardiogram and heart rate were observed in dogs at doses up to 300 mg/kg bw. Pyridaben did not influence contraction of the gastrocnemius muscle after electric stimulation of the sciatic nerve in rats at doses up to 300 mg/kg bw. Pyridaben did reduce food consumption after 24 hours at 30 and 300 mg/kg bw in rats, but not after 6 hours. Pyridaben reduced traction force at 300 mg/kg bw in rats; coordinative motion was not influenced. No reduction in corneal reflex was seen after ocular administration in guinea pigs, while skin twitch reflex was reduced in 1 guinea pig after intradermal application. A reduced reflex after tail pinching was seen in mice and reflex was almost nullified after intraperitoneal injection of acetic acid at 100 mg/kg bw. Pyridaben did increase the pH of gastric juice at 300 mg/kg bw, but not the volume produced in rats. Mobility through the small intestine was not influenced by pyridaben in mice. Bile volume was not influenced by pyridaben in rats. Pyridaben resulted in a reduced urine volume at 30 and 300 mg/kg bw in rats.

Pyridaben did inhibit contraction of isolated guinea pig ileum, especially histamine-induced contractions. Pyridaben did inhibit noradrenalin-induced contraction of the isolated vas deferens from guinea pigs, but not electrically stimulated contraction. Pyridaben did not influence coagulation of rabbit blood, but it did influence haemolysis. The flow rate in an isolated rabbit ear vein was not influenced by pyridaben. Chronotropic effects of isoproterenol on contractions of atria preparations from guinea pigs were negatively influenced by pyridaben. Pyridaben did reduce contractile force of the isolated rat fundus. Pyridaben induced reduced oxytocin-induced contractions of the uterus from non-pregnant rats, while spontaneous contractions of the uterus from pregnant rats were not influenced.

B.6.9 Medical data and information (Annex IIA 5.9)

B.6.9.1 Medical surveillance on manufacturing

STUDY 1

Characteristics

Reference	: Ikai, 1992	Examinations	: ca. twice a year
Type of study	: medical surveillance plant workers		
year of execution	: 1985-1992	GLP statement	: no
test substance	: pyridaben	guideline	: not available
species	: workers	acceptability	: acceptable
group size	: variable		

Study design

In two pyridaben plants, medical surveillances of all workers involved in handling pyridaben were held from 1985-1992. General health checks (medical interview, height, weight, vision and hearing testing, roentgenography, blood pressure, blood test, urine test) were performed on all workers twice a year.

Additionally, once in three years the onset of pneumoconiosis was investigated with roentgenography. In workers handling powder, a blood test and urine test were performed in workers handling hazardous materials twice a year and a blood test was performed in workers handling organic solvents (with handling pyridaben technical) twice to four times a year.

Results

None of the workers involved in handling pyridaben or formulations of pyridaben showed any health effect.

Conclusion

In a production plant of pyridaben technical and formulations, no health effects were noted among workers.

STUDY 2

Characteristics

Reference	: Oki, 2004	Examinations	: ca. twice a year
Type of study	: medical surveillance plant workers	GLP statement	: no
year of execution	: 1998-2002	guideline	: not available
test substance	: pyridaben	acceptability	: acceptable
species	: workers		
group size	: 7-16		

Study design

In a pyridaben plant, medical surveillances of all workers involved in handling pyridaben were held from 1998-2002. General health checks (medical interview, height, weight, vision and hearing testing, sputum examination, blood pressure, blood test (hepatic function, blood fat and glucose examination), urine test, electrocardiography, X-ray examination of the chest (1/year)) were performed on all workers handling pyridaben technical or pyridaben 200 g/kg wettable powder twice a year and all workers handling pyridaben 200 g/L suspension concentrate once a year. Additionally, once in three years the onset of pneumoconiosis was investigated with roentgenography in workers handling pyridaben 200 g/kg wettable powder, and a urine test was performed in workers handling organic solvents (with handling pyridaben technical) twice a year.

Results

During 1998-2002, 16 workers were engaged in the manufacturing of pyridaben technical for 39-79 days per year, 7 workers were engaged in manufacturing pyridaben 200 g/kg wettable powder for 97-137 days/year and 7 workers were engaged in manufacturing of pyridaben 200 g/L suspension concentrate for 11-28 days per year (it is not indicated whether workers are involved in one manufacturing process are also involved in one of the other processes). None of the workers involved in handling pyridaben or formulations of pyridaben showed any health effect.

Conclusion

In a production plant of pyridaben technical and formulations, no health effects were noted among workers.

B.6.9.2 Clinical cases of poisoning incidents

No data or reports on personnel involved in the manufacturing of the technical and/or formulated product are known to the notifier.

B.6.9.3 Observations on exposure of the general population

There is no information available on the exposure of the general population to pyridaben.

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests

No data or reports were on specific signs of toxicity in humans or specific clinical test methods are known to the notifier.

B.6.9.5 First aid measures and therapeutic regimes

For first aid measures the notifier refers to the Material Safety Data Sheet, which showed the following text:

Inhalation:	Move to fresh air. If not breathing, give mouth-to-mouth resuscitation (or an artificial respiration). Keep warm with blanket and keep at rest. Seek emergency medical advice.
Skin contact:	Remove all contaminated clothing, shoes and socks from the affected area. Immediately rinse with running water. Wash skin thoroughly with soap and water. If irritation persists, seek medical advice.
Eye contact:	Rinse immediately with running water for at least 15 minutes and seek medical advice.
Ingestion:	Rinse mouth with water. Give one or two glasses of water and induce vomiting by touching the back of the throat with a finger. Seek medical advice immediately. Do not give anything by mouth or induce vomiting to an unconscious person.

As no specific effects are anticipated, no specific treatment is given by the notifier. A note to the physician is included in the MSDS:

Treat according to symptoms (decontamination, visual function), no known specific antidote.

B.6.9.6 Expected effects of poisoning

Effects of pyridaben poisoning in man are not known.

B.6.10 Summary of mammalian toxicology and proposed adi, ael arfd and drinking water limit (Annex IIA 5.10)

This toxicological dossier contains studies with the test substance pyridaben, which is also known under the name NC-129, Sanmite or Nexter.

B.6.10.1 Toxicokinetics

In all studies available [3,6-pyridazinone-ring¹⁴C]-pyridaben and [benzyl ring¹⁴C]-pyridaben were studied in parallel. In case no differences between the findings for both compounds were present the text gives a single range and/or refers to ¹⁴C-pyridaben.

Absorption

In three studies with Sprague Dawley rats 168 hours after a single oral dose of 3 and 30 mg/kg bw, or 14-day repeated oral dosing at 3 mg/kg bw, 80-97% AR was excreted with faeces. In urine concentrations of applied radioactivity were 3-4% after [3,6-pyridazinone-ring¹⁴C]-pyridaben and 11-19% after low dose [benzyl ring¹⁴C]-pyridaben and slightly higher after 30 mg/kg bw [benzyl ring¹⁴C]-pyridaben (13-24%). In expired air very low amounts of radioactivity were recovered (<0.07% AR). In tissues 5-11% AR was retained after 2 hours and <1.4% AR 168 hours after a single dose ¹⁴C-pyridaben. Peak ¹⁴C-concentrations in blood were observed after 12 hours in both sexes after an oral single or repeated dose of 3 mg/kg bw, but only after 24 hours after a single oral dose of 30 mg/kg bw. Oral absorption was found to be 3-5% and 11-19% after low dose [3,6-pyridazinone-ring¹⁴C]-pyridaben and [benzyl ring¹⁴C]-pyridaben, respectively, and 3-7% and 13-24% after high dose [3,6-pyridazinone-ring¹⁴C]-pyridaben and [benzyl ring¹⁴C]-pyridaben, respectively. Actual absorption might have been higher because of the fact that excretion via the entero-hepatic circulation was not taken into account (no bile-duct cannulation and analysis of radioactivity in bile). No differences between the sexes were observed.

In another study with Sprague Dawley rats 67-84% AR was retained from faeces 72 hours after a single dose of 30 mg/kg bw ¹⁴C-pyridaben, whilst radioactivity in urine accounted for 4-6% AR after dosing [3,6-pyridazinone-ring¹⁴C]-pyridaben and 14-24% after dosing [benzyl ring¹⁴C]-pyridaben. Oral absorption based on recovered radioactivity in urine, liver, skin and carcass is estimated to be 5-7% and 15-27% for [3,6-pyridazinone-ring¹⁴C]-pyridaben and [benzyl ring¹⁴C]-pyridaben, respectively. Bile-duct cannulation was not included in the study design. After cannulation of the bile-duct of additional groups of rats in the same study (single dose 30 mg/kg bw), radioactivity in bile accounted for 22-30% AR. In faeces (and the gastro-intestinal tract) and in urine 56-72% AR and 1.6-4.0% AR, respectively, were recovered. A statistical significance difference between males and females for urine and bile (benzyl ring label) (p<0.01-0.05) and between both radiolabels for urine and faeces (p<0.01) was found. Based on the results of this study it can be concluded that absorption via the gastro-intestinal tract is 26-39% AR, based on %AR in bile, urine, skin and carcass.

A much higher absorption (49-57%) was found in another study with Wistar rats receiving a single dose of 3 mg/kg bw. In this study the bile-duct of the rats was cannulated. After 48 hours biliary and faecal excretion accounted for 47-56% and 45-51% of the applied radioactivity, respectively. Urinary excretion was 1-5.7% AR.

From the studies with bile-duct cannulation it can be concluded that oral absorption after a single low dose is 49-57% and 26-39% after a single high dose. However, it is not clear whether the difference in absorption rate was caused by the height of the dose or by the use of two different strains of rats with a strain dependent

different absorption. In these studies no consistent differences between the sexes or both radiolabels were reported.

As the liver is not the main target organ, the %AR in bile should not be included in the final oral absorption rate. Therefore, based on %AR in urine, skin and carcass, oral absorption amounts to 11-19%. Although a direct effect on liver was not seen in the repeated dose toxicity studies with pyridaben, the working mechanism of pyridaben is very aspecific (inhibition of mitochondrial electron transport) and an effect on liver cannot completely be ruled out. Data on elimination through bile were available. Based on %AR in bile, urine, skin and carcass oral absorption amounts to 26-39% for 30 mg/kg bw, and 49-57% for 3 mg/kg bw, the latter being measured over 48 hours, whereas the other studies had a duration of 168 hours. The majority of %AR passed the bile within the first 6 hours.

Considering the fact that the working mechanism is aspecific, it is considered too worst-case to take the lowest level for oral absorption. However, it is also concluded that the amount excreted in bile should not be included in the total oral absorption, as the liver is not the critical effect. Relevant absorption for risk assessment purposes will be between 10 and 30%. Therefore, the highest amount of oral absorption of the low dose based on urine, skin and carcass will be considered for risk assessment purposes, being 20%.

Elimination

In three studies with Sprague Dawley rats 168 hours after a single oral dose of 3 and 30 mg/kg bw, or repeated oral dosing at 3 mg/kg bw, the majority of administered radioactivity was excreted in faeces (80-97% AR), whilst radioactivity in urine accounted for 3-19% AR and in expired air for <0.07% AR. Pre-treatment for 14 days did not affect the pattern of excretion and retention.

In another study 72 hours after a single dose of 30 mg/kg bw ¹⁴C-pyridaben 67-84% AR was retained from faeces and 4-6% AR from urine after dosing [3,6-pyridazinone-ring-¹⁴C]-pyridaben and 14-24% from urine after dosing [benzyl ring-¹⁴C]-pyridaben.

The elimination half-life of [3,6-pyridazinone-ring-¹⁴C]-pyridaben was longer than that for [benzyl ring-¹⁴C]-pyridaben: 23 hours versus 9-10 hours after a single dose of 3 mg/kg bw, 60-70 hours versus 12 hours after repeated dosing and 49-56 hours versus 18-24 hours after a single dose of 30 mg/kg bw. No differences between the sexes became apparent.

Distribution

In one study, at 2 hours after a single oral dose of 3 mg/kg bw, low radioactivity concentrations were found in tissues (4-10% and 5-6% AR after [3,6-pyridazinone-ring-¹⁴C]-pyridaben and [benzyl ring-¹⁴C]-pyridaben, respectively). The highest amounts were recovered from liver (563 and 1150 ng eq./g (1.0-2.1 % AR), 1250-1330 ng eq./g (2.0-2.9% AR) after [3,6-pyridazinone-ring-¹⁴C]-pyridaben and [benzyl ring-¹⁴C]-pyridaben, respectively). In other organs radioactivity ranged from 256-392 ng eq./g (kidneys), 1110 and 2170 ng eq./g (uterus), 896-3290 ng eq./g (ovary) and 849-1390 ng eq./g (lymph nodes). Concentrations decreased rapidly thereafter.

In a second study 24 hours after a single dose of 30 mg/kg bw a comparable pattern of distribution to that after a single oral dose of 3 mg/kg bw was shown. The concentrations in high dose rats were on average a factor of 12 and 17 for [3,6-pyridazinone-ring-¹⁴C]-pyridaben and [benzyl ring-¹⁴C]-pyridaben, respectively,

higher than in low dose rats (hence roughly proportional to the dose). In a separate study with 30 mg/kg bw (single dose) radioactivity recovered from liver and kidney was 6 and 11 times higher than that recovered after a single dose of 3 mg/kg bw, again reasonably proportional to dose.

After repeated oral administration of 3 mg/kg bw radioactivity in tissues 168 after the last dose was below 0.1% AR.

There were no remarkable differences between tissue levels and depletion rates of male and female rats.

Metabolism

Three studies are available using TLC analysis and co-elution of reference compounds as analytical method. The analyses were stated to be confirmed by two-dimensional TLC, but the results were not adequately included in the reports. The fourth study uses either HPLC or TLC to separate the metabolites and confirmed metabolites by co-elution of reference compounds or LC-MS.

All metabolites identified were <5% AR except for the parent in faeces, P-9 and B-11 in urine and two unknowns.

In 0-48 hour urine of rats treated with [3,6-pyridazinone-ring-¹⁴C]-pyridaben (single oral dose or repeated dosing of 3 mg/kg bw), which contained 2-3% AR, no metabolites were identified and confirmed. Metabolites identified by TLC included parent, P-9, P-1, P-3 and PB-12. After a single dose of 30 mg/kg bw [3,6-pyridazinone-ring-¹⁴C]-pyridaben P-9 was identified and confirmed by LC-MS in 0-72 hour urine (total 2-6% AR). In addition parent, P-1, P-3, P-7, PB-3, PB-9, PB-11 and PB-12 were identified by TLC or HPLC. The metabolite pattern was essentially the same for both sexes.

In urine of rats treated with a single dose of 3 or 30 mg/kg bw [benzyl ring-¹⁴C]-pyridaben or treated repeatedly with 3 mg/kg bw (0-48 hour after low dose (6-14% AR) and 0-72 hour after high dose (12-24% AR)) B-11 was identified and confirmed by LC-MS. Several other metabolites were present and identified by TLC or HPLC including B-1 and B-8 and in addition parent, B-3, B-7, B-15, PB-4, PB-7, PB-8, PB-12. The metabolite pattern was essentially the same for both sexes and dosing regimes.

In faeces of rats treated with [3,6-pyridazinone-ring-¹⁴C]-pyridaben (total radioactivity 45-54% AR low dose, 61-91% AR high dose) the parent and PB-12 were identified. At 30 mg/kg bw the parent (confirmed by LC-MS) was the main component accounting for >10% AR. In addition PB-3, PB-4, PB-7, PB-8, PB-9, PB-11, P-1 and P-3 were identified by TLC or HPLC, but not further confirmed. No major differences between sexes or dosing regimes could be found.

After treatment with [benzyl ring-¹⁴C]-pyridaben TLC or HPLC analysis showed the parent to be the main component of faeces (13-32%). In addition B-1, B-3, B-7, B-8, B-11, B-15, PB-3, PB-4, PB-7, PB-9, PB-11, PB-12 and D-1 were found, but not confirmed adequately. Total radioactivity in faeces was 57-68% AR after a single dose and 35-42% AR after repeated dosing.

In liver the parent was recovered after HPLC analysis after a single dose at 30 mg/kg bw [3,6-pyridazinone-ring-¹⁴C]-pyridaben (total radioactivity 0.7% AR). TLC analysis did not show any metabolites. After [benzyl ring-¹⁴C]-pyridaben (single dose of 3 or 30 mg/kg bw) B-1 was identified after TLC and HPLC analysis. In addition parent, B-3, B-7, B-8, B-11, PB-4, PB-9, PB-12 and PB-13 were found. In bile PB-8 was found after a

single dose of 30 mg/kg bw [3,6-pyridazinone-ring¹⁴C]-pyridaben. No metabolites were found after [benzyl ring¹⁴C]-pyridaben.

In three studies, metabolites were poorly separated on the TLC plates and not accurately confirmed. This renders the studies supplementary. However, all studies combined do provide information on the metabolic pattern of pyridaben. In the fourth study (at 30 mg/kg bw ¹⁴C-pyridaben) metabolites separated by HPLC could not be sufficiently identified by co-chromatography of referent compounds. In this study accurate confirmation of identity by LC-MS was available for P-9 and B-11 in urine and the parent compound in faeces.

B.6.10.2 Toxicodynamics

Acute toxicity

Pyridaben needs to be classified as toxic if swallowed (T, R25) on the basis of its acute oral toxicity in rats and as toxic after inhalation (T, R23) on the basis of its acute inhalatory toxicity in rats. Pyridaben does not need to be classified on the basis of its acute dermal toxicity and is considered not irritating to the skin and eyes. Pyridaben does not have sensitising properties in a Maximisation nor in a Buehler test.

Short-term and semichronic toxicity

Three weeks of dermal exposure of rats to 0, 30, 100, 300 and 1000 mg/kg bw/day resulted in decreased body weight gain at 300 and 1000 mg/kg bw/day. The NOAEL for systemic effects was set at 100 mg/kg bw/day.

After subacute inhalation exposure of rats to 0, 1, 3 and 10 mg/m³, reduced body weight was observed at 10 mg/m³. Based on these effects a NOAEL of 3.0 mg/m³ was established for systemic effects.

Dietary exposure of rats to 0, 30, 65, 155 and 350 mg/kg food/day of pyridaben (equal to 0, 2.3, 4.9, 11.6 and 25.7 mg/kg bw/d for males and 0, 2.6, 5.5, 12.8 and 27.7 mg/kg bw/day for females) for 13 weeks caused decreased body weight gain at 65 mg/kg food in females and at 155 and 350 mg/kg food in both sexes. Food and water consumption were decreased at 155 and 350 mg/kg food. Food conversion efficiency was also decreased at 155 and 350 mg/kg food in both sexes and at 65 mg/kg food in females. No toxicological significant changes were noted in haematology. At clinical biochemistry, gamma glutamyltransferase was increased at 350 mg/kg food in both sexes. Alkaline phosphatase was increased at 350 mg/kg food in females at the end of the exposure period and in males at the end of the recovery period. Total protein was decreased at 65 mg/kg food in females and at 155 and 350 mg/kg food in both sexes. Albumin was decreased in females at 65, 155 and 350 mg/kg food. Albumin/globulin ratio was slightly increased in males at 350 mg/kg food. α 1-Globulin concentration was increased and α 2- and β -globulin concentrations were decreased in males at 350 mg/kg food. The NOAEL is established at 30 mg/kg food (equal to 2.7 mg/kg bw/d for females) based on decreased body weight gain and changes at clinical biochemistry (decreased protein and albumin).

Dietary exposure of mice to 0, 30, 90, 270 and 810 mg/kg food (equal to 0, 4.1, 13.0, 40.1 and 119.2 mg/kg bw/d for males and 0, 4.9, 14.6, 43.1 and 124.6 mg/kg bw/d for females) for 13 weeks, caused reduced body weight gain and water consumption at 90 mg/kg food and above. Food consumption and food conversion efficiency were decreased from 180 and 90 mg/kg food onwards, respectively. Changes in haematology (a.o. decreased haematocrit and haemoglobin) were noted at 810 mg/kg food. Changes in clinical biochemistry were noted at 270 mg/kg food and above and included changes in ALP, ASAT, OCT, urea, total protein, chloride and phosphorus. Changes in relative organ weights were noted at 270 and 810 mg/kg food, but were considered to be due to the decreased body weight gain. Histopathology revealed acute inflammation and necrosis of the liver in all groups including control. A NOAEL of 30 mg/kg food is established (equal to 4.1 mg/kg bw/day in males and 4.9 mg/kg bw/day in females) based on decreased body weight gain.

Dogs exposed to 0, 0.5, 1.0, 4.0 and 16.0 mg/kg bw/d of pyridaben in a gelatin capsule for 3 months showed a dose-related increased incidence of food-like emesis and ptyalism at 4.0 and 16.0 mg/kg bw/d. Body weight gain was reduced at 4.0 and 16.0 mg/kg bw/d in males. Food consumption was reduced in all treated males but this was not dose-related. Mild lymphoid hyperplasia was noted in 2/4 males at 16.0 mg/kg bw/d. The NOAEL is set at 1.0 mg/kg bw/d based on decreased body weight gain, food-like emesis and ptyalism.

Dogs exposed to 0, 0.5, 1.0, 4.0, 16.0 and 32.0 mg/kg bw/d of pyridaben in a gelatin capsule for 12 months showed a reduced body weight gain at 1.0-32.0 mg/kg bw/d in both sexes and at 0.5 mg/kg bw/d in females. A high variation in body weight and body weight gain was observed within groups and between groups, also between the two control groups. The variation in food consumption was also high. An increased incidence of soft stool was noted at 4.0 mg/kg bw/d and above. Food-like emesis and ptyalism were observed at all dose levels, including control group, with an increased incidence and higher severity at higher dose levels.

In view of the large variation in body weights at the start of the experiment, the decrease in body weight gain at 0.5 and 1.0 mg/kg bw/d is considered to be no adverse effect. The NOAEL is set at 1.0 mg/kg bw/d based on decreased body weight gain.

In conclusion, in all rodent and dog studies with oral, dermal and inhalation exposure decreased body weight (gain) was the adverse effect.

Considering the identity of the observed effects at the LOAELs after oral, dermal and inhalation repeated exposure, classification with R48 is not considered necessary.

Genotoxicity

Pyridaben did not induce point mutations in *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and in the *Escherichia coli* tester strain WP₂uvrA, both with and without metabolic activation. Pyridaben was negative in an *in vitro* chromosome aberration test with a Chinese hamster ovary cell line CHO. Pyridaben was negative in a gene mutation test using Chinese hamster cells. In addition, pyridaben was negative in an

in vitro DNA repair study using male rat hepatocytes, pyridaben was also negative in an *in vivo* mouse micronucleus test. In conclusion, pyridaben is considered non-genotoxic.

Long-term toxicity

In a 2-year combined toxicity/carcinogenicity study in rats, administration of pyridaben at 0, 4, 10, 28 and 80 (oncogenicity group)/120 (toxicity group) mg/kg food (equal to 0, 0.2, 0.4, 1.1 and 3.2-5.0 mg/kg bw/day in males and 0, 0.2, 0.5, 1.5 and 4.2-6.5 mg/kg bw/day in females) resulted in reduced body weight and food consumption at 80 and 120 mg/kg food. Leukocyte count was decreased at 10-120 mg/kg food in females. Neutrophils were decreased at 4 mg/kg food in females and at 10-120 mg/kg food in both sexes. Observed changes in leucocytes and neutrophils were considered to be due to bacterial infection and not test substance related. Urine volume was decreased and specific gravity was increased in males at 120 mg/kg food. Protein content was increased at 120 mg/kg food in females and increased in both sexes in the course of the study. Absolute testes weight was increased at 120 mg/kg food in the toxicity group and ovary weight was increased at 80 mg/kg food in the oncogenicity group.

Based on reduced body weight, food consumption and food conversion, the NOAEL is set at 28 mg/kg food (equal to 1.1 mg/kg bw/day in males and 1.5 mg/kg bw/day in females). No oncogenic potential of the test substance in rats was observed.

In a 78-week carcinogenicity study in mice, pyridaben was administered via the diet at 0, 2.5, 8.0, 25 or 80 mg/kg food (equal to 0, 0.27, 0.81, 2.78 and 8.88 mg/kg bw/day for males and 0, 0.29, 0.91, 2.78 and 9.74 mg/kg bw/day for females). Treatment at 80 mg/kg food resulted in slightly reduced survival in males. Body weight gain was decreased at 80 mg/kg food in both sexes and at 25 mg/kg food in males. Food conversion efficiency during the first 14 days of the study was lower at 80 mg/kg food in both sexes.

No oncogenic potential was observed in mice. Based on reduced body weight gain a NOAEL of 8.0 mg/kg food (equal to 0.81 and 0.91 mg/kg bw/d for males and females, resp.) is established.

Reproduction and developmental toxicity

In an oral 2-generation reproduction study in rats (0, 10, 28 and 80 mg/kg food), a decrease in body weight gains and food consumption was noted among males and females from the F₀- and F₁-generation at 80 mg/kg food. An increase in absolute ovary weight was observed at 28 and 80 mg/kg food and accompanied by an increase in relative ovary weight at 80 mg/kg food in the F₀-generation. There were no changes observed between parental animals of the treated and control groups in mating indices, pregnancy rates, fertility, oestrus cycle, post-implantation loss and macroscopic findings.

Examination of the F₀ and F₁-offspring revealed decreased body weights of pups at 80 mg/kg food in weeks 3 and 4 post partum. No treatment-related changes were detected in clinical signs, litter size, birth weight, sex ratio, litter survival, pup development, auditory and visual responses or macroscopic observations of the F₀ and F₁-offspring. Based on the data presented in this study, the NOAEL for parental toxicity was 28 mg/kg food (equivalent to 1.87 mg/kg bw/day). The NOAEL for developmental toxicity was 28 mg/kg food

(equivalent to 5 mg/kg bw/day). The NOAEL for reproductive toxicity was considered to exceed 80 mg/kg food (equivalent to 5.33 mg/kg bw/day).

In a teratogenicity study in rats (0, 2.5, 5.7, 13.0 and 30.0 mg/kg bw/day) the NOAEL for maternal effects was 5.7 mg/kg bw/day (equal to 4.7 mg/kg bw/day after correction for measured concentration of the test substance), based on decreased body weight gain and food consumption. The NOAEL for developmental effects was set at 13.0 mg/kg bw/day (equal to 12.5 mg/kg bw/day after correction for measured concentration of the test substance), based on decreased mean foetal weight, and retarded ossification of various bones. No treatment-related effects were observed on the number of corpora lutea or implantations, the number of live foetuses, and the sex ratio. There were no morphological changes observed in foetuses that could be attributed to treatment. Therefore, the NOAEL for teratogenicity was considered to exceed 30.0 mg/kg bw/day (equal to 26.7 mg/kg bw/day after correction for measured concentration of the test substance).

In an oral teratogenicity study in rabbits (0, 1.5, 5 and 15 mg/kg bw/day), a NOAEL for maternal effects of 5 mg/kg bw/day was derived, based on body weight loss and decrease in body weight gain, slightly reduced food consumption and reduced faeces. Since no effects on foetal development and no irreversible structural effects were noted, the NOAEL for developmental and teratogenic effects was set at 15 mg/kg bw/day.

In a dermal teratogenicity study in rabbits (0, 70, 170 and 450 mg/kg bw/day), the NOAEL for local maternal effects was <70 mg/kg bw/day, based on moderate to severe erythema and/or slight eschar formation at 70 mg/kg bw/day. The NOAEL for systemic maternal effects was set at 70 mg/kg bw/day, based on decreased body weight and food consumption. Since no effects on foetal development and no irreversible structural effects were noted, the NOAEL for developmental and teratogenic effects was set at 450 mg/kg bw/day.

Neurotoxicity

In an acute neurotoxicity study in rats (0, 50, 100 and 200 mg/kg bw by gavage), no mortality was observed. Animals given 100 and 200 mg/kg bw showed piloerection, hypoactivity, tremors and partially closed eyes, mainly on the day of administration. Thereafter occasional piloerection was observed in males given 100 and 200 mg/kg bw and in one female given 200 mg/kg bw, and tremors were observed in one male given 200 mg/kg bw. No clinical signs were observed from day 4 onwards. Body weight gains were reduced in males and females given 100 and 200 mg/kg bw. Food consumption was reduced on the day of dosing in all treated males and females. In the functional observation battery a lower activity, weak startle reflex, weak flexor responses were noted at day 1 in males at 100 and 200 mg/kg bw. Furthermore, an increased incidence of slow or poorly coordinated righting reflex was noted in males at 200 mg/kg bw and reduced body temperature in females at 100 mg/kg bw and both sexes at 200 mg/kg bw. No changes in landing foot splay, grip strength and motor activity were observed. Macroscopic post mortem examination and histopathological examination of the central and peripheral nervous system did not reveal any abnormalities.

Based on the clinical observations and changes in FOB at 100 and 200 mg/kg bw, the NOAEL for acute toxicity is set at 50 mg/kg bw. Observed changes are likely to result from overt toxicity, rather than from specific neurotoxicity.

Dietary exposure of rats to 0, 30, 100 or 350 mg/kg food (equal to 0, 2.5, 8.5 and 28.8 mg/kg bw/day in males, and 0, 2.8, 9.3 and 31.1 mg/kg bw/day in females) for 13 weeks, caused reduced body weight gains in males and females given 350 mg/kg food. Food consumption was reduced in males and females given 350 mg/kg food. No treatment-related findings were noted in the functional observation battery, grip strength and motor activity tests. Macroscopic post mortem examination and histopathological examination of the central and peripheral nervous system did not reveal any abnormalities.

Based on the changes in body weight at 350 mg/kg food, the NOAEL is set at 100 mg/kg food (equal to 8.5 mg/kg bw/day in males and 9.3 mg/kg bw/day in females). No signs of neurotoxicity were observed, hence to the NOAEL for neurotoxicity is 350 mg/kg food (28.8 mg/kg bw/d).

Mechanistic data

To further evaluate the effect of pyridaben on several body functions additional tests were conducted.

The first study was conducted to clarify why salivation was seen in the dog studies performed. From this 13-week study, in which 300 mg/kg bw was administered by stomach-soluble or small intestine-soluble capsule. Pyridaben causes decreased food consumption and body weight loss when given in a stomach-soluble capsule, but not in a small intestine-soluble capsule. In none of the animals salivation was observed. However, the effects observed regarding salivation do not coincide with the 90-day and 1-year dog studies (paragraph 6.3.4). Moreover, mortality was high at 300 mg/kg bw in an earlier 13-week study (Project no. IRDC 563-005, 1989), while no mortality was observed at this level in the current study. Therefore, no conclusion can be drawn on salivation.

In the other two studies a number of body functions were studied in several animal species after administration of a single dose intragastrically, ocular or intradermal, or in *in vitro* experiments.

Pyridaben induced behavioural changes in rats at 100 mg/kg bw and higher and in mice at 30 mg/kg bw and higher. Pyridaben resulted in reduced brain activity, reduced body temperature, in rats at 300 mg/kg bw, increased sleeping time at 100 mg/kg bw in mice. A decreased respiration rate and heart rate and decreased blood pressure compared to the control group was found at 30 and 300 mg/kg bw in rats. No change in blood pressure, blood flow, electrocardiogram and heart rate were observed in dogs at doses up to 300 mg/kg bw. Pyridaben did not influence contraction of the gastrocnemius muscle after electric stimulation of the sciatic nerve in rats at doses up to 300 mg/kg bw. Pyridaben did reduce food consumption after 24 hours at 30 and 300 mg/kg bw in rats, but not after 6 hours. Pyridaben reduced traction force at 300 mg/kg bw in rats; coordinative motion was not influenced. No reduction in corneal reflex was seen after ocular administration in guinea pigs, while skin twitch reflex was reduced in 1 guinea pig after intradermal application. A reduced reflex after tail pinching was seen in mice and response reflex was almost nullified after intraperitoneal injection of acetic acid at 100 mg/kg bw. Pyridaben did increase the pH of gastric juice at 300 mg/kg bw, but

not the volume produced in rats. Mobility through the small intestine was not influenced by pyridaben in mice. Bile volume was not influenced by pyridaben in rats. Pyridaben resulted in a reduced urine volume at 30 and 300 mg/kg bw in rats.

Pyridaben did inhibit contraction of isolated guinea pig ileum, especially histamine-induced contractions. Pyridaben did inhibit noradrenalin-induced contraction of the isolated vas deferens from guinea pigs, but not electrically stimulated contraction. Pyridaben did not influence coagulation of rabbit blood, but it did influence haemolysis. The flow rate in an isolated rabbit ear vein was not influenced by pyridaben. Chronotropic effects of isoproterenol on contractions of atria preparations from guinea pigs were negatively influenced by pyridaben. Pyridaben did reduce contractile force of the isolated rat fundus. Pyridaben induced reduced oxytocin-induced contractions of the uterus from non-pregnant rats, while spontaneous contractions of the uterus from pregnant rats were not influenced.

Toxicological data with metabolites

An acute oral toxicity study and genotoxicity studies were performed with the metabolite PB-7.

The acute oral LD₅₀ of PB-7 was found to be 2728 mg/kg bw in male rats and 3086 mg/kg bw in female rats.

PB-7 did not induce point mutations in *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and in the *Escherichia coli* tester strain WP₂uvrA, both with and without metabolic activation. PB-7 was negative in a lethal DNA damage test in strains of *Escherichia coli* (WP2, WP67 and CM87).

The available genotoxicity package on PB-7 is considered insufficient to conclude on the genotoxic properties of PB-7.

B.6.10.3 ADI

The ADI has to be derived from the results of toxicity studies with experimental animals. The calculation of the ADI is based on the highest dose at which no adverse effect is observed in the most appropriate study in the most sensitive species. Pyridaben was tested in several subacute, semi-chronic and chronic studies in dogs, rats and mice, in a reproduction study in rats, and in teratogenicity studies in rats and rabbits. Furthermore, it was established in several *in vitro* and *in vivo* genotoxicity tests that pyridaben does not have genotoxic potential.

The overall critical effect of pyridaben was considered to be reduced body weight (gain). Several repeated dose studies, including the 2-generation reproduction toxicity study and teratogenicity studies were considered for the establishment of the ADI (Table 6.10.3-01).

Table 6.10.3-01 Studies considered for the establishment of the ADI

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Semichronic toxicity studies			
13-week, oral, rat (diet)	2.6 (F)	5.5 (F)	Holmes, 1988a
13-week, oral, mouse (diet)	4.1 (M)	13.0 (M)	Holmes, 1988b
3-month, oral, dog (capsule)	1.0	4.0	Spicer, 1989b
1-year, oral, dog (capsule)	1.0	4.0	Spicer, 1990, 1991 incl additional study
Chronic toxicity studies			
2-year, oral, rat	1.1 (M)	5.0 (M)	Holmes, 1990a
78-week, oral, mouse	0.81 (M)	2.78 (M+F)	Holmes, 1990b
Reproduction and teratogenicity studies			
2-generation, oral, rat	Parental: 1.87 developmental: 5	5.33 15	Willoughby, 1990
Teratogenicity, oral, rat	Maternal: 4.7	12.5	Willoughby, 1990
Teratogenicity, oral, rabbit	Maternal: 5	15	Ross, 1988

In all semichronic, chronic and reproduction and teratogenicity studies the critical effect is reduced body weight (gain). The NOAELs for all studies – independent of exposure duration - are comparable (about 1 mg/kg bw/d), differences only appear because of the choice of dose levels. Therefore, the relevant NOAEL for derivation of the ADI is 1 mg/kg bw/d. A safety factor of 100 for inter- and intraspecies differences is applied, resulting in an ADI of 0.01 mg/kg bw/day.

Note: Since similar effects were observed in rats, mice, rabbits and dogs, one could consider applying only a safety factor of 10 for intraspecies differences. However, in the absence of relevant human data (see also Guidance for the setting of AOELs, 5 July 2005, European Commission), a safety factor of 100 for inter- and intraspecies differences is applied.

B.6.10.4 ARfD (acute reference dose)

According to the Guidance for the setting of an Acute Reference Dose (Document 7199/VI/99 rev. 5) an ARfD should not be allocated if:

- the pesticide has shown a very low acute oral toxicity (e.g. no adverse clinical signs and deaths have been observed at the limit dose for acute LD₅₀ testing), and
- the toxicological profile was based only on e.g. mild effects, which are not relevant for acute intake (e.g. adaptive liver enlargement, chronic body weight reduction, reduced food intake), and
- the toxicological profile has no other alerts for acute toxicity.

Furthermore, residue data should be taken into account to conclude on the necessity of an ARfD.

Based on the submitted data on pyridaben, the following considerations were made:

- The lowest acute oral LD₅₀ of pyridaben was found to be 161 mg/kg bw in male rats and 181 mg/kg bw in females rats.
- In the acute neurotoxicity study, overt signs of toxicity were noted at doses of 100 mg/kg bw/day, including clinical signs, reduced body weight, lower activity, weak startle reflex and flexor responses.
- In mechanistic studies, a.o. effects on activity, respiration rate, blood pressure, heart rate, were observed after single doses of 30 mg/kg bw in rats and mice.

Based on the above data, it can be concluded that the criteria for not allocating an ARfD are not met, since the LD₅₀ value is <2000 mg/kg bw and alerts for acute toxicity were observed. From the acute LD₅₀ studies in mice, the acute neurotoxicity study in rats and the acute mechanistic studies in mice (and rats showed similar effects) it is clear that acute exposure to pyridaben results in acute effects, including body weight changes, changes in activity and reflexes, changes in respiration rate, heart rate and blood pressure. Therefore, the allocation of an ARfD is considered justified.

The calculation of the acute reference dose is based on the highest dose at which no adverse effect is observed in the most appropriate acute toxicological endpoint of relevance to humans, derived from the most appropriate study in the most appropriate species.

The following studies were considered relevant for the establishment of the ARfD:

- The acute oral LD₅₀ studies in mice in which a NOAEL of 31 mg/kg bw could be established. Signs of toxicity observed in both sexes at 50 mg/kg bw (LOAEL) were mortality, decreased locomotor movement, prone and/or crouching posture and ataxia.
- The acute neurotoxicity study in rats: a NOAEL of 50 mg/kg bw/day was established. At 100 (LOAEL) and 200 mg/kg bw clinical signs (piloerection, hypoactivity, tremors and closed eyes), reduced body weight, no locomotion and weak reflexes were noted.
- Acute mechanistic studies in mice with a NOAEL of 10 mg/kg bw. Females at 30 mg/kg bw (LOAEL) showed diarrhoea, abnormal body posture, decreased response to touch, decreased motor activity, abnormal gait, decreased muscle tone and abnormal respiration within 1 hour after administration and the effects disappeared within 24 hours. Males showed similar clinical signs and loss of startle reflex at 100 mg/kg.
- Acute body function study in rats with a NOAEL of 3 mg/kg. At 30 mg/kg a decreased respiration rate, heart rate and blood pressure was seen. A similar experiment in dogs showed no effect on heart rate, electrocardiogram, blood flow and blood pressure up to 300 mg/kg bw.
- The teratogenicity study in rats: a NOAEL of 4.7 mg/kg bw/d was established for maternal effects and 12.5 mg/kg bw/d for developmental effects. Only a non-specific effect of decreased body weight gain and food consumption in dams were noted at the LOAEL of 12.5 mg/kg bw/d. Decreased foetal weight and retarded ossification of various bones was observed at 26.7 mg/kg bw/d.

- The teratogenicity study in rabbits: a NOAEL of 5 mg/kg bw/day was established for maternal effects and 15 mg/kg bw/d for developmental effects. Only a non-specific effect of decreased body weight gain in dams was noted at the LOAEL of 15 mg/kg bw/d and no effects were observed in offspring.

The most relevant NOAEL for the derivation of the ARfD is considered the NOAEL of 5 mg/kg bw/day, based on a comparison of NOAELs and LOAELs of the studies described above. Application of a safety factor of 100 for inter- and intraspecies differences results in an ARfD of 0.05 mg/kg bw/day.

B.6.10.5 AOEL

Nexter Pro is used on citrus in the field and on tomato in glasshouses as a spray.

Application of Nexter Pro on citrus and tomato takes place once during or before growth stage BBCH 83 and 89, respectively. As multiple cultures might be produced within one year in glasshouses, chronic use of Nexter Pro cannot be excluded.

Exposure of bystanders can be excluded for use on tomatoes, since Nexter Pro is used in glasshouses. Exposure of bystanders cannot be excluded for use on citrus fruit.

For the use of Nexter Pro, re-entry activities should be considered for citrus and tomato. For tomato crops in glasshouses, it cannot be excluded that exposure will be several times per year.

Pyridaben was tested in several semi-chronic and chronic oral studies in dogs, rats, and mice, in a reproduction study in rats and in teratogenicity studies in rats and rabbits (see table 6.10.5-01).

Table 6.10.5-01 Studies considered for the establishment of the AOEL

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Dermal and inhalation toxicity studies			
28-day, inhalation, rat	0.84 ¹ (3.0mg/m ³)	2.7 ¹ (10 mg/m ³)	Hoffman, 1989
21-day, dermal, rat	100	300	Blaszczak, 1992
Semichronic toxicity studies			
13-week, oral, rat (diet)	2.6 (F)	5.5 (F)	Holmes, 1988a
13-week, oral mouse (diet)	4.1 (M)	13.0 (M)	Holmes, 1988b
3-month, oral, dog (capsule)	1.0	4.0	Spicer, 1989b
1-year, oral, dog (capsule)	1.0	4.0	Spicer, 1990,1991 incl additional study
Chronic toxicity studies			
2-year, oral, rat	1.1 (M)	5.0 (M)	Holmes, 1990a
78-week, oral, mouse	0.81 (M)	2.78 (M+F)	Holmes, 1990b
Reproduction and teratogenicity studies			
2-generation, oral, rat	Parental and developmental: 1.87	5.33	Willoughby, 1990
Teratogenicity, oral, rat	Maternal: 4.7	12.5	Willoughby, 1988
Teratogenicity, oral, rabbit	Maternal: 5	15	Ross, 1988
Teratogenicity, dermal, rabbit	Maternal: 70	170	Hellwig, 1995

¹ based on an respiratory volume of 9 L/h and body weight of 200 g (TNO-report V98/390 d.d. December 1998, Default values in occupational risk assessment)

As pyridaben is extensively metabolised, route-specific toxicity cannot be excluded. Therefore, if available, toxicity studies for the route concerned should be considered to calculate route-specific AOELs. In case of pyridaben subacute dermal and inhalation studies were available.

In a 28-day inhalation study, a NOAEL of 3.0 mg/m³ (equivalent to 0.84 mg/kg bw/day) was established for systemic effects. The observed effects at the LOAEL of 10 mg/m³ (2.7 mg/kg bw/day) were equivalent to the effects observed after oral exposure, mainly body weight effects. The effect levels for both oral and respiratory route are in the same order of magnitude. Therefore, it is concluded that derivation of a specific AOEL for respiratory exposure is not considered necessary.

In a 21-day dermal toxicity study, a NOAEL of 100 mg/kg bw/day was derived, based on decreased body weight gain. As the application area was 25 cm², the area concentration is 4 mg/cm² at the NOAEL, hence a dermal absorption of 2.7% is applicable. Based on this dermal absorption an internal dose of 2.7 mg/kg bw/day can be calculated for the dermal study, which is higher than the internal dose for the oral route. Therefore, as a worst-case, the oral NOAEL is used for the establishment of the AOEL.

The working mechanism of pyridaben is very aspecific, namely inhibition of mitochondrial electron transport. Considering the effect levels in subacute, semichronic and chronic oral toxicity studies in rats, dogs and mice, there seems to be no effect of exposure duration after oral administration of pyridaben. Therefore, all repeated dose studies can be considered as starting point for the derivation of the AOEL. The overall NOAEL for pyridaben is considered to be approximately 1 mg/kg bw/day for both semichronic and chronic exposure of operators, bystander and/or workers.

Application of a safety factor of 100 for intra- and interspecies differences, results in an external AOEL of 0.01 mg/kg bw/day. As oral absorption is assumed to be 20% (see B.6.1.4), correction is made for oral absorption, resulting in a systemic AOEL of 0.002 mg/kg bw/day (0.12 mg/day for a 60 kg person, and 0.14 mg/day for a 70 kg person).

Note: Since similar effects were observed in rats, mice, rabbits and dogs, one could consider applying only a safety factor of 10 for intraspecies differences. However, in the absence of relevant human data (see also Guidance for the setting of AOELs, 5 July 2005, European Commission), a safety factor of 100 for inter- and intraspecies differences is applied.

B.6.10.6 Drinking water limit

According to Council Directive 97/57/EC, exposure to pyridaben through the drinking water should account for not more than 10% of the ADI. If it is assumed that the average daily consumption of water amounts to 2 litre per person of 60 kilogram, a drinking water limit of $(60 \times 0.01) / 10 = 0.06$ mg/l, i.e. 0.03 mg/l can be calculated. However, according to Document 8064/VI/79 of the European Commission, the EU drinking water limit for pesticides of 0.1 µg/l is applicable for pyridaben.

Since aquatic metabolite PB-7 is less toxic than pyridaben, no drinking water limit for PB-7 is established.

B.6.11 Acute toxicity including irritancy and skin sensitisation of the preparation

B.6.11.1 Acute toxicity

STUDY 1

Characteristics

reference	: Kirsch, 1992a	exposure	: Once by gavage
type of study	: Acute oral toxicity study	doses	: 200, 1000 and 2200 mg/kg bw
year of execution	: 1991-1992	vehicle	: Aqua bidest
test substance	: BAS 300 11 I, batch no. AF-137-50, 75.8% pyridaben	GLP statement	: Yes
route	: Oral	guideline	: In accordance with OECD 401 (1987)
species	: Rat, Wistar, CHBB: THOM (SPF)	acceptability	: Acceptable
group size	: 5/sex/dose	LD ₅₀	: 1930 mg/kg bw

Study design

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

Results

Mortality: 1/5 males given 1000 mg/kg was found dead on day 13. In the 2200 mg/kg dose group, 3/5 males (day 0, 1 and 5) and 3/5 females (day 1, 5 and 14) were found dead.

Symptoms of toxicity: In the 200 mg/kg dose group, no symptoms were noted. Impaired/poor general state, dyspnoea, apathy, staggering and piloerection were noted in nearly all 1000 mg/kg and 2200 mg/kg dose group animals. Other symptoms occurring included among others smeared fur, exsiccosis, red discoloured urine and red smeared ano-genital fur. These clinical signs generally disappeared before the end of the study.

Body weight: Mean body weights in the 1000 mg/kg dose group were decreased on day 7 in both males and females. In males, body weights were increased on days 13 and 20. In females, body weights remained the same on day 13, and were increased on day 20. In both males and females of the 2200 mg/kg dose group, body weights decreased up to day 13, and increased thereafter.

Pathology: Surviving animals showed no pathological changes. Findings in the animals from the 1000 mg/kg and 2200 mg/kg dose groups included general congestion and dark-red/bloody contents of the stomach and small intestines.

Acceptability

This study is considered acceptable. No individual data on pathology findings were present though.

Conclusions

The acute oral LD₅₀ of BAS 300 11 I was calculated to be 1930 mg/kg bw in both male and female rats.

STUDY 2

Characteristics

reference	: Kirsch, 1992b	exposure	: 24 hours on a 50 cm ² skin area (semi-occlusive exposure).
type of study	: Acute dermal toxicity study	doses	: 2000 mg/kg bw (both sexes)
year of execution	: 1991	vehicle	: Aqua bidest
test substance	: BAS 300 11 I; Batch no.: AF-137-50; 75.8% pyridaben	GLP statement	: Yes
route	: Dermal	guideline	: In accordance with OECD 402 (1987)
species	: Rat, Wistar, CHBB: THOM (SPF)	acceptability	: Acceptable
group size	: 5/sex/dose	LD ₅₀	: > 2000 mg/kg bw

Study design

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

Results

Mortality: No mortality occurred.

Symptoms of toxicity: All males and females showed very slight to well-defined erythema on day 1, which were fully reversible within 7 days.

Body weight: No treatment related findings.

Pathology: No treatment related findings.

Acceptability

This study is considered acceptable.

Conclusions

The acute dermal LD₅₀ of BAS 300 11 I in rats was found to be greater than 2000 mg/kg bw in males and females.

STUDY 3

Characteristics

reference	: Gamer, 1992	exposure	: 4 hours; nose only
type of study	: Acute inhalation toxicity study	doses	: 50, 108 and 640 µg/l (actual concentrations); MMAD 1.3-1.5 µm; GSD 3.3-4.7 µm
year of execution	: 1991	vehicle	: None
test substance	: BAS 300 11 I; Batch no.: AF-137-50; 75.8% pyridaben	GLP statement	: Yes
route	: Inhalation	guideline	: In accordance with OECD 403 (1981)
species	: Rat, Wistar (Chbb: THOM)	acceptability	: Acceptable
group size	: 5/sex/dose	LC ₅₀	: ± 90 µg/l

Study design

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

Results

Mortality: In the 108 µg/l dose group, 4/5 males and 4/5 females died during or on the day of exposure. In the 640 µg/l dose group, all animals died during exposure.

Symptoms of toxicity: All animals showed symptoms of changed respiration, eyelid closure and paleness. Animals at 108 and 640 µg/l also showed a decreased pain reflex. Surviving animals also showed symptoms of piloerection. All symptoms generally had disappeared after day 1.

Body weight: No treatment-related effects on body weight (gain) were seen in the low-dose group. Due to poor survival, body weight (gain) could not be evaluated in the other two groups.

Pathology: No treatment-related macroscopic observations were seen in surviving animals. Animals that died during the study showed general congestion, and intensified hyperaemia with slight to moderate oedema in the lungs.

Acceptability

The study is considered acceptable.

Conclusions

The acute inhalation LD₅₀ of BAS 300 11 I in rats was found to be approximately 90 µg/l for both males and females.

B.6.11.2 Irritation and sensitization

STUDY 1

Characteristics

reference	: Rossbacher, 1992a	exposure	: 4 hours, semi-occlusive, application area 6.25 cm ²
type of study	: Skin irritation study	doses	: 0.5 g
year of execution	: 1991-1992	vehicle	: None
test substance	: BAS 300 11 I; Batch.nr.: AF-137-50; 75.8% pyridaben	GLP statement	: Yes
route	: Dermal	guideline	: In accordance with OECD 404 (1981)
species	: Rabbit, White Vienna	acceptability	: Acceptable
group size	: 5 males and 1 female	Effect	: Not irritating

Study design

The study was performed in accordance with OECD 404 (1981).

Results

The results are summarised in tables 6.11.2-01 and 6.11.2-02.

Table 6.11.2-01 Individual irritation scores

Scores observed after	1 hours	24 hours	48 hours	72 hours	8 days	15 days
Erythema	0, 2, 1, 1, 1, 1	1, 2, 1, 1, 2, 1	1, 2 ^E , 1, 2, 2, 1	1, 2 ^E , 1, 2, 1, 1	1, 1 ^S , 1, 1, 1 ^S , 0	0 ^S , 0 ^S , 0, 0, 0, 0
Oedema	0, 1, 0, 0, 0, 0	0, 1, 0, 0, 1, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0

E: erythema extending beyond the area of exposure, 48 and 72 hours after exposure.

S: scaling

Table 6.11.2-02 Mean value irritation scores

Animal	mean 24-72 hrs	
	erythema	oedema
1 (♂)	1	0
2 (♂)	2	0.3
3 (♂)	1	0
4 (♂)	1.7	0
5 (♂)	1.7	0.3
6 (♀)	1	0

Acceptability

The study is considered acceptable.

Conclusions

In this study BAS 300 11 I is considered to be non-irritating to rabbit skin.

STUDY 2**Characteristics**

reference	: Rossbacher, 1992b	exposure	: Single instillation
type of study	: Acute eye irritation study	doses	: About 24 mg (100 µl)
year of execution	: 1991-1992	vehicle	: None
test substance	: BAS 300 11 I; Batch no.: AF-137-50, 75.8% pyridaben	GLP statement	: Yes
route	: Ocular	guideline	: In accordance with OECD 405 (1987)
species	: Rabbit, White Vienna	acceptability	: Acceptable
group size	: 6 females	Effect	: Irreversible structural effects

Study design

The study was performed in accordance with OECD 405 (1987), No washing of the eyes was performed, even though OECD 405 indicates that for some solid substances this may be appropriate.

Results

The results are summarised in tables 6.11.2-03 and 6.11.2-04.

Table 6.11.2-03

Scores observed after	1 hour	24 hours	48 hours	72 hours	8 days	15 days	21 days
Cornea/opacity	0, 1 ^C , 0, 0, 0, 1 ^C	0, 1 ^{P,C} , 0, 0, 0, 1 ^C	0, 1 ^{S,C} , 0, 0, 0, 1 ^{R,S,C}	0, 1 ^C , 0, 0, 0, 1 ^{R,C}	0, 1 ^C , 0, 0, 0, 1 ^{R,C}	0, 2 ^C , 0, 0, 0, 0 ^R	0, 2 ^{V,C} , 0, 0, 0, 0
Iris	0,0,0,0,0,0	0,1,0,0,0,0	0,1,0,0,0,0	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	2,2,2,2,2,2	2,2,2,1,2,2	1,2,1,1,2,2	0,1,0,0,0,2	0,0,0,0,0,1	0,0,0,0,0,0	0,0,0,0,0,0
Conjunctiva chemosis	2,3,2,0,1,2	0,1,1,0,0,2	0,1,0,0,0,1	0,0,0,0,0,1	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0
Conjunctiva discharge	2,1,2,2,1,2	0,1,1,0,0,2	0,1,0,0,0,2	0,0,0,0,0,1	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0

R = small retractions in the eyelids

S = suppuration

P = pupil contracted

V = marginal vascularization of the cornea

C = loss of corneal tissue

Table 6.11.2-04 Mean value irritation scores

Animal	mean 24-72 hrs			
	corneal opacity	Iris	Conjunctiva redness	Conjunctiva chemosis
1	0	0	1	0
2	1	0.7	1.7	0.7
3	0	0	1	0.3
4	0	0	0.7	0
5	0	0	1.3	0
6	1	0	2	1.3

Acceptability

The study is considered acceptable.

Conclusions

Based on the vascularization of the cornea and loss of corneal tissue in one of the animals at day 21, BAS 300 11 I should be labelled with "Risk of serious damage to eyes".

STUDY 3**Characteristics**

reference	: Rossbacher, 1995	exposure	: Single instillation
type of study	: Acute eye irritation study	doses	: About 24 mg (100 µl)
year of execution	: 1994/95	vehicle	: None
test substance	: BAS 300 11 I; Batch no.: AF-137	GLP statement	: Yes
	50, 75.8% pyridaben		
route	: Ocular	guideline	: Not in accordance with OECD 405 (1987)
species	: Rabbit, White New Zealand	acceptability	: Acceptable
group size	: 3 animals/sex	Effect	: Not irritating to eyes

Study design

The study was performed in accordance with OECD 405 (1987). Washing of the eyes was performed 7 hours after application.

Results

The results are summarised in tables 6.2.2-05 and 6.2.2-06.

Table 6.2.2-05

Scores observed after	1 hour	24 hours	48 hours	72 hours	8 days
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	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	0,0,0,0,0,0
Conjunctiva redness	2,1,2,2,2,2	2,2,2,2,2,2	1,1,2,1,1,2	0,0,2,0,0,1	0,0,0,0,0,0
Conjunctiva chemosis	1,1,0,0,1,1	0,1,1,0,1,1	0,0,1,0,0,0	0,0,1,0,0,0	0,0,0,0,0,0
Conjunctiva discharge	2,1,2,1,2,2	1,1,1,0,1,1	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0

Table 6.2.2-06 Mean value irritation scores

Animal	mean 24-72 hrs			
	corneal opacity	Iris	Conjunctiva redness	Conjunctiva chemosis
1	0	0	1	0
2	0	0	1	0.3
3	0	0	2	1
4	0	0	1	0
5	0	0	1	0.3
6	0	0	1.7	0.3

Acceptability

The study is considered acceptable.

Conclusions

In this study BAS 300 11 I is considered to be not irritating to the rabbit eyes, when washed within 7 hours after application.

STUDY 4**Characteristics**

reference	: Rossbacher, 1992c	exposure	: Three topical inductions, topical challenge (occlusive, 6h).
type of study	: Skin sensitisation study (Buehler)	doses	: 50% topical induction (days 0, 7, 14) 25% topical challenge (day 28)
year of execution	: 1992	vehicle	: Aqua bidest water
test substance	: BAS 300 11 I, Batch no. AF-137-50; 75.8% pyridaben	GLP statement	: Yes
route	: Dermal	guideline	: In accordance with OECD 406 (1981)
species	: Guinea-pig, Pirbright White, Dunkin/Hartley HOE DHPK	acceptability	: Acceptable
group size	: 2 x 10 controls (females) 20 test animals (females)	Effect	: Not sensitising

Study design

The study was performed in accordance with OECD 406 (1981) and conducted according to Buehler. 1-Chlor-2,4-dinitro-benzol was used as the positive control.

Dose levels were based on the results of a pre-test. The minimum irritant concentration was found to be 50% and the maximum non-irritant concentration was found to be 25%.

In the main test, 20 animals were topically induced with 50% test compound on days 0, 7 and 14 for 6 hours. The control animals were left untreated. A topical challenge with 25% test substance for 6 hours was carried out 14 days after the third induction. Ten control animals were also challenged, while ten other control animals were left untreated.

Results

After the first induction, 18/20 animals showed very slight to well-defined erythema and 5/20 animals showed very slight oedema. Due to severe irritation (scabbing), the application area for the 2nd induction was moved. After the second induction, all animals showed well-defined erythema and very slight oedema. Due to severe irritation (scabbing), the application area for the 3rd induction was moved to the first position. After the third induction, all animals showed very slight to well-defined erythema mostly with scaling. 17/20 animals showed very slight oedema and one showed well-defined oedema. Control animals showed no erythema and no oedema.

After the challenge with 25% test substance, no erythema and no oedema were noted in any of the treated animals, nor in the control groups.

Sensitisation of this strain of guinea-pigs was positively tested with 1-chlor-2,4-dinitro-benzol.

Acceptability

The study is considered acceptable. A Buehler test is considered less sensitive than a Maximization test or LLNA. However, since pyridaben is not a skin sensitizer in a Maximisation study, the present study with BAS 300 11 I is considered acceptable.

Conclusions

In this study, BAS 300 11 I has been found to be not sensitising to the skin of guinea-pigs.

B.6.11.3 Summary

The results of the acute toxicity, irritation and sensitisation studies are presented in table 6.11.3-01, 6.11.3-02, and 6.11.3-03, respectively.

Table 6.11.3-01 Acute toxicity studies, LD50/LC50 values

Test substance	LD ₅₀ /LC ₅₀	Species	Route	Vehicle	Reference
BAS 300 11 I	1930 mg/kg bw	Rat	Oral	Aqua bidest	Kirsch, 1992a
BAS 300 11 I	> 2000 mg/kg bw	Rat	Dermal	Aqua bidest	Kirsch, 1992b
BAS 300 11 I	± 90 µg/l	Rat	Inhalation	None	Gamer, 1992

Table 6.11.3-02 Skin and eye irritation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
BAS 300 11 I	Not irritating	Rabbit	Dermal	None	Rossbacher, 1992a
BAS 300 11 I	Risk of serious damage to eyes	Rabbit	Ocular	None	Rossbacher, 1992b
BAS 300 11 I	Not irritating	Rabbit	Ocular	None	Rossbacher, 1995

Table 6.11.3-03 Sensitisation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
BAS 300 11 I	Not sensitising	Guinea-pig	Dermal (Buehler)	Aqua bidest	Rossbacher, 1992c

According to the Directive 2001/59/EC, BAS 300 11 I needs to be classified as harmful if swallowed (Xn, R22) and as very toxic after inhalation (T+, R26).

BAS 300 11 I is not irritating to the skin. Irreversible effects on the cornea in one eye irritation study were considered to be due to mechanical effects of the powder, as this was not observed when the eyes were washed 7 hours after instillation in a similar eye irritation test. Since washing is permissible for solids, according to OECD 405, BAS 300 11 I is considered not irritating to the eye. . In a Buehler test BAS 300 11 I is not a skin sensitizer.

B.6.12 Dermal absorption

STUDY 1 Characteristics

reference	: Kemp <i>et al.</i> 2003a	exposure	: 6h (semi-occlusive) 12 cm ² . Absorption was followed after washing at 24 and 144 h after application.
type of study	: <i>In vivo</i> dermal absorption	doses	: 7.5 µg/cm ² and 5 mg/cm ² ^a . Low dose representative for spray dilution of product. High dose representative for exposure during mixing/loading of the product.
year of execution	: 2002-2003	vehicle	: BAS 300 11 I Bland (matrix used for Nexter Pro)
test substance	: Pyridaben NC-129, i.e. 2-tert-butyl-5-(4-tert-butylbenzylthio)-4-chloropyridazin-3(2H)-one. Lot no. 129T950808, chemical purity 99.2%. ¹⁴ C labelled pyridaben, batch no. COL081002/F6/7, radiochemical purity >98%, specific activity 2.69 MBq/mg.	GLP statement	: Yes
route	: Dermal	guideline	: Draft OECD guideline 427
species	: Rat, Sprague-Dawley (CrI:CD®BR), male	acceptability	: acceptable
group size	: 4/dose/sacrifice time	Result	: Low dose: 8.9% High dose: 2.7%

^a the actual achieved doses were 2.4 µg/cm² and 4.71 mg/cm².

Study design

The percutaneous absorption of pyridaben was studied in groups of 4 male rats. In the low dose group, rats received a single topical application of 7.5 µg/cm² (120 µL to an area of 12 cm²). This dose represents the maximal attainable dilution using [¹⁴C]-pyridaben exclusively, and the one in-use rate of the product. The rats

in the high dose group received a single topical application of 5000 µg/cm². This dose is equivalent to the concentration to which the operator is exposed during mixing/loading activity. After dosing, a protective device consisting of a detachable saddle covered with stainless steel metal gauze was taped into place with tape and bandage (semi-occlusive conditions). The animals were exposed under these conditions for 6 hours. During the total observation period, the animals were kept in metabolism cages to allow the collection of urine and faeces.

After 6 hours, the cover was removed and retained for analysis. After shaving if necessary, the treated area was washed with a mild detergent solution. The shavings, razor heads and swabs were retained for analysis. Animals destined to provide 24 and 144 h samples were given a clean gauze cover and bandage and placed back in the metabolism cage. At the end of the collection period, the cages were rinsed with water/acetone (1:1 v/v). Cage wash was kept for analysis.

At the defined time points (6, 24, and 144 h after application, determined on the basis of a preliminary study at both dose levels), 4 animals per dosing group were sacrificed. A terminal blood sample was collected via the orbital sinus. Skin stripping was performed: the stratum corneum was removed at the application area. In addition, the remaining treated skin, a small area of skin surrounding the dose site and untreated skin and the carcass were retained for analysis.

The levels of total radioactivity were determined by (solubilisation) LSC in all samples of blood, urine, faeces, cage wash, skin wash, shavings, razor heads, cotton swabs, tape strips, protective device, dose site, non-treated skin area and carcass.

Results

Total recovery (group means) was 85-103% AR. Results are presented in Tables 6.12-01, 6.12-02 and 6.12-03.

Table 6.12-01 Excretion and retention in rats of total radioactivity after single dermal exposure

Absorption, excretion and distribution are expressed as percentage of the administered radiolabel.

Dose Level	Low dose			High dose		
	2.4 µg a.s./cm ²			4710 µg a.s./cm ²		
Sacrifice time (h)	6	24	144	6	24	144
Urine	0.03	0.2	0.8	0.0	0.01	0.4
Faeces	0.04	0.5	6.7	0.3	0.04	0.7
Cage Wash	0.3	0.1	0.3	0.1	0.2	0.8
Total Excretion	0.4	0.8	7.8	0.4	0.2	1.9
Residues Tissues	1.4	2.8	0.6	1.6	0.5	0.6
Systemic Absorption	1.8	3.6	8.4	2.0	0.7	2.5
Stratum Corneum ¹	23	25	6.6	4.5	5.6	4.9
Remaining treated skin	0.4	1.1	0.5	0.2	0.1	0.2
Scissor wash	n.s.*	0.02	0.05	0.02	0.03	0.04
Application site	23	26	7.2	4.7	5.7	5.1
Skin Wash (Surface dose) ²	15	20	9.5	2.7	6.7	6.1
Skin swabs	48	47	65	74	75	76
Gauze wash	11	6.1	12	1.6	1.7	4.5
Dislodgeable Dose	74	73	87	78	83	87
Total Recovery	99	103	103	85	89	94

¹ excluding the first two tape strips.

² including the first two tape strips.

* n.s. = no sample.

Table 6.12-02 Distribution in stratum corneum

Distribution is expressed as percentage of the administered radiolabel.

Dose Level	Low dose			High dose		
	2.4 µg a.s./cm ²			4710 µg a.s./cm ²		
Sacrifice time (h)	6	24	144	6	24	144
Tape Strip numbers						
1-2	15.3	15.9	5.4	2.7	6.2	3.3
3-5	11.8	16.8	4.3	2.8	4.0	3.3
6-8	8.9	6.5	1.8	1.5	1.1	1.4
9-11	2.1	1.5	0.4	0.2	0.3	0.2
12-14	0.3	-	0.04	-	0.1	-
15-17	-	-	-	-	0.1	-
Total in stratum corneum¹	23	25	6.6	4.5	5.6	4.9

¹ excluding the first two tape strips.

- no sample.

Table 6.12-03 Mean systemically available dose and potentially available dose at different time points

Absorption is expressed as percentage of the administered radiolabel.

Dose Level	Low dose		High dose	
	2.4 µg a.s./cm ²		4710 µg a.s./cm ²	
Time	systemically available ¹	potentially available ²	systemically available ¹	potentially available ²
6 h	1.8	2.5	2.0	6.7
24 h	3.6	30	0.7	6.3
144 h	8.4	16	2.5	7.6

¹ systemically available dose: urine, faeces, blood, cage wash, carcass.

² potentially absorbed dose = systemically available dose + application site

At the end of the exposure period of 6 hours, 1.8% and 2.0% of AR in the low and high dose groups respectively had been systemically absorbed. After the washing procedure, the radioactivity still present in/on the skin amounted to 23% and 4.7% in the low and high dose groups respectively. The amount of radioactivity systemically absorbed increased to 3.6 and 0.7 at 24 hours and to 8.4 and 2.5% AR at 144 h after application in the low and high dose groups, respectively. The majority of the absorbed radioactivity had already been excreted by 144 h, amounting to 7.8 and 1.9% AR, respectively. The amount remaining in the treated skin (without stratum corneum) at 144h amounted to 0.5% and 0.2% AR, respectively. The amount of radioactivity remaining in the tissues by 144 h were 1.1% and 0.8% (residues in tissues and skin without stratum corneum), respectively.

At all time points, the majority of the radioactivity present in the stratum corneum was present in the upper layers of the stratum corneum, indicating that there is little migration of the test substance through the skin. However, after skin wash, both in the low and the high dose groups, systemic absorption increased over time, indicating that following cessation of the exposure, the amount remaining on and retained in the treated skin might become systemically available.

However, for occupational risk assessment purposes dermal absorption after 24 hours should be considered, since a daily acceptable exposure limit is derived. Therefore it is questionable whether or not the stratum corneum should be included in the dermal absorption values for pyridaben. The amount of radioactivity in the stratum corneum was mainly recovered from the upper layers (see table 6.12.1.2). Within time, no increase in radioactivity in the lower layers of the stratum corneum was observed, indicating little migration of the test substance through the skin. Furthermore, for operators no repeated dermal exposure is to be expected, since Nextra Pro is only applied once per season. However, repeated exposure to pyridaben cannot be excluded for workers (re-entry activities like binding and harvesting).

Overall it is concluded that it is not considered suitable to include the stratum corneum in the dermal absorption values for pyridaben, as this would result in extreme worst-case dermal absorption values. However, as a reasonable worst-case approach, one could consider to take for dermal absorption based on the radioactivity recovered in urine, faeces, cage wash, tissue residues, remaining treated skin site and scissor wash. No justification could be given for the low systemic absorption value at the high dose at 24 hours, when compared to the values at 6 and 144 hours. Furthermore, as repeated dermal exposure of workers cannot be excluded and no data on dermal absorption after repeated exposure are available, as a reasonable worst case the systemic absorption values (including %AR in urine, faeces, cage wash, residues in tissues) at 144 hours, including remaining treated skin site and scissor wash are taken as a measure for the dermal absorption of pyridaben, which results in 8.9% and 2.7% for the low and high dose, respectively.

Acceptability

The study was performed in accordance with draft OECD guideline 427. The period of sampling was too short (144 hours). Absorption and excretion were not completed after 144 hours. However, since the potentially available dose could be taken as a measure for the dermal absorption of pyridaben, the study is considered acceptable.

Conclusion

After 144 hours, dermal absorption of pyridaben in rats *in vivo* is 8.9% and 2.7% after a single dermal application of respectively 7.5 µg/cm² (actual 2.4 µg/cm²) and 5 mg/cm² (actual 4.71 mg/cm²).

STUDY 2

Characteristics

reference	: Kemp <i>et al.</i> 2003b	exposure	: 6h, non-occlusive
type of study	: <i>in vitro</i> dermal absorption	doses	: nominally 7.5 µg a.i./cm ² ; representing spray dilution of the product.
year of execution	: 2003	vehicle	: BAS 300 11 I Bland (matrix used for Nexter Pro) and water
test substance	: Pyridaben NC-129, i.e. 2-tert-butyl-5-(4-tert-butylbenzylthio)-4-chloropyridazin-3(2H)-one. Lot no. 129T950808, chemical purity 99.2%. ¹⁴ C labelled pyridaben, batch no. COL081002/F6/7, radiochemical purity >98%, specific activity 2.69 MBq/mg.	GLP statement	: yes
route	: dermal	guideline	: Draft OECD guideline 428
species	: Rat, Sprague-Dawley (CD), 5 males Human, 1 male and 1 female	acceptability	: acceptable
group size	: Rat: 10/dosing group Human:12/dosing group	Result	: Ratio <i>in vitro</i> dermal absorption human/rat: 1:2 for low doses (7.5 µg/cm ²)

Study design

The percutaneous absorption of ¹⁴C-Pyridaben was studied *in vitro*. Split thickness skin membranes from rats and humans were exposed to 7.5 µg a.i. cm⁻². The integrity of the skin membranes was established prior to the application of the test substance, by measurement of the permeation of tritiated water. Skin membranes with a permeability coefficient (K_p) > 3.5·10⁻³ cm·h⁻¹ were considered unsuitable for testing. The application suspension was applied at a rate of 10 µL/cm². The donor chambers were left open. The test substance remained in contact with the skin for 6 hours in a flow-through diffusion cell. Six hours after application, the skin was washed with 1% v/v Tween 80/water until no further radioactivity was removed (confirmed with a Geiger-Müller mini-monitor). Samples of the receptor fluid were taken at 1-hour intervals from the application until the end of the study (24h). The skin membranes were then tape-stripped. Strips 1 and 2 represented the residual surface (non-absorbed) dose. Subsequent tape-strips containing the stratum corneum were analysed separately, as well as the remaining treated skin. The exposure chambers were washed. The receptor fluid, skin washings, skin tape-strips, the remaining treated skin and the exposure chamber washes were analysed using (solubilisation) LSC.

Results

The preparation of the test substance was not homogeneous, probably due to a combination of the very small quantities of active ingredient and blank formulation required for the dose level used, the physico-chemical characteristics of Pyridaben and the low volumes applied to the cells (6.4 µL). The inhomogeneity of the prepared dose is reflected in the total recoveries obtained for the individual cells, ranging from 25-180% for human skin, and 48-195% for rat skin.

However, the mean distributions of radioactivity obtained were similar to those cells which showed recoveries close to 100%. The mean results are given in Table 6.12-04.

After 24h, 12 and 0.5% of AR had penetrated through rat and human skin respectively. The majority, respectively 95% and 92% of AR had not been absorbed after 24h. The test substance has affinity for the

stratum corneum, regarding the fact that 31% and 22% of AR was recovered from the stratum corneum. The amount of material located in the remaining skin sample after tape stripping was 0.3% of AR in human skin and 10% for rat skin.

The total absorbed dose over 24 hours can be considered as the sum of the amount in the remaining skin (absorbable at 24 hours) and that present in the receptor fluid (cumulative amount absorbed by 24 hours). This corresponds to 0.5% and 12% for human and rat skin, respectively. Since pyridaben is a highly lipophilic substance (low solubility in receptor fluid), the amount of radiolabel retained in the skin should be included to estimate dermal absorption.

In the present study skin stripping was performed. The first two skin strippings are considered not absorbed, and based on the conclusions in the in vivo study, the remaining skin strippings, the stratum corneum, should also be considered not absorbed.

Table 6.12-04 Mean recovery of radioactivity for rat and human skin membranes

Absorption is expressed as percentage of the administered radiolabel.

Dose level	Low dose	
	Human skin membrane 2.16 µg/cm ²	Rat skin membrane 1.5 µg/cm ²
Test system		
Receptor fluid		
0-24 h	0.2	2.2
Remaining skin	0.3	10
Absorbed	0.5	12
Stratum Corneum ¹	22	31
Skin surface ²	68	64
Cell wash	3.8	0.9
Recovery	94	108

1 excluding the first two tape strips

2 skin swabs and the first two tape strips

At steady-state conditions, the flux amounted to 0.231 ng·cm⁻²·h⁻¹ for human skin and to 1.663 ng·cm⁻²·h⁻¹ for rat skin. Dermal penetration at different time points could not be presented, since in the study report only cumulative data per hour were presented in figures. However, increasing levels of radiolabel in the receptor fluid from 1 to 24 hours were noted for both rat and human skin. There was some variability between the skin of different rats and between skin cells from the same rat, which simply reflects individual animal and laboratory variation. OECD 428 specifies that the data from a minimum of 4 cells should be reported, but in this study values reported for the rat were the means of 10 separate cells from 5 different rats. Thus, the RMS considers that the reported mean steady state absorption rate data for the rat is valid, and indicates that rat skin is 7.2 fold more permeable than human skin. It should also be considered that the dermal absorption data based on % absorption in this study indicate that rat skin is 25 fold more permeable than human skin.

Acceptability

The study was performed without occlusion, whereas (semi)occlusion is preferred for occupational settings. However, since the study was performed at a constant exposure chamber temperature of 32°C, pyridaben has a low vapour pressure (< 1 · 10⁻⁵ Pa), and since the skin will not have been dehydrated due to the application of an aqueous test substance solution, the study is considered acceptable.

The poor homogeneity of the dosing preparation in combination with the very poor recoveries of AR in individual cells make that this study does not meet the OECD criteria for *in vitro* dermal absorption studies. However, the mean distributions of radioactivity obtained were similar to those cells which showed recoveries close to 100%. In addition, when the cell data were normalised to 100% recovery, individual and mean distributions of radioactivity were very similar to the mean data. Therefore, the results obtained from this *in vitro* study may be considered indicative for risk assessment purposes.

Conclusion

The objective of the study was to determine dermal absorption through rat and human skin *in vitro* and to compare the rate data with the human data, in conjunction with the results of the *in vivo* dermal absorption study in rats. Although increasing levels of radiolabel were found in receptor fluid for 1 to 24 hours, hence a maximum flux was possibly not reached, using the fluxes to compare rat and human data is considered more realistic than using the dermal absorption data based on % absorption from this *in vitro* study. Comparison of rat and human skin data indicate that dermal absorption through rat skin is about 7.2 fold greater than through human skin, for the low dose level (spray dilution). For the high dose level (concentration at mixing and loading), no data were available.

Overall conclusion dermal absorption

The dermal absorption of Pyridaben in rats *in vivo* was 8.9% and 2.7% after a single dermal application of respectively 7.5 µg/cm² (actual 2.4 µg/cm²) and 5 mg/cm² (actual 4.71 mg/cm²). Comparison of rat and human skin data indicate that dermal absorption through rat skin is about 7.2 fold greater than through human skin, for the low dose level (spray dilution). For the high dose level (concentration at mixing and loading), no data were available.

Considering the results from the *in vivo* and *in vitro* dermal absorption studies, for occupational risk assessment purposes, a dermal absorption of 1.2% should be considered for test substance concentration of approximately 1.5-7.5 µg/cm² (e.g. spray dilution of BAS 300 11 I (i.e. Nexter Pro)) and 0.4% for test substance concentrations of approximately 5 mg/cm² (e.g. concentrated formulation during mixing and loading).

B.6.13 Toxicological data on non active substances

MSDSes for the formulants of NEXTER PRO were submitted by the notifier. From the provided MSDSes no conclusion could be drawn on the toxicological data on the formulants. The MSDSes were not in accordance with 91/155/EEC.

However, the acute endpoints, including skin and eye irritation and skin sensitisation are covered in B.6.11. Other endpoints may be reviewable after submission of MSDSes in accordance with 91/155/EEC.

B.6.14 Exposure data

Nexter Pro is used as an insecticide in citrus and tomato, and contains 750 g pyridaben/kg product. In citrus, Nexter Pro is applied outdoors, by mechanical upward spraying (mist blower, motor pump spray gun or high pressure rotary sprayer). In tomato, Nexter Pro is applied indoors, by manual up- and downward spraying.

B.6.14.1 Exposure studies

Several studies on dislodgeable foliar residues (DFR) are submitted by the notifier. These studies were summarized in Appendix 3.

B.6.14.2 Exposure Calculations

Mechanical upward spraying on citrus (outdoor), operator exposure

Internal operator exposure values without and with personal protective equipment (PPE) were calculated using the UK and the German model. For risk assessment purposes, the 75th percentile of the UK-model was used (UK-75th) and the geometric mean of the German model (DE-GM). These calculations are presented in appendix 3.

Manual up- and downward spraying on tomatoes (indoor), operator exposure

Both the UK and the German model have no data available for indoor applications. Therefore, the Dutch Glasshouse model was used. The Dutch model for manual up and downward spraying indoors is based on studies with ornamentals published in the scientific literature and on studies carried out in The Netherlands. Indicative 90th percentiles are deduced from the various exposure databases. These calculations are presented in appendix 3.

Bystander exposure

As no bystanders should be allowed in greenhouses during the application of Nexter Pro, therefore, no estimation of bystander exposure for manual up- and downward spraying in greenhouses will be made (tomato). For Nexter Pro, bystander exposure should be considered for application in citrus. For bystander exposure during mechanical upward spraying, no formally approved models exist. As an estimate, the draft values proposed for the EUROPOEM II, 2002 model were used. These values represent the 90th percentile exposure values for bystanders. Since bystanding should as much as possible be prevented and will usually occur incidentally, it cannot be assumed that bystanders will be using any kind of personal protective equipment, therefore the use of this equipment is not considered in bystander risk assessment.

Calculations for bystander exposure are presented in appendix 3.

Worker exposure

For the use of Nexter Pro, re-entry activities should be considered for harvesting and sorting of citrus and tomatoes. Exposure during re-entry activities in tomato is estimated using the Dutch model and for citrus by dislodgable foliar residue calculations (Popendorf and Leffingwell (1982); Popendorf (1985, 1992); Van Hemmen *et al.* (1995); EUROPOEM II). For dislodgable foliar residue calculation, data from DFR studies were used.

The calculations of worker exposure are presented in appendix 3.

Based on the *in vivo* and *in vitro* dermal absorption data described in section B.6.12., a dermal absorption of pyridaben in humans was estimated to be 1.2% for the diluted formulation and 0.4% for the undiluted formulation. For inhalation exposure a default value of 100% was used.

B.6.14.3 Internal exposure and risk assessment

Internal exposures and risk-indices are specified in Table 6.14.3-01, 6.14.3-02 and 6.14.3-03.

Table 6.14.3-01 Operator internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL-systemic (mg a.s./day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Mechanical upward spraying on citrus						
UK- 75 th	Respiratory	0.12	0.12	0.12	50	50
	Dermal	0.58	0.41	0.12	242	167
	Total exposure	0.70	0.53	0.12	292	217
DE- GM	Respiratory	0.06	0.06	0.14	45	45
	Dermal	0.35	0.03	0.14	250	22
	Total exposure	0.41	0.09	0.14	295	67
Manual up- and downward spraying on tomato (indoor)						
NL-glasshouse	Respiratory	0.03	0.003	0.14	18	2
	Dermal	0.06	0.006	0.14	43	4
	Total exposure	0.09	0.009	0.14	61	6

Table 6.14.3-02 Bystander internal exposure and risk assessment

Route	Estimated internal exposure (mg a.s./day)	AOEL systemic (mg a.s./day)	% AOEL
<i>Exposure during mechanical upward spraying on citrus</i>			
Respiratory	0.015	0.14	11
Dermal	0.036	0.14	26
Total exposure	0.051	0.14	36
<i>Exposure during manual up- and downward spraying on tomato</i>			
should be prohibited			

Table 6.14.3-03 Worker internal exposure and risk assessment

Route	Estimated internal exposure		AOEL	% AOEL	
	(mg a.s./day)		Systemic		
	without PPE	with PPE	(mg a.s./day)	without PPE	with PPE
Mechanical upward spraying on citrus					
Respiratory	-	-	0.14	-	-
Dermal	0.042	0.004	0.14	30	3
Total exposure	0.042	0.004	0.14	30	3
Manual up- and downward spraying on tomato (indoor)					
Respiratory	0.0825	0.0083	0.14	59	6
Dermal	0.225	0.023	0.14	161	16
Total exposure	0.308	0.031	0.14	220	22

Conclusions

- For mechanical upward spraying on citrus, it is concluded that a safe use was identified for operators with PPE for mechanical upward spraying on citrus, using the German model.
- For manual up- and downward spraying on tomato (indoor), it is concluded that a safe use was identified for operators without PPE for manual up- and downward spraying on tomato, using the Dutch Glasshouse model.
- For bystander exposure during mechanical upward spraying on citrus, it is concluded that a safe use was identified for bystanders for mechanical upward spraying on citrus, using the EUROPOEM II model (90th percentile).
- No bystanders should be allowed in greenhouses during the application of Nexter Pro on tomatoes.

- For worker exposure during re-entry activities in citrus it is concluded that a safe use was identified for workers without PPE during re-entry activities in citrus, using the EUROPOEM II.
- For worker exposure during re-entry activities in tomato it is concluded that a safe use was identified for workers with PPE during re-entry activities in tomato, using the EUROPOEM II.

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B.6.15 References relied on

References for the active substance

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
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IIA, 5.1/03	Rhenius, S. T.	1989b	NC-129: Absorption, distribution and excretion in the rat: Single oral dose, 30 mg/kg (+ amendments no. 1 and 2) [REDACTED] Nissan Chemical Industries, Ltd. Report no. M2b GLP, Unpublished	Y	NCI
IIA, 5.1/04	Hallifax, D.	1994b	NC-129: Metabolic transformation in the rat: single oral dose, 30 mg/kg [REDACTED] Nissan Chemical Industries, Ltd. Report no. M110 GLP, Unpublished	Y	NCI

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
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IIA, 5.2.1/02	Kawakami, Y., Sunaga, M.	1990b	Acute toxicity study by oral administration of NC-129 in mice [REDACTED] Nissan Chemical Industries, Ltd. Report no. T1b GLP, Unpublished	Y	NCI
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IIA, 5.2.1/04	Rees, P. B.	1994	NC-129: Acute oral toxicity study in the rat [REDACTED] Nissan Chemical Industries, Ltd. Report no. T13 GLP, Unpublished	Y	NCI

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IIA, 5.2.1/06	Hayashi, K.	1989b	NC-129: Acute oral toxicity study in female mice [REDACTED] Report no. T2bf GLP, Unpublished	Y	NCI
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IIA, 5.2.2/02	Cummins, H. A.	1987	NC-129: Acute percutaneous toxicity study in the rabbit [REDACTED] Nissan Chemical Industries, Ltd. Report no. T4 GLP, Unpublished	Y	NCI
IIA, 5.2.3	Iwasaki, M.	1987	NC-129: Acute inhalation toxicity study in rats [REDACTED] Nissan Chemical Industries, Ltd. Report no. T6 GLP, Unpublished	Y	NCI
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IIA, 5.2.5	Cummins, H. A.	1986b	NC-129: Acute eye irritation/corrosion test in the rabbit [REDACTED] Nissan Chemical Industries, Ltd. Report no. T8 GLP, Unpublished	Y	NCI
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IIA, 5.3.2/01	Holmes, P.	1988a	NC-129: Toxicity study by dietary administration to CD rats for 13 weeks followed by a 4 week reversibility period (+ amendments no.1 and 2) [REDACTED] Nissan Chemical Industries, Ltd. Report no. T22 GLP, Unpublished	Y	NCI
IIA, 5.3.2/02	Holmes, P.	1988b	NC-129: Toxicity study by dietary administration to CD-1 mice for 13 weeks (+ amendments no.1 and 2) [REDACTED] Nissan Chemical Industries, Ltd. Report no. T24 GLP, Unpublished	Y	NCI
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