NOVALURON

First draft prepared by
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Explanation

Novaluron is the provisionally approved International Organization for Standardization (ISO) common name for (±)-1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea, a racemic compound. Novaluron is an insecticide of the benzoylphenyl urea class that inhibits chitin synthesis, affecting the moulting stages of insect development. It acts by ingestion and contact, and causes abnormal endocuticular deposition and abortive moulting. Novaluron has not been evaluated previously by the JMPR.

For technical-grade novaluron, the FAO specification was established by the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS) and published as FAO Specification 672/TC (December 2004).

All pivotal studies with novaluron were certified to be compliant with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

The absorption, distribution, metabolism and excretion of novaluron has been investigated in Sprague-Dawley CD rats treated orally with [chlorophenyl-^{14}C(U)]-labelled or [difluorophenyl-^{14}C(U)]-labelled novaluron (Figure 1).

1.1 Absorption, distribution and excretion

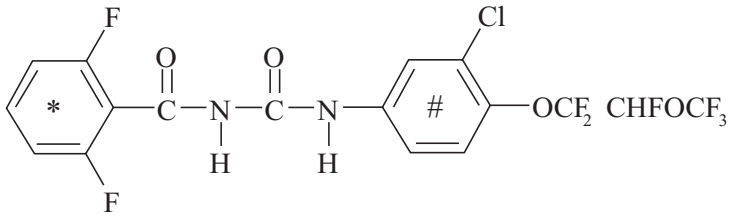
In a preliminary study of absorption, distribution and excretion, groups of two male and two female Sprague-Dawley CD rats received [chlorophenyl-^{14}C(U)]novaluron and [difluorophenyl-^{14}C(U)]novaluron (purity of unlabelled test substance, 99.3%; radiochemical purity, > 98% or > 99%, respectively; see Figure 1) orally by gavage as a single dose at 5 mg/kg bw. The animals were housed individually in metabolism cages from which urine and faeces were collected at 24 h intervals until 120 h, and expired air was collected until 48 h. Additional groups of two male and two female rats each were used for the kinetic studies, with blood samples taken from the tail vein at 0.5, 1, 2, 5, 8 and 24 h after dosing. Radioactivity in collected samples was determined by liquid scintillation counting.

After a single low oral dose of either [chlorophenyl-^{14}C (U)]novaluron or [difluoropheny-^{14}C (U)]novaluron at 5 mg/kg bw in male and female rats, most of the dose (> 80%) was excreted in the faeces within 48 h after dosing; urinary excretion was a minor route (Table 1).

Relatively low concentrations of radioactivity were detected in the plasma of rats dosed with novaluron radiolabelled at either position (mean C_{max}, < 0.60 µg equivalents/g). The observed T_{max} in plasma from rats dosed with [chlorophenyl-^{14}C (U)]novaluron or [difluoropheny-^{14}C (U)]novaluron was 5 h and 8 h, respectively; however, the variation within a group for the plasma samples collected at 5 h and 8 h was generally greater than the difference between the mean values for these time-points (Table 2).

Overall, the results indicate poor absorption of radioactivity (up to about 6% or 12%, respectively) after a single oral low dose of either [chlorophenyl-^{14}C (U)]novaluron or [difluoropheny-^{14}C (U)]novaluron in male and female rats (Bounds, 1998).

Figure 1. Positions of radiolabel on novaluron



* Position of radiolabel in [difluorophenyl-14C(U)] novaluron

\# Position of radiolabel in [chlorophenyl-14C(U)] novaluron

In a study of absorption, distribution, excretion and biotransformation, groups of four male and four female Sprague-Dawley CD rats received [chlorophenyl-^{14}C(U)]- or [difluorophenyl-^{14}C(U)]novaluron (Figure 1; purity of unlabelled test substance, 99.3%; radiochemical purity, > 98% or > 99%, respectively) orally by gavage according to the following dosing regimen: a single low dose (2 mg/kg bw), a single high dose (1000 mg/kg bw) or 14 consecutive low doses (2 mg/kg bw) of chlorophenyl-labelled novaluron, and a single low dose (2 mg/kg bw) of difluorophenyl-labelled novaluron. Animals of these groups were used for investigations of excretion balance, plasma and blood kinetics and tissue distribution. Additional groups of four male and four female rats that received a single low dose (2 mg/kg bw) or a single high dose (1000 mg/kg bw) of chlorophenyl-labelled novaluron and a single low dose (2 mg/kg bw) of difluorophenyl-labelled novaluron were used for studies of biliary excretion. Whole-body autoradiography was performed for additional groups of four male and four female rats that received a single low dose (2 mg/kg bw) of either chlorophenyl- or difluorophenyl-labelled novaluron. The animals for studies of excretion balance were housed individually in metabolism cages, while those used for blood and tissue studies were maintained in standard cages. Urine and faeces were collected at 24 h intervals up to 168 h for intact animals and up to 48 h for bile-duct cannulated animals, with bile samples being taken at 3, 6, 12, 24 and 48 h after dosing. Blood samples were taken from the tail vein at 0, 0.5, 1, 2, 5, 8, 24, 48, 72, 96, 120 and 168 h after dosing. At termination, selected organs and tissues were removed. Radioactivity in collected samples was determined by liquid scintillation counting.

Table 1. Recovery of radioactivity (percentage of administered dose at 120 h) from rats given radiolabelled novaluron as a single oral dose at 5 mg/kg bw by gavage

Medium	[Chlorphenyl-^{14}C(U)]novaluron		[Difluorophenyl-^{14}C(U)]novaluron	
	Males	Females	Males	Females
Urine	2.24	2.86	8.34	6.38
Cage wash	0.11	0.16	0.53	0.57
Faeces	92.5	91.5	86.1	87.5
Expired air	ND	ND	ND	ND
Carcass	1.99	2.11	2.90	1.35
Skin	0.85	0.70	0.98	0.64
Total recovery	97.7	97.3	98.8	96.5

From Bounds (1998)

ND, not detected (results within the background range)

Table 2. Mean concentration of radioactivity (ppm) in plasma samples taken from rats given radiolabelled novaluron as a single oral dose at 5 mg/kg bw by gavage

Time-point (h)	[Chlorphenyl-^{14}C(U)]novaluron		[Difluorophenyl-^{14}C(U)]novaluron	
	Males	Females	Males	Females
0.5	ND	ND	ND	ND
1	ND	ND	ND	0.028
2	0.026	0.022	0.032	0.039
5	0.044	0.040	0.051	0.046
8	0.042	0.030	0.056	0.046
24	0.027	ND	0.011	0.018

From Bounds (1998)

ND, not detected (results within the background range)

After oral administration, [chlorophenyl-^{14}C (U)]novaluron was poorly absorbed (about 6–7% of the administered dose) after a single low dose (2 mg/kg bw) and about 10-fold less after a single high dose (1000 mg/kg bw). After a single low dose (2 mg/kg bw) of [difluoropheny-^{14}C (U)]novaluron, absorption was approximately 20%; however, this value may be an overestimate owing to cleavage of novaluron in the gastrointestinal tract before absorption.

Whole-blood and plasma analyses indicate that the rate and extent of systemic exposure of rats to novaluron, as measured by C_{max} and $AUC_{168\ h}$ was greater at 1000 mg/kg bw than at 2 mg/kg bw; however, while the dose increased 500-fold, the increase in C_{max} and $AUC_{168\ h}$ was much less (Tables 3 and 4). This is consistent with the lower percentage absorption seen at 1000 mg/kg than at 2 mg/kg bw. Comparison of $AUC_{168\ h}$ values for blood and plasma indicated accumulation of [chlorophenyl-^{14}C (U)]novaluron into erythrocytes after single or repeated lower doses.

Whole-body autoradiography demonstrated that mean concentrations of radioactivity were greatest in the liver, kidneys, fat, adrenals, pancreas and mesenteric lymph nodes. The lowest mean concentrations were detected in brain, testes, thymus, eyes, bone, bone marrow, muscle, blood and plasma. Retention in tissues and carcass 7 days after dosing was low (< 1.6% after a single dose, and < 4.8% after multiple dosing) (Table 5). In selected tissues sampled at 168 h after dosing, the distribution of radioactivity was similar in males and females, but the concentrations in tissues from females were slightly greater than those from males (Table 6). The concentrations of radioactivity in tissues after administration of the higher dose were 50- to 100-fold greater than those from animals given the lower dose (compared with a 500-fold increase in dose). Modest accumulation in these tissues was evident from tissue concentrations of radioactivity in animals receiving 14 repeated daily doses, with concentrations being three- to fivefold greater than those in animals receiving a single dose. The terminal half-life for the decline of radioactivity in fat after the final repeat dose was 52 h and 56 h for male and female rats respectively.

Table 3. Concentrations of radioactivity in blood (ppm) from rats given radiolabelled novaluron by gavage

Time-point (h)	[Chlorphenyl-^{14}C(U)]-novaluron						[Difluorophenyl-^{14}C(U)]-novaluron	
	2 mg/kg bw, single dose		1000 mg/kg bw, single dose		2 mg/kg bw, repeated dose		2 mg/kg bw, single dose	
	Males	Females	Males	Females	Males	Females	Males	Females
0	ND	ND	ND	ND	0.07	0.10	ND	ND
0.5	ND	ND	0.60	0.88	0.07	0.07	ND	ND
1	ND	ND	1.03	1.35	0.08	0.09	0.00	0.02
2	0.02	0.01	1.96	1.06	0.07	0.10	0.02	0.03
5	0.03	0.03	1.89	1.58	0.08	0.09	0.04	0.04
8	0.03	0.03	1.70	ND	0.08	0.10	0.04	0.05
24	0.02	0.03	ND	0.48	0.07	0.10	0.01	0.01
48	0.01	0.02	ND	ND	0.06	0.07	ND	ND
72	ND	0.01	0.73	ND	0.06	0.07	ND	0.00
96	ND	0.01	ND	0.43	0.05	0.07	ND	ND
120	0.00	ND	ND	ND	0.05	0.05	ND	ND
168	ND	ND	ND	ND	0.04	0.04	ND	ND
AUC 168 h	1.08	1.98	26.8	8.31	9.52	11.26	0.85	0.88

From O'Connor (2000)

AUC, area under the curve; ND, not detected (results within the background range).

The major route of elimination of radioactivity after oral dosing with either chlorophenyl- or difluorophenyl-labelled novaluron was by excretion in the faeces (Table 5). After single or repeated low or single high doses of [chlorophenyl-^{14}C(U)]novaluron, excretion via faeces over 7 days accounted for approximately 86–95% of the administered dose, while excretion via urine (including cage wash) was about 5–9% after low doses and about 10-fold less after a higher dose. After a single low dose of [difluorophenyl-^{14}C(U)]novaluron, excretion via faeces over 7 days was

Table 4. Concentrations of radioactivity in plasma (ppm) from rats given radiolabelled novaluron by gavage

Time-point (h)	[Chlorphenyl-^{14}C(U)]novaluron						[Difluorophenyl-^{14}C(U)]novaluron	
	2 mg/kg bw, single dose		1000 mg/kg bw, single dose		2 mg/kg bw, repeated dose		2 mg/kg bw, single dose	
	Males	Females	Males	Females	Males	Females	Males	Females
0	ND	ND	ND	ND	0.03	0.03	ND	ND
0.5	ND	ND	1.07	1.67	0.03	0.02	ND	ND
1	ND	0.00	2.01	1.74	0.04	0.03	0.00	0.01
2	0.02	0.02	3.01	1.86	0.04	0.04	0.02	0.03
5	0.04	0.03	2.81	1.86	0.05	0.03	0.04	0.04
8	0.04	0.03	2.64	1.54	005	0.03	0.04	0.05
24	0.02	0.01	1.42	1.27	0.03	0.03	0.01	0.01
48	ND	ND	ND	ND	0.03	0.02	ND	ND
72	ND	ND	1.22	ND	0.02	0.02	ND	0.00
96	ND	ND	ND	0.61	0.01	0.01	ND	ND
120	ND	ND	ND	ND	0.01	0.01	ND	ND
168	ND	ND	ND	ND	0.01	ND	ND	ND
AUC 168 h	0.08	0.58	69.97	51.43	3.73	2.78	0.81	0.92

From O'Connor (2000)

AUC, area under the curve; ND, not detected (results within the background range).

Table 5. Recovery of radioactivity (percentage of administered dose) at 168 h in excreta and tissues from rats given radiolabelled novaluron by gavage

Medium	[Chlorphenyl-^{14}C(U)]novaluron						[Difluorophenyl-^{14}C(U)]novaluron	
	2 mg/kg bw, single dose		1000 mg/kg bw, single dose		2 mg/kg bw, repeated dose		2 mg/kg bw, single dose	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine	4.75	4.62	0.56	0.49	5.73	8.49	18.0	15.7
Cage wash	0.37	0.46	0.03	0.06	0.63	0.86	1.92	1.79
Faeces	94.3	95.3	93.8	95.4	90.2	85.9	76.0	79.3
Tissues	1.0	1.4	0.1	0.1	3.1	4.3	0.7	0.9
Gastrointestinal tract	0.1	0.2	< 0.1	< 0.1	0.3	0.5	0.1	0.1
Total recovery	101	102	94.4	95.9	100	100	96.7	97.8

From O'Connor (2000)

about 76–79%, while excretion via urine (including cage wash) accounted for about 18–20% of the administered dose. The proportion of dose excreted in urine was considerably greater and more rapid than seen for the animals dosed with [chlorophenyl-^{14}C(U)]novaluron at the same level. This was due to differences in the metabolic fate of the difluorophenyl and chlorophenyl moieties after cleavage of the urea bridge, which is a known phenomenon for this type of molecule (Koerts et al., 1997). Consequently, the occurrence of any cleavage of novaluron in the

Table 6. Mean tissue concentration of radioactivity (ppm) at 168 h for rats given a single dose or repeated doses of radiolabelled novaluron by gavage

Tissue	[Chlorphenyl-14C(U)]novaluron						[Difluorophenyl-14C(U)]novaluron	
	2 mg/kg bw, single dose		1000 mg/kg bw, single dose		2 mg/kg bw, repeated dose		2 mg/kg bw, single dose	
	Males	Females	Males	Females	Males	Females	Males	Females
Liver	0.08	0.10	4.76	4.82	0.23	0.29	0.03	0.03
Kidneys	0.05	0.06	2.49	2.75	0.14	0.16	0.01	0.02
Spleen	0.00	0.01	ND	ND	0.04	0.04	ND	ND
Pancreas	0.03	0.04	1.7	3.60	0.12	0.14	0.02	0.03
Lung	0.02	0.03	0.76	0.99	0.08	0.13	0.00	0.01
Brain	ND	0.00	ND	ND	0.02	0.03	ND	0.00
Heart	0.01	0.03	ND	0.88	0.06	0.11	0.00	0.01
Thymus	ND	0.01	ND	ND	0.04	0.06	ND	0.02
Submandibular glands	ND	ND	ND	1.27	0.04	0.07	0.00	0.02
Epididymis	0.06	0.02	5.40	ND	0.18	0.12	0.09	0.05
Testes	0.01	0.09	ND	7.17	0.03	0.34	0.00	0.08
Adrenals	0.05	0.13	1.21	8.13	0.24	0.38	0.05	0.05
Eye	0.00	0.00	ND	ND	0.01	0.02	0.00	0.00
Lymph nodes (mesenteric)	0.05	0.07	4.28	6.58	0.24	0.22	0.04	0.07
Thyroid	ND	0.01	ND	ND	0.07	0.12	ND	ND
Bone marrow	ND	ND	ND	ND	0.01	0.01	ND	ND
Bone (femur)	0.11	0.02	ND	ND	ND	ND	ND	ND
Sternum	0.01	0.02	0.13	0.51	0.04	0.10	0.01	0.02
Muscle	0.00	0.00	ND	ND	0.02	0.03	0.00	0.01
Fat (mesenteric)	0.12	0.22	9.92	21.4	0.41	0.70	0.08	0.18
Fat (perirenal)	0.17	0.32	13.3	28.4	0.65	0.84	0.10	0.19
Fat (subcutaneous)	0.11	0.19	10.7	18.1	0.36	0.47	0.12	0.19
Skin	0.02	0.03	0.79	2.36	0.05	0.10	0.02	0.02
Carcass	0.02	0.02	ND	ND	0.06	0.09	0.01	0.02
Gastrointestinal tract & contents	0.02	0.03	1.51	1.63	0.07	0.10	0.01	0.02
Blood	0.00	0.01	ND	ND	0.03	0.05	ND	ND
Plasma	ND	ND	ND	ND	ND	0.00	ND	ND

From O'Connor (2000)

ND, not detected (results within the background range)

gastrointestinal tract before absorption would mean that the absorption value of 20% after dosing with [difluorophenyl-^{14}C(U)]novaluron may be an overestimate.

After a single low dose of either chlorophenyl- or difluorophenyl-labelled novaluron to bile-duct cannulated rats, the total recovery of radioactivity in urine and bile (Table 7) was approximately half that detected during 48 h in urine from intact animals given a similar dose of novaluron with the same label. This indicated that surgical alteration may have had an effect on absorption and excretion of radioactivity. As these animals were sacrificed at 48 h after dosing, the excretion of radioactivity, including unabsorbed dose, was incomplete in most animals. Consequently, the proportion of the administered dose that was recovered in the carcass (including the gastrointestinal tract and contents) provided data to complete the mass balance for these animals, but did not provide data on the proportion of dose absorbed nor the concentration in the tissues (O'Connor, 2000).

1.2 Biotransformation

In a study of absorption, distribution, excretion and biotransformation, groups of four male and four female Sprague-Dawley CD rats received [chlorophenyl-^{14}C(U)]- or [difluorophenyl-^{14}C(U)]novaluron (Figure 1; purity of unlabelled test substance, 99.3%; radiochemical purity, > 98% or > 99%, respectively) orally by gavage according to the study design described in detail above for the toxicokinetic part of the study (O'Connor, 2000). For the identification and quantification of parent compound and metabolites in urine, faeces, bile, tissues and organ samples, chromatographic (thin-layer chromatography, high-performance liquid chromatography) and spectroscopic (mass spectroscopy) techniques were used.

Absorbed novaluron was extensively metabolized, and up to 14 or 15 components were detected in the urine and bile, respectively. The main metabolic pathway was cleavage of the urea bridge between the chlorophenyl and difluorophenyl moieties. After a low dose of [difluoropheny-^{14}C(U)]novaluron, the major metabolite excreted in the urine was 2,6-difluorobenzoic acid (up to 12% of the administered dose), while after a low dose of [chlorophenyl-^{14}C(U)]novaluron, single metabolites accounted for ≤ 1% of the dose, with traces (0.7%) of the metabolite 3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) aniline ("chlorophenyl aniline"). Most of the faecal radioactivity (> 86% or > 72% of a single or repeated

Table 7. Recovery of radioactivity in excreta and tissues (percentage of administered dose) at 48 h from bile-cannulated rats given radiolabelled novaluron by gavage

Medium	[Chlorphenyl-^{14}C(U)]novaluron				[Difluorophenyl-^{14}C(U)]novaluron	
	2 mg/kg bw, single dose		1000 mg/kg bw, single dose		2 mg/kg bw, single dose	
	Males	Females	Males	Females	Males	Females
Urine	1.04	0.45	0.03	0.03	2.76	3.99
Cage wash	0.24	0.92	0.02	0.00	1.96	0.71
Faeces	75.9	68.6	72.3	95.4	75.1	89.6
Bile	0.92	0.93	0.08	0.08	0.44	0.97
Skin	0.48	0.45	ND	ND	0.94	0.88
Carcass [a]	13.8	27.0	25.3	2.51	12.1	5.78
Total recovery	92.3	98.3	97.7	98.1	93.3	102

From O'Connor (2000)

ND, not detected (results within the background range).

[a] Including contents of the gastrointestinal tract

low dose, respectively) consisted of unchanged novaluron, with few metabolites detected in later samples, all of which were ≤ 1.2% of the administered dose. The parent material was also the major component present in extracts from fat, liver and kidneys, with low concentrations of the chlorophenyl aniline and urea derivatives of novaluron also present in the liver and kidneys (O'Connor, 2000).

The proposed metabolic pathway is shown in Figure 2.

2. Toxicological studies

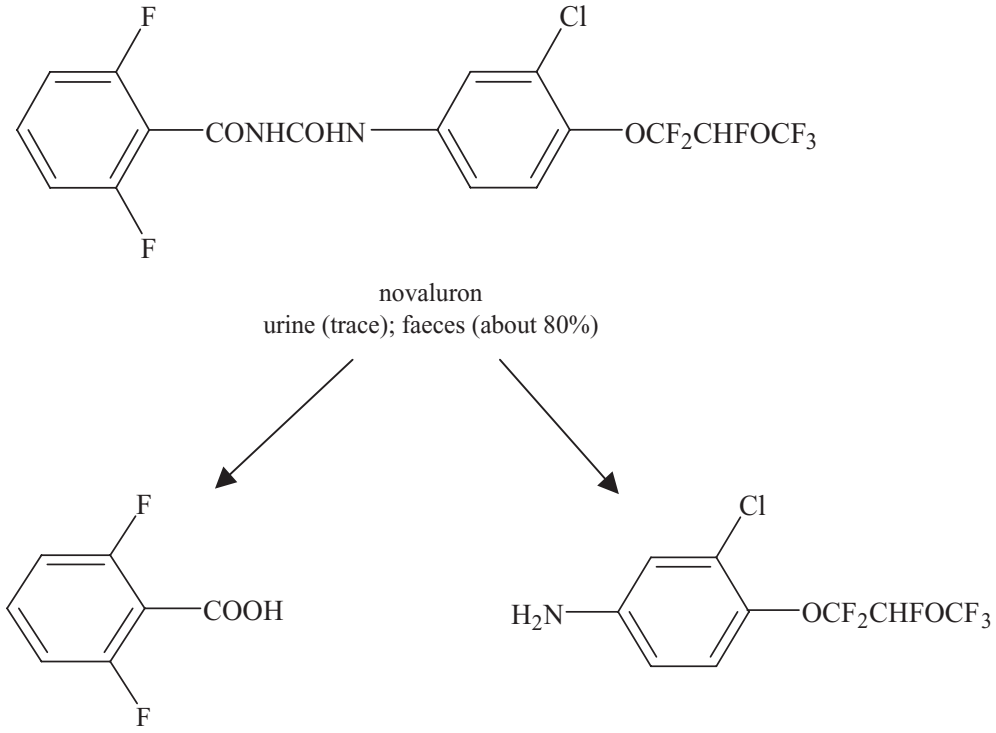
The results of studies of acute toxicity of novaluron administered orally, dermally or by inhalation are summarized in Table 8.

2.1 Acute toxicity

(a) Lethal doses

In a test for acute oral toxicity, five male and five female mice (strain Hsd/Ola:ICR) were given novaluron (purity, 99.3%) at a limit dose of 5000 mg/kg bw (20 ml/kg) by gavage in distilled water. The study was certified to comply with GLP and was conducted in accordance with United States Environmental Protection Agency (USEPA) guidelines, and the Meeting considered that it satisfied all the essential criteria of Organisation for Economic Co-operation and Development (OECD) guideline 401. There were no mortalities or clinical signs of toxicity.

Figure 2. Proposed metabolic pathway of novaluron



2,6-difluorobenzoic acid
(275-158-I)
urine

3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxy ethoxy) aniline
(275-309-I)
urine (trace); faeces (trace)

Table 8. Acute toxicity of novaluron

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw) LC_{50} (mg/l)	Reference
Mouse	ICR	Male & female	Oral	99.3	> 5000	Stocker (1998b)
Rat	Sprague-Dawley	Male & female	Oral	Not specified	> 5000	Cuthbert & D'Arcy-Burt (1986)
Rat	Sprague-Dawley	Male & female	Oral	99.3	> 5000	Stocker (1998a)
Rat	CFY	Male & female	Dermal	94.3	> 2000	Liggett (1988a)
Rat	Sprague-Dawley	Male & female	Inhalation	Not specified	> 5.15	Robinson (1992)

All animals gained in body weight during the study, except for three mice that did not gain in body weight by day 15. Gross examination at necropsy revealed no abnormalities in organs and tissues. The acute oral median lethal dose (LD_{50}) for novaluron in male and female mice was > 5000 mg/kg bw (Stocker, 1998b).

In a test for acute oral toxicity, five male and five female Sprague-Dawley rats were given novaluron (purity not specified) at a limit dose of 5000 mg/kg bw (10 ml/kg) by gavage in 0.5% carboxymethylcellulose and Tween 80. The study was certified to comply with GLP, was conducted in accordance with USEPA FIFRA guideline 81-1, and satisfied the essential requirements of OECD guideline 401. No mortalities were observed during observation for 14 days. No treatment-related clinical findings or effects on body weight were noted in any of the animals. No abnormal findings were observed in any of the animals at necropsy. The acute oral LD_{50} for novaluron in male and female rats was > 5000 mg/kg bw (Cuthbert & D'Arcy-Burt, 1986).

In a subsequent study of acute oral toxicity, five male and five female Sprague-Dawley rats were each given novaluron (purity, 99.3%) at a limit dose of 5000 mg/kg bw (20 ml/kg bw) by gavage in distilled water. The study was conducted according to GLP and satisfied the essential requirements of OECD guideline 401. No mortalities were observed during the 14-day observation period. Clinical signs of reaction to treatment were confined to piloerection and hunched posture, seen in all rats on day 1 of the study. Recovery was complete in affected animals within 5 h after dosing. No effects on body weight were noted in any of the animals. No abnormal findings were observed in any of the animals at necropsy. The acute oral LD_{50} for novaluron in male and female rats was again > 5000 mg/kg bw (Stocker, 1998a).

In a test for acute dermal toxicity, five male and five female CFY rats received novaluron (purity, 94.3%) as single dose at 2000 mg/kg bw (4 ml/kg) in distilled water (50% w/v) administered under an occlusive dressing to the clipped dorso–lumbar skin of animals for 24 h. The study was certified to comply with GLP and satisfied the essential criteria of OECD guideline 402. There were no mortalities or other treatment-related abnormality. The acute dermal LD_{50} for novaluron in male and female rats was > 2000 mg/kg bw (Liggett, 1988a).

A group of five male and five female Sprague-Dawley rats was exposed (nose-only) for 4 h to a dust aerosol of novaluron (purity, not specified) at a gravimetric (nominal) concentration of 5.15 mg/l ± 0.19 (32.8 mg/l). The study was designed in accordance with OECD 403 and USEPA guidelines and was certified to comply with GLP. There were no mortalities during the study. Clinical signs in both sexes included slow, laboured breathing during exposure and all animals

appeared unkempt immediately after dosing, with red staining on the nose in two females. However, these observations are typical after "nose-only" inhalation dosing procedures. All animals appeared normal 1–2 h after dosing, and throughout the 14-day observation period. Necropsy revealed dark patches on the kidneys of one male and two female animals. In addition, white fatty deposits were observed on the spleen of one male animal. The relative lung weights were considered to be within normal limits. The acute inhalation median lethal concentration (LC_{50}) for novaluron in male and female rats was > 5.15 mg/l (Robinson, 1992).

(b) Dermal and ocular irritation and dermal sensitization

The results of studies of irritation and skin sensitization potential with novaluron are summarized in Table 9.

The skin irritation potential of novaluron (purity, 94.3%) was investigated in one male and five female New Zealand White rabbits. The study was conducted in accordance with OECD 404 and was certified to comply with GLP. About 0.5 g of the test material moistened with 0.5 ml of distilled water was applied topically to the clipped dorso–lumbar skin under a semi-occlusive dressing. No skin irritation or dermal reaction was observed from 30 min to 3 days after exposure. Novaluron was not irritating to the skin of the rabbit under the conditions of this test (Liggett, 1988b).

The eye irritation potential of novaluron (purity, 94.3%) was investigated in six male New Zealand White rabbits. The study was conducted in accordance with OECD guideline 405 and was certified to comply with GLP. Approximately 72 mg (weight of 0.1 ml) of undiluted test material was applied into the lower everted eyelid of one eye of each animal, the untreated eye served as control. Transient mild conjuctival irritation was observed in all animals at 1 h after instillation only. No ocular effects were observed at 24 h or until the end of the study at 7 days. Novaluron is not considered to be an irritant under the conditions of this test (Liggett, 1988c).

The skin sensitization potential of novaluron (purity, 96.7%) was investigated using the Buehler test in male albino Dunkin/Hartley guinea-pigs. In a preliminary screening, no signs of dermal irritation were observed at test concentrations of 2.5, 5 and 10% (w/v), in dimethylsulfoxide (DMSO). The study was certified to comply with GLP and was conducted in accordance with OECD guideline 406. However, only 10 animals were used in the treatment group, while a minimum of 20 animals is required by the guideline. One animal in the negative control group died during the third week of the experiment. Pneumonia was seen at the post-mortem examination. Test animals showed no positive dermal reactions at the challenge. No skin

Table 9. Irritation and skin sensitization potential of novaluron

Species	Strain	Sex	End-point (method)	Purity (%)	Result	Reference
Rabbit	NZW	Male & female	Skin irritation	94.3	Not irritating	Liggett (1988b)
Rabbit	NZW	Male	Eye irritation	94.3	Not irritating	Liggett (1988c)
Guinea-pig	Dunkin/Hartley	Male	Skin sensitization (Buehler)	96.7	Not sensitizing	Di Giovanni (1993)
Guinea-pig	Dunkin/Hartley	Male	Skin sensitization (Magnusson & Kligman)	99.3	Not sensitizing	Coleman (1997)

NZW, New Zealand White

reactions were observed in the negative control group. Novaluron was not sensitizing to the skin according to the Buehler test under the conditions of this study (Di Giovanni, 1993).

The skin sensitization potential of novaluron (purity, 99.3%) was investigated using the Magnusson & Kligman maximization method. The study was certified to comply with GLP and was conducted in accordance with OECD guideline 406. Groups of 20 male albino Dunkin/Hartley guinea-pigs were tested accordingly to the following dosing regimen: (a) induction by intradermal injection of 5% acetone in Alembicol D (10% w/v); (b) induction by topical application of 80% w/v in acetone; then (c) topical challenge with 40% w/v and 80% w/v in acetone. Groups of 10 animals were similarly treated in positive control tests with hexyl cinnamic aldehyde (HCA). There were no dermal reactions seen in any of the test or control animals challenged with novaluron at 80% and 40% w/v in acetone. Dermal reactions were seen in all of the ten animals in the positive control group compared with none in the controls. Novaluron was not sensitizing to skin in the Magnusson & Kligman test in guinea-pigs (Coleman, 1997).

2.2 Short-term studies of toxicity

Mice

Groups of six male and six female Crl:CD-1(ICR)BR mice were given diets containing novaluron (purity, 96.73%) at a concentration of 0, 70, 700 or 7000 ppm (corresponding to mean achieved compound intakes of 11.7, 114.7 and 1162.5 mg/kg bw per day in males and 16.4, 153.0 and 1371.8 mg/kg bw per day in females) for 4 weeks. Investigations included pre-termination blood sampling for haematology and clinical chemistry assessments. All animals were examined grossly at necropsy and selected organ weights were recorded. Histopathological examinations were undertaken for animals in the control groups, at the highest dose, and all decedents. The spleen and liver at all doses were examined microscopically. The study was certified to comply with GLP and was considered to be in accordance with OECD test guideline 407.

There were no deaths or clinical signs of toxicity. There were no treatment-related differences in feed consumption. Mean body weight after 4 weeks was statistically significantly greater (> 10%) in females only at doses of 70 ppm and above (Table 10). Body-weight gain was statistically significantly increased during week 1 only in males at 7000 ppm and in females at 700 ppm and above.

Haematology showed varying degrees of treatment-related changes in erythrocyte parameters in both sexes; reduced erythrocyte counts, increased haemoglobin concentration (Hb), reduced erythrocyte volume fraction (EVF), increased mean cell haemoglobin concentration (MCHC) and mean cell haemoglobin (MCH). Mean corpuscular volume (MCV) was increased at all doses, but showed no clear dose–response relationship (Table 10). Clinical chemistry findings that were considered to be treatment-related included increases of slight degree, concerning creatinine concentrations in females at 700 ppm and in both sexes at 7000 ppm; aspartate aminotransferase (AST) activity at 7000 ppm; total protein and calcium concentrations in females at 70 ppm and above; total bilirubin concentrations in females at 7000 ppm; gamma globulin concentrations in both sexes at 7000 ppm; cholesterol concentrations in females at 70 ppm and above; and triglycerides and cholinesterase levels in females at 700 and 7000 ppm.

Organ weights in females showed a slight, dose-related increase in absolute liver weight at doses of 700 ppm and above and in relative liver weight at 7000 ppm only (Table 10). A moderate dose-related, statistically significant, increase in absolute and relative spleen weights was observed in both sexes at doses of 700 ppm and above. The slight increase in the absolute kidney (statistically significant at 7000 ppm) and heart weights in females could be related to the increased terminal body weights.

Table 10. Summary of selected findings in a 28-day study in mice given diets containing novaluron

Parameter	Dietary concentration (ppm)							
	0	70	700	7000	0	70	700	7000
	Males				Females			
Body-weight gain (g)								
Week 1	1.61	1.62	2.99	3.31*	0.00	1.02	2.16*	3.11**
Week 4	36.55	37.38	38.65	38.01	27.53	30.36*	30.87**	33.89**
Haematology								
EC (10^{12}/l)	7.64	7.40	7.16	6.68**	7.58	7.72	7.76	6.99
Hb (g/dl)	12.23	12.35	12.80	12.72	12.58	12.38	14.07**	13.82
EVF (l/l)	0.402	0.408	0.392	0.367*	0.397	0.412	0.422	0.374
MCHC (g/dl)	30.43	30.35	32.73**	34.63**	31.72	30.10**	33.45*	36.93**
MCH (pg)	16.03	16.70	17.93**	19.02**	16.60	16.05*	18.17**	19.75**
MCV (fl)	52.67	55.00	54.83	55.00	52.33	53.33	54.33	53.50
Blood chemistry								
Creatinine (mg/dl)	0.46	0.46	0.49	0.61*	0.30	0.35	0.47**	0.46
AST (IU/l)	108.81	96.11	86.04	146.72	95.84	102.39	115.89	117.24
Total cholesterol (mg/dl)	104.05	135.47	133.43	143.68	78.69	109.91*	118.41**	130.01**
Triglycerides (mg/dl)	74.00	76.10	108.09*	79.41	74.42	82.87	106.27	122.90
Cholinesterase (mEq/l)	298.81	323.66	432.33*	293.95	559.81	643.64	755.79**	732.89*
Total protein (g/dl)	5.12	5.41	5.46	5.44	5.44	5.83*	5.77	6.10**
Total bilirubin (mg/dl)	0.74	0.53	0.75	0.67	0.50	0.51	0.49	0.64*
Gamma globulins (%)	2.83	3.59	2.73	4.61**	3.41	3.17	3.57	4.34
Beta globulins (%)	22.87	22.11	20.20	21.71	21.12	19.57	18.28*	23.08
Calcium (mg/dl)	9.48	10.03	10.05	10.10	9.53	10.03*	9.93	10.21**
Organ weights								
Liver (mg)	1991	1807	2058	2137*	1343	1432	1555*	1825**
Spleen (mg)	110	127	153**	168**	108	132	197**	241**
Liver (% of bw)	5.535	5.078*	5.413	5.747	4.991	4.964	5.322	5.708**
Spleen (% of bw)	0.306	0.356	0.403*	0.454**	0.401	0.457	0.662**	0.757**
Gross pathology								
Spleen: enlarged	0/6	0/6	0/6	0/6	0/6	0/6	1/6	1/6
Histopathology								
Liver: centrilobular hypertrophy	0/6	0/6	0/6	5/6	1/6	1/6	0/6	3/6
Spleen:								
Haematopoiesis [a]	6/6 (1.2)	6/6 (1.2)	6/6 (2.0)	6/6 (2.0)	5/6 (1.2)	5/6 (1.2)	6/6 (2.0)	6/6 (2.0)
Pigment increased [a]	0/6	3/6 (1.0)	6/6 (1.7)	6/6 (1.3)	0/6	0/6	4/6 (1.0)	6/6 (1.0)
Red pulp, congestion [a]	1/6 (1.0)	0/6	0/6	6/6 (1.0)	0/6	0/6	0/6	3/6 (1.0)

From Ammannati (1993a)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

[a] Values in parenthesis: severity: 1 (slight); 2 (moderate); 3 (severe)

AST, aspartate aminotransferase; EC, erythrocyte count; EVF, erythrocyte volume fraction; Hb, haemoglobin; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume

Gross examination of organs and tissues revealed an enlarged spleen in one female each from the groups at 700 and 7000 ppm. Histopathological examination of tissues and organs revealed a slight increase in degree of haematopoiesis and pigments in the spleen, without clear signs of a dose–response relationship in animals at doses of 700 ppm and above. Increased pigments were of the same nature as those normally present in the controls (haemosiderinic, as shown by specific staining). A slight trend towards an increase of pigments in the spleen was noted in some males. In the spleen, increased frequency of slight red pulp congestion was observed at doses of 7000 ppm compared with controls. Slight centrilobular hypertrophy of the liver was observed in some mice at 7000 ppm, but a similar change was also noted in one female in the control group.

The NOAEL was 70 ppm (equal to 11.7 mg/kg bw per day) on the basis of changes in erythrocyte parameters and increased splenic weights in both sexes at 700 ppm (equal to 114.7 mg/kg bw per day) and above, (Ammannati, 1993a).

In a further study, groups of eight male and eight female CD-1 mice were given diets containing novaluron (purity, 99.5–99.8%) at a concentration of 0, 50, 100, 1000 or 7000 ppm (corresponding to mean achieved compound intakes of 7.3, 15.4, 150.9 and 1237.2 mg/kg bw per day in males and 9.0, 19.1, 172.7 and 1278.7 mg/kg bw per day in females) for 4 weeks. All mice were observed daily for mortality and signs of ill health or toxicity. Body weight and feed consumption were determined weekly. During week 4 before scheduled termination, blood samples were taken from non-fasted animals for haematology. Blood samples for clinical chemistry assessments were taken at postmortem. Urine analysis was not performed. All animals were grossly examined at necropsy and selected organ weights were recorded. Histopathological examinations were not undertaken. Stability, homogeneity and verification of achieved concentrations were conducted and found to be acceptable. The study was compliant with GLP.

There were no deaths or clinical signs of toxicity. There were no treatment-related differences in feed consumption. No treatment-related deaths or clinical signs of toxicity were observed. Feed consumption was increased in males at 7000 ppm. After 4 weeks of treatment, body-weight gain was increased (≥ 40%) in females at doses of 50 ppm and above, and in males at 1000 (13.5%) and 7000 (46%) ppm (Table 11).

Reduced EVF, Hb and erythrocyte counts were observed in males at doses of 100 ppm and above. Reduction in erythrocyte count and MCHC were observed in females at doses of 1000 and above, or 7000 ppm. Slightly low MCHC was also observed in females at 50 and 100 ppm, but in the absence of corresponding changes in primary erythrocyte parameters was not considered to be treatment-related. Males had increased MCH and MCV, and decreased MCHC at 7000 ppm. Increased MCV was observed in females at 7000 ppm and polychromatic and hypochromatic erythrocytes were observed on morphological examination.

A limited range of clinical chemistry investigations, mainly for liver toxicity, revealed only a slight increase in AST. However, the remaining parameters investigated (alkaline phosphatase, alanine aminotransferase, and gamma-glutamyl transpeptidase activity) did not show any treatment-related differences.

Gross examination post-mortem revealed increased swollen spleens in some animals of all treated groups, including females in the control group. Organ weight measurements showed increased absolute and relative spleen weights in both sexes at 1000 ppm and above and increased absolute and relative kidney and liver weights in males at 7000 ppm.

The NOAEL was 50 ppm (equal to 7.3 mg/kg bw per day) on the basis of statistically significant reduction in EVF, reduced EC and Hb in males at doses of 100 ppm (equal to 15.4 mg/kg bw per day) and above (East, 1997).

Table 11. Selected findings in a 28-day study in mice given diets containing novaluron

	Dietary concentration (ppm)									
	0	50	100	1000	7000	0	50	100	1000	7000
	Male					Female				
Body-weight gain (g)										
Weeks 0–1	3.4	3.6	3.6	4.5	5.1*	0.5	1.2	2.0	2.3*	3.2**
Weeks 0–4	7.4	7.0	7.7	8.4	10.8*	3.7	5.2	5.6	5.8	6.9
Feed consumption (g/animal)										
Weeks 1–4	149	143	148	147	177	132	128	138	132	136
Feed conversion (%)										
Weeks 1–4	5.0	4.9	5.2	5.7	6.1	2.8	4.1	4.1	4.4	5.1
Haematology										
EC (10^{12}/l)	9.35	9.25	8.97	8.90*	8.13[c]	9.15	9.30	8.90	8.70	8.43*
EVF (l/l)	0.45	0.45	0.43*	0.43**	0.42***	0.44	0.46	0.43	0.42	0.43
Hb (g/dl)	14.6	14.5	14.1	13.7**	13.3***	14.6	14.9	13.9	13.6*	13.9
MCV (fl)	48.2	48.3	48.2	48.0	51.5***	47.9	49.4	47.9	48.8	51.7***
MCH (pg)	15.6	15.7	15.7	15.4	16.3*	16.0	16.1	15.6	15.7	16.6
MCHC (g/dl)	32.4	32.4	32.7	32.1	31.8*	33.3	32.6*	32.6*	32.1**	32.0***
Polychromasia—slight	NT	NT	NT	NT	8/8	NT	NT	NT	3/8	8/8
Hypochromasia—slight	NT	NT	NT	NT	7/8	NT	NT	NT	1/8	8/8
Blood chemistry										
AST (IU/l)	65	87	75	69	69	75	79	71	95	112**
Organ weights										
Spleen (% of bw)	0.297	0.332	0.361	0.401*	0.465**	0.563	0.559	0.559	0.678	0.707
Liver (% of bw)	4.753	4.590	4.511	4.800	5.117	4.862	4.555	4.841	4.845	4.914
Kidney (% of bw)	1.388	1.459	1.541	1.408	1.599*	1.217	1.172	1.218	1.203	1.232
Macroscopic pathology										
Spleen: swollen	0/8	2/8	3/8	4/8	7/8**	4/8	5/8	6/8	6/8	7/8

From East (1997)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

AST, aspartate aminotransferase; EC, erythrocyte count; Hb, haemoglobin; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume; NT, not tested

Groups of 12 male and 12 female CD-1 mice were given diets containing novaluron (purity, 99.5–99.8%) at a concentration of 0, 30, 100, 1000 or 10 000 ppm (corresponding to mean achieved compound intakes of 4.2, 12.8, 135.9 and 1391.9 mg/kg bw per day for males and 4.7, 15.2, 135.6 and 1493.1 mg/kg bw per day for females) for 13 weeks. Additional groups of six male and six female rats received treatment with novaluron at dietary concentrations of 0, 30 or 10 000 ppm for 13 weeks and were observed for 8 weeks to investigate recovery from treatment-related effects. The mice were observed daily for clinical signs. All animals were assessed for clinical signs, body-weight gains, feed consumption and feed conversion efficiency, ophthalmoscopy, haematology (including methaemoglobin and sulfhaemoglobin), blood chemistry, urine analysis, organ weights, macroscopic pathology and histopathology. The study was certified to comply with GLP and designed to meet the requirements of OECD test guideline 408.

There were no treatment-related deaths. One male at 30 ppm was killed for humane reasons during week 8 after signs which included a swollen area on the head, with eyes closed, which on histopathological examination was identified as a mycotic abscess. One female at 10 000 ppm died during week 3 of recovery, with no signs of toxicity before death. There were signs of increased incidence of ungroomed coat in males at 1000 ppm and above and piloerection at 10 000 ppm (Table 12). Ophthalmoscopy did not reveal any ocular effects. Feed intake was not affected, but body-weight gain was increased in males at 1000 ppm and above, and in females at 100 ppm and above. Feed conversion efficiencies for animals at 100 ppm and above were marginally higher than those of controls, with animals at 1000 ppm showing the highest values.

Haematological changes included reduced EC in males at 100 ppm and above (statistically significant at 1000 ppm and above); reduction in EVF in females at 100 ppm and above and in males at 1000 ppm and above (Table 12). Males showed increased incidence of high sulfhaemoglobin concentration at 100 ppm and above with only slightly increased incidences in females at 1000 ppm and above. There was an increase in the inclusion bodies (in the blood and reticulocyte smears, probably Heinz bodies) in the erythrocytes, observed in all animals at doses of 100 ppm and above, and this was not resolved in the group receiving the highest dose at week 4 of reversibility. Other findings, which were not resolved during week 4 of reversibility in the highest dose were reduced EVF and Hb in females. However, all changes that had not been resolved at week 4 of the reversibility phase were completely resolved by week 8 of the reversibility phase.

The only apparent blood chemistry change was slightly high bilirubin concentrations in all animals at 100 ppm and above. It was noted by the investigators that the differences from controls, although statistically significant were small and not strictly dosage-related. The changes in bilirubin were fully reversible after 8 weeks of recovery. Urine analysis did not reveal any abnormalities.

Gross observations of swollen spleen at necropsy were confirmed by increase in the absolute and relative spleen weight at doses of 1000 ppm and above. However, histopathological examinations of organs and tissues did not reveal any significant treatment-related abnormalities.

The NOAEL in the 13-week dietary study in mice with reversibility observations after 8 weeks was 30 ppm (equal to 4.2 mg/kg bw per day) on the basis of haematology changes at 100 ppm (equal to 12.8 mg/kg bw per day) and above (East, 1998a).

Rats

Groups of 10 male and 10 female Crl:CD(SD)BR rats were given diets containing novaluron (purity, 94.3%) at a concentration of 0, 20, 160, 1280 or 10 280 ppm (corresponding to mean achieved compound intakes of 2.1, 16.7, 136, and 1131 mg/kg bw per day respectively in males, and 0, 2.2, 17.0, 137, and 1072 mg/kg bw per day in females) for 4 weeks All rats were observed daily for signs of ill health or reaction to treatment. Body weight and feed consumption were determined weekly. Haematology and clinical chemistry tests were conducted on blood samples taken during week 4 from animals fasted overnight. Urine analysis was not performed. All animals were grossly examined at necropsy and selected organ weights were recorded. No histopathological examinations were undertaken. The study was certified to comply with GLP, but a quality assurance (QA) audit was not conducted. The significant deviation from OECD test guideline 407 is the lack of histopathological examinations. However, it was noted that the study was designed as a range-finding for the 90-day study in rats.

There were no mortalities or clinical signs of toxicity during the study period. Feed consumption was significantly increased in males at 1280 and 10 280 ppm compared with controls and was associated with increased body-weight gain (Table 13). Body-weight gain was increased (> 10%) in males at 160 ppm and above and in females at 1280 ppm and above. However the increases in body-weight gain did not show a dose–response relationship in males, and in females was not statistically significant.

Table 12. Summary of selected findings of a 13-week study in mice given diets containing novaluron

Finding	Dietary concentration (ppm)									
	0	30	100	1 000	10 000	0	30	100	1 000	10 000
	Male					Female				
Clinical signs										
Piloerection	1/18	1/18	1/12	1/12	9/18	0/18	1/18	0/12	0/12	0/18
Ungroomed fur	2/18	1/18	3/12	5/12	9/18	0/18	0/18	0/12	0/12	0/18
Cumulative body-weight gain (g)										
Week 13	15.9	16.0	16.9	20.4**	17.3	10.0	11.0	12.0	15.7**	13.0
Week 8 recovery	0.0	1.7	NT	NT	2.2	2.8	1.9	NT	NT	1.1
Feed conversion efficiency (%)										
Week 1–13	3.0	3.1	3.4	3.7	3.2	2.2	2.5	2.7	3.8	3.0
Haematology										
EVF (l/l)										
Week 13	0.37	0.37	0.36	0.35*	0.35*	0.41	0.40	0.38**	0.39*	0.37***
Week 4 recovery	0.40	0.39	NT	NT	0.40	0.42	0.41	NT	NT	0.39**
EC (10^{12}/l)										
Week 13	8.02	8.10	7.67	7.53*	7.51**	8.68	8.41	7.96**	8.28	7.75***
Week 4 recovery	8.52	8.25	NT	NT	8.46	9.22	8.83	NT	NT	8.26**
MCHC (g/dl), week 13	34.0	33.9	33.8	34.5	35.2***	33.7	33.5	33.2	34.5	35.4***
MCH (pg), week 13	15.7	15.4	15.8	16.1	16.5**	15.8	15.9	16.1	16.2	17.0***
Reticulocyte counts (%)										
Week 13	1.5	1.0	0.7	0.9	1.8	1.2	1.4	1.7	1.8*	2.5***
Week 4 recovery	0.9	1.7*	NT	NT	1.8*	1.1	1.2	NT	NT	3.6***
Inclusion bodies										
'few', week 13	0	11[a]	5	0	0	0	0	4	8	8
'several', week 13	0	1	5	0	0	0	0	0	4	2
'many', week 13	0	0	2	12	18	0	0	0	0	8
'several', week 4 recovery	0/6	0/6	NT	NT	0/6	0/6	0/6	NT	NT	1/5
'many', week 4 r	0/6	0/6	NT	NT	6/6	0/6	0/6	NT	NT	4/5
Met-Hb (%)										
Week 13	0.82	0.63	0.29***	0.28***	0.70	0.67	0.74	0.67	0.82	0.70
Week 4 recovery	0.66	0.67	NT	NT	0.28**	0.92	0.83	NT	NT	0.92
Sulfhaemoglobin										
'high', week 13	0/18	0/17	6/12	7/12	11/18	0/18	0/18	0/12	2/12	2/18
'not high', week 13	18/18	17/17	6/12	5/12	7/18	18/18	18/18	12/12	10/12	16/18
Blood chemistry										
Bilirubin, total (µmol/l), week 13	2	2	4***	4***	4***	2	2	3**	4***	3***
Organ weights										
Spleen (g):										
Absolute, week 13	0.153	0.144	0.161	0.223**	0.239**	0.145	0.121*	0.154	0.224**	0.249**
Relative (% of bw), week 13	0.358	0.328	0.355	0.462*	0.537**	0.443	0.362*	0.434	0.583**	0.720**

Finding	Dietary concentration (ppm)									
	0	30	100	1 000	10 000	0	30	100	1 000	10 000
	Male					Female				
Macroscopic pathology										
Spleen: swollen										
Week 13	6/12	6/11	7/12	11/12	11/12	6/12	2/12	4/12	11/12	9/12
Week 8 recovery	0/6	1/6	NT	NT	2/6	0/6	1/6	NT	NT	2/5

From East (1998a)

EC, erythrocyte count; EVF, erythrocyte volume fraction; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; Met-Hb, methaemoglobin concentration; NT, not tested.

[a] Occasional small inclusion bodies present

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Haematology showed changes in erythrocyte parameters, which was more pronounced in females at doses of 1280 ppm and above. In males, statistically significant reductions were observed in EVF at 160 ppm and above, erythrocyte count and Hb at 10 280 ppm. In females, statistically significant reductions were observed in EVF, erythrocyte count and Hb at 1280 ppm and above. Platelet counts were increased in males and females at 1280 ppm and above. There were no treatment-related changes in clinical chemistry parameters. Organ weight measurements showed statistically significant increase in spleen weights at doses of 1280 ppm and above in males and 160 ppm and above (≥ 15%) in females. There were no observations of treatment-related macroscopic pathology.

The NOAEL in this 28-day range-finding study in rats was 20 ppm (equal to 2.1 mg/kg bw per day) on the basis of increased spleen weights in females at doses of 160 ppm (equal to 17 mg/kg bw per day) and above (Hopkins, 1989).

Groups of 10 male and 10 female Crl:CD(SD)BR rats were given diets containing novaluron (purity, 94.3%) at a concentration of 0, 10, 320 or 10 000 ppm (corresponding to mean achieved compound intakes of 0.7, 22.2 and 713 mg/kg bw per day in males and 0.8, 24.3 and 754 mg/kg bw per day in females) for 13 weeks. All animals were assessed for clinical signs, body-weight gains, feed and water consumption, ophthalmoscopy, haematology, clinical chemistry, urine analysis, organ weights, macro- and microscopic pathology. The study was certified to comply with GLP and designed to meet the requirements of USEPA test guidelines.

There were no treatment-related deaths or signs of toxicity during the study. One male receiving 100 ppm died during week 13. Incidental findings included yellow staining of the fur, scabbing, hair loss, ulceration, and weight loss in dosed and control animals. Ophthalmoscopic examinations did not reveal any treatment-related ocular findings. Feed and water consumption and body-weight gain did not show any treatment-related differences compared with controls.

Significant reductions in mean EVF, Hb and erythrocyte count were observed in females at 320 ppm and in both sexes at 10 000 ppm (Table 14). Slight polychromasia and slight anisocytosis were observed at 10 000 ppm. Females at 10 000 ppm also showed a slight, statistically significant increase in the MCV and an increase in the white blood cell count (WBC), which is associated with an elevated lymphocyte count. There were no toxicologically significant changes in clinical chemistry parameters. There was a significant increase in the absolute and relative spleen weights in both sexes at 10 000 ppm, but histopathological examination of the spleen revealed no abnormalities. Changes in kidney weights at 320 ppm and above were noted to be not dose-related and were not accompanied by any histopathological findings.

The NOAEL was 10 ppm (equal to 0.8 mg/kg bw per day) on the basis of reductions in erythrocyte parameters in females at 320 ppm (equal to 24.3 mg/kg bw per day) and above (Kirk, 1990).

Table 13. Summary of selected findings from a 4-week study in rats given diets containing novaluron

Parameter	Dietary concentration (ppm)									
	0	20	160	1280	10 280	0	20	160	1280	10 280
	Male					Female				
Body-weight gain (g), weeks 0–4	151	162	168	184**	169**	68	71	68	81	81
Feed consumption (g/rat), weeks 1–4	706	742	743	777*	777*	514	544	518	536	535
Feed conversion ratio, weeks 1–4	4.7	4.6	4.4	4.2	4.6	7.6	7.7	7.6	6.7	6.6
Haematology										
EVF (l/l)	0.53	0.53	0.50*	0.51*	0.50**	0.51	0.50	0.51	0.45**	0.44**
EC (10^{12}/l)	7.4	7.3	7.2	7.3	6.9**	7.2	7.1	7.2	6.3**	6.1**
Hb (g/dl)	15.6	15.8	14.9	15.1	14.6**	15.4	14.9	15.2	13.4**	13.0**
Platelets (10^9/l)	1045	1070	1113	1207*	1179*	1006[a]	1052	1043	1227**	1263**
Polychromasia—minimal	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10
Anicytosis—minimal	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
Organ weights										
Spleen (g) —absolute	0.68	0.70	0.72	0.81**	0.79**	0.50	0.54	0.58	0.70	0.74
—adjusted for bw	NC	NC	NC	NC	NC	0.52	0.54	0.60*	0.69**	0.71**

From Hopkins (1989)

EC, erythrocyte count; EVF, erythrocyte volume fraction; Hb, haemoglobin; NC, not calculated.

[a] Analysis performed on log-transformed data.

* $p < 0.05$; ** $p < 0.01$.

Table 14. Summary of the relevant findings from a 13-week study in rats given diets containing novaluron

Parameter	Dietary concentration (ppm)							
	0	10	320	10 000	0	10	320	10 000
	Male				Female			
Haematology								
EC (10^{12}/l)	7.9	8.1	8.2	7.5**	7.4	7.3	7.1*	6.4**
EVF (l/l)	0.55	0.56	0.55	0.52**	0.53	0.53	0.50**	0.47**
Hb (g/dl)	16.4	16.5	16.2	15.2**	15.9	15.8	15.1**	14.5**
MCV (fl)	70	70	68	70	72	72	71	74**
WBC (10^9/l)	15.7	17.0	14.6	16.3	10.1	8.4	8.2	13.3**
Polychromasia—slight	2/10	NT	1/10	7/10	1/10	NT	1/10	4/10
Anisocytosis—slight	2/10	1/10	4/10	7/10	NT	NT	NT	2/10
Organ weights								
Spleen (g) —absolute	0.94	1.00	1.04	1.12**	0.61	0.59	0.65	0.77**
—adjusted for bw	0.93	1.00	1.04	1.12	0.62	0.58	0.66	0.77
Kidneys (g) —absolute	3.62	3.65	4.07	3.93	2.42	2.21	2.32	2.17**
—adjusted for bw	3.62	3.65	4.07*	3.93*	2.44	2.16	2.38	2.15

From Kirk (1990)

EC, erythrocyte count; EVF, erythrocyte volume fraction; Hb, haemoglobin; MCV, mean corpuscular volume; NT, not tested.

* $p < 0.05$; ** $p < 0.01$

Groups of 10 male and 10 female Crl:CD(SD)BR rats received diets containing novaluron (purity, 96.7%) at a concentration of 0, 50, 100, 200 or 400 ppm (corresponding to mean achieved compound intakes of 3.52, 6.93, 13.83 and 27.77 mg/kg bw per day for males and 4.38, 8.64, 17.54 and 34.39 mg/kg bw per day for females) for 13 weeks. All animals were assessed for clinical signs, body-weight gains, feed and water consumption, ophthalmoscopy, haematology, clinical chemistry, urine analysis, organ weights, macro- and microscopic pathology. The study was certified to comply with GLP and satisfied the requirements of OECD guideline 408.

There were no treatment-related deaths or signs of toxicity. However, two control animals died. Feed consumption, body-weight gain and ophthalmoscopy showed no treatment-related intergroup differences. Hb and EVF were significantly reduced in females at doses of 100 (week 14 only) and at 200 and above (week 7 and 14) ppm (Table 15). Erythrocyte count was significantly reduced at 200 ppm and above in females. Prothrombin time was significantly reduced in females during week 7 only at 200 ppm and above. Platelets were increased in females at doses of 100 ppm and above, although a clear dose–response relationship was not observed. In males, statistically significant reduction in Hb, neutrophils, EVF and prothrombin time and an increase in lymphocytes was observed during week 7, but not during week 14. Blood chemistry showed a trend of increased glucose in both sexes at doses of 200 ppm and above. Urine analysis showed no treatment-related differences. Organ weights showed a trend of increased spleen weights in females at 100 and 200 ppm and in both sexes at 400 ppm, which did not attain statistical significance.

Females showed a dose-related increase in the frequency and/or degree of haemosiderinic pigments in the spleen at doses of 100 ppm and above, but the increase at 100 ppm was considered to be limited. In the liver increased incidence of pigment laden macrophages were observed in females at 100 ppm and above, and individual incidences in males at 100 ppm and above.

The NOAEL was 50 ppm (equal to 4.38 mg/kg bw per day) on the basis of haematological changes in females at 100 ppm (equal to 8.64 mg/kg bw per day) and above (Ammannati, 1993b).

Groups of 10 male and 10 female CD rats were given diets containing novaluron (purity, 99.8%) at a concentration of 0, 50, 100, 10 000 or 20 000 ppm (corresponding to mean achieved compound intakes of 4.2, 8.3, 818.5 and 1666.9 mg/kg bw per day for males and 4.7, 8.9, 871.0 and 1820.6 mg/kg bw per day for females) for 13 weeks. Dose selection was based on the findings of a preceding 28-day study in rats. Additional groups of five male and five female rats received treatments at concentrations of 0, 50 or 20 000 ppm for 13 weeks and were observed for 4 weeks of recovery. The rats were observed daily for clinical signs. All animals were assessed for clinical signs, body-weight gains, feed consumption and feed conversion efficiency, ophthalmoscopy, haematology (including methaemoglobin and sulfhaemoglobin), blood chemistry, urine analysis, organ weights, macroscopic pathology and histopathology. The study was certified to comply with GLP and designed to meet the requirements of OECD test guideline 408.

There were no treatment-related deaths and or clinical signs of toxicity. One male at 100 ppm died 5 h after routine blood sampling, having shown no previous signs of toxicity. Ophthalmological examinations of animals at the highest dose did not reveal any treatment-related ocular defects. There were no treatment-related differences in feed consumption. Body-weight gain in males was consistently higher at 10 000 ($p < 0.05$) and 20 000 ($p < 0.01$) ppm than in controls throughout the study period, but for the first 4 weeks only in females at the same doses. During the reversibility period, body-weight gain was slightly lower in males (statistically not significant) and females ($p < 0.01$) at 20 000 ppm.

There were treatment-related haematological changes at doses of 10 000 ppm and above, which included reduced Hb, erythrocyte counts and mean corpuscular haemoglobin concentrations. Increased MCV and concentrations of methaemoglobin were observed in both sexes at 10 000 ppm and above. Reticulocyte counts were significantly increased in both sexes at

Table 15. Summary of selected findings in a 13-week study in rats given diets containing novaluron

Finding	Dietary concentration (ppm)									
	0	50	100	200	400	0	50	100	200	400
	Male					Female				
Haematology										
EC (10^{12}/l):										
Week 7	7.55	7.57	7.43	7.28	7.36	7.44	7.23	7.21	6.95**	6.85**
Week 14	7.71	8.03	8.06	7.63	7.62	7.48	7.31	7.12	6.97*	6.76**
Hb (g/dl):										
Week 7	15.84	15.63	15.43	14.85	15.14	15.60	15.05	15.26	14.36**	14.64**
Week 14	14.92	15.53	15.54	15.02	14.97	15.29	14.79	14.21**	13.79**	13.91**
EVF (l/l):										
Week 7	0.460	0.457	0.445	0.437*	0.447	0.465	0.449	0.452	0.430**	0.441*
Week 14	0.441	0.446	0.448	0.431	0.437	0.453	0.442	0.424*	0.415**	0.421*
MCV (fl):										
Week 7	60.9	60.4	60.0	60.1	60.8	62.5	62.0	62.8	61.8	64.3*
Week 14	57.2	55.6	55.6	56.6	57.3	60.7	60.5	59.6	59.5	62.2
Platelets (10^9/l):										
Week 7	759	778	768	798	786	685	734	772**	741	754*
Week 14	890	794	804	772	743	673	674	767*	691	699
Prothrombin time (s):										
Week 7	13.4	13.3	13.3	13.2	12.8*	14.1	13.6	13.7	13.1**	13.2**
Week 14	12.7	12.6	12.9	12.2	12.1	12.7	12.6	12.2	12.0	12.3
Blood chemistry										
Glucose (mg/dl), week 14	112.3	111.0	114.7	126.3	137.8**	105.5	112.1	113.1	118.4	123.0
Organ weights										
Spleen (g):										
Absolute (g)	0.79	0.75	0.81	0.80	0.85	0.47	0.48	0.52	0.51	0.52
Relative (% of bw)	0.16	0.15	0.16	0.15	0.17	0.18	0.18	0.20	0.19	0.19
Histopathology										
Liver—pigment laden macrophages[a]	0/9	0/10	0/10	1/10 (1.0)	1/10 (1.0)	1/9 (1.0)	1/10 (1.0)	3/10 (1.0)	6/10 (1.0)	6/10 (1.0)
Spleen—extramedullary haematopoiesis[a]	4/9 (1.0)	4/10 (1.0)	3/10 (1.0)	7/10 (1.0)	9/10 (1.0)	1/9 (1.0)	2/10 (1.0)	8/10 (1.0)	5/10 (1.0)	4/10 (1.0)
Spleen—red pulp, pigment increased[a]	0/9	0/10	0/10	1/10 (1.0)	9/10 (1.0)	3/9 (1.0)	2/10 (1.0)	5/10 (1.0)	10/10 (1.0)	10/10 (1.8)

From Ammannati (1993b)

EC, erythrocyte count; EVF, erythrocyte volume fraction; Hb, haemoglobin; MCV, mean corpuscular volume.

[a] Values in parentheses: severity: 1 (slight): 2 (moderate): 3 (severe)

* $p < 0.05$; ** $p < 0.01$

10 000 ppm and above. MCH was increased at 10 000 ppm and above, and EVF was reduced at 100 ppm and above in females only. Platelet counts were increased in females at 20 000 ppm. Changes at 100 ppm were limited to low Hb and erythrocyte counts in females only. By week 4 of the reversibility period, there was full recovery for all changes, except for high concentrations of methaemoglobin in both sexes previously given 20 000 ppm, where there was partial recovery. Blood chemistry parameters and urine analysis did not reveal any treatment-related differences (Table 16).

At necropsy, there were no gross abnormalities at any doses. Organ weights showed increased absolute and relative spleen weights in females only at 10 000 ppm and above and in both sexes at 20 000 ppm, but the effect in males was less marked (Table 16). At the end of the recovery period, relative spleen weights were still slightly high in females at 20 000 ppm, but there was some evidence of recovery. Males showed complete recovery.

Histopathological examination revealed extramedullary haematopoiesis and pigment-laden Kupffer cells were present after 13 weeks in the livers of several females at doses of 10 000 ppm and above. Extramedullary erythropoiesis was observed in the spleen in the majority of animals given 50 or 100 ppm and in all animals at 10 000 ppm or 20 000 ppm. Increased haemosiderosis was observed in most males at doses of 10 000 ppm and above, and in all treated females. After 4 weeks of recovery, increased haemosiderosis was still present in the spleen of one male and in all females that had received 20 000 ppm. The changes in the liver showed almost full recovery. Owing to the relatively slow mechanism of haemosiderin metabolism (via the endoplasmic reticulum), incomplete recovery over a relatively short ecovery period is not wholly unexpected and it is likely that full recovery from increased haemosiderosis would be achieved given a longer reversibility period.

The NOAEL was 50 ppm (equal to 4.2 mg/kg bw per day) on the basis of haematological effects at 100 ppm, equal to 8.3 mg/kg bw per day (East, 1998b).

In a range-finding study of dermal toxicity, groups of three male and three female Crl:CD BR rats received novaluron (purity, 99.7%) in 1% w/v methylcellulose by dermal application at a dose of 0, 10, 30 or 100 mg/kg bw per day, 6 h/day for 14 days. Animals were assessed for signs of toxicity, feed consumption and body-weight gain. Haematology was restricted to assessments of methaemoglobin and sulfhaemoglobin from samples taken before and after treatment. The study was certified to comply with GLP.

There were no deaths or systemic signs considered to be attributable to treatment. Local signs at the treated skin sites were restricted to isolated cases of barely perceptible erythema in one female receiving 30 mg/kg bw per day and one female receiving 100 mg/kg bw per day during the first week of treatment, which were considered by the investigators to be related to the bandaging procedure. Overall group mean feed consumption for the treated animals was generally similar to the controls. Overall group mean body-weight gain for males given 100 mg/kg bw per day and for all treated groups of females were slightly higher than those of the controls (Table 17). The body-weight gain of males given 10 or 30 mg/kg bw per day was similar to that of the controls, but it is unclear whether the observed differences were treatment-related, because of the small group sizes.

Methaemoglobin concentrations did not show any significant intergroup differences. Organ weights were not reported. Gross examination of organs and tissues did not reveal any treatment-related changes (Rees, 1998a).

Table 16. Summary of selected findings in a 13-week study in rats given diets containing novaluron

Finding	Dietary concentration (ppm)									
	0	50	100	10 000	20 000	0	50	100	10 000	20 000
	Male					Female				
Cumulative bw gain (g):										
Week 4	172	185	180	198*	199**	83	83	87	91	99**
Week 13	300	325	318	343*	341*	152	158	145	154	167*
Week 4 reversibility	49	51	NT	NT	37	12	7	NT	NT	–4**
Haematology										
EVF (l/l), week 13	0.44	0.45	0.44	0.43	0.43	0.42	0.41	0.39***	0.39***	0.39***
Hb (g/dl), week 13	15.3	15.4	15.2	14.5**	14.6**	14.8	14.4	13.9***	13.4***	13.3***
EC (1012/l), week 13	8.62	8.59	8.48	8.05***	8.19**	7.93	7.65*	7.33***	6.75***	6.73***
MCHC (g/dl), week 13	34.5	34.4	34.4	33.7***	33.7***	35.2	35.2	35.4	34.6**	34.3***
MCH (pg), week 13	17.7	18.0	17.9	18.1	17.8	18.7	18.9	19.0	19.8***	19.8***
MCV (fl) , week 13	51.4	52.3	52.1	53.7*	52.9	53.2	53.6	53.6	57.2***	57.9***
Met-Hb (%):										
Week 13	0.56	0.48	0.67	1.08***	1.11***	0.60	0.76*	0.74	1.79***	2.07***
Week 4 reversibility	0.70	0.66	NT	NT	0.90*	0.70	0.75	NT	NT	1.04***
Reticulocyte counts (%), week 13	0.8	1.2	0.9	2.2***	2.2***	1.4	1.4	1.1	3.1*	3.8***
Platelets (109/l), week 13	979	957	1014	962	1028	1012	1044	1041	1078	1150**
Organ weights										
Spleen (g):										
Absolute, week 13	0.719	0.759	0.786	0.855	0.873*	0.609	0.621	0.616	0.795**	0.856**
% of bw, week 13	0.158	0.152	0.167	0.171	0.181	0.207	0.208	0.219	0.276**	0.277**
% of bw, week 4 reversibility	0.149	0.154	NT	NT	0.150	0.171	0.176	NT	NT	0.203*
Histopathology:										
Spleen:										
Extramedullary erythropoiesis, week 13										
Minimal	3/10	2/10	0/9	0/10	1/10	2/10	2/10	4/10	0/10	2/10
Slight	1/10	4/10	7/9	4/10	2/10	2/10	4/10	4/10	6/10	4/10
Moderate	0/10	2/10	0/9	6/10	7/10	0/10	1/10	1/10	4/10	4/10
Total	4/10	8/10	7/9	10/10*	10/10*	4/10	7/10	9/10	10/10*	10/10*
Increased haemosiderosis, week 13										
Slight	1/10	2/10	2/9	9/10	9/10	0/10	4/10	8/10	3/10	10/10
Moderate	0/10	0/10	0/9	0/10	0/10	0/10	1/10	0/10	7/10	0/10
Total	1/10	2/10	2/9	9/10**	9/10**	0/10	5/10*	8/10***	10/10***	10/10***
Increased haemosiderosis, week 4 reversibility										
Slight	0/5	NT	NT	NT	1/5	0/5	NT	NT	NT	3/5
Moderate	0/5	NT	NT	NT	0/5	0/5	NT	NT	NT	2/5
Total	0/5	NT	NT	NT	1/5	0/5	NT	NT	NT	5/5**

Finding	Dietary concentration (ppm)									
	0	50	100	10 000	20 000	0	50	100	10 000	20 000
	Male					Female				
Liver:										
Extramedullary haematopoiesis, week 13										
Minimal	1/10	2/10	1/10	0/10	0/10	3/10	0/10	0/10	5/10	2/10
Slight	0/10	1/10	0/10	2/10	0/10	1/10	2/10	3/10	2/10	2/10
Total	1/10	3/10	1/10	2/10	0/10	4/10	2/10	3/10	7/10	4/10
Pigment laden Kupffer cells, week 13										
Minimal	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	4/10	4/10
Slight	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	4/10
Total	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	5/10*	8/10***

From East (1998b)

EC, erythrocyte count; EVF, erythrocyte volume fraction; Hb, haemoglobin; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean corpuscular volume; Met-Hb, methaemoglobin; NT, not tested.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

In a subsequent study, groups of 10 male and 10 female Crl:CD BR rats were given novaluron (purity, 99.7%) at a dose of 0, 75, 400 or 1000 mg/kg bw per day in 1% w/v methylcellulose applied topically under a semi-occlusive dressing to the clipped intact skin (an area of approximately 10% of the total body surface area) for 6 h/day, for 28 consecutive days. After the exposure period, the dressing was removed and the exposure site of each animal was washed. The dose was adjusted weekly, based on the most recently recorded body weight. Animals were observed twice daily for signs of toxicity or dermal irritation. Feed consumption and body weight were recorded. Ophthalmoscopy was performed on all animals at the start of treatment and on controls and at 1000 mg/kg bw per day animals during week 4. Peripheral blood was obtained from the retro-orbital sinus of animals during week 4 for extensive haematology and blood chemistry assessments. Bone marrow samples were obtained at termination for cellularity, composition and assessments of myeloid : erythroid ratio. All rats were subjected to a detailed necropsy. The weights of selected organs, including the adrenals, kidneys, liver, spleen and testes were recorded. The adrenals, heart and testes of animals in the control group and in the group receiving the highest dose, and the spleen, kidneys, liver, lungs, treated and naïve skin were examined microscopically at all doses. The study was certified to comply with GLP and satisfied the essential requirements of OECD guideline 410.

There were no deaths or signs of toxicity. The treated skin site showed no signs of local reaction to treatment. Ophthalmoscopy at week 4 did not reveal any treatment-related abnormalities. Overall feed consumption was noted to be slightly lower (12%) in males at the highest dose than in controls. Body-weight change suggested a dose-related reduction in body-weight gain ranging from 15% to 26% in treated males. However, the apparent effect on body weight was attributed to two atypically heavy animals among control males, marginally lower body-weight gain in individual animals and the small population size (Table 18). There were no treatment-related effects on body weight in females. It is noted that evidence from dietary studies does not suggest impaired body-weight development as a typical response to treatment with novaluron. In this study of dermal administration, comparatively lower feed consumption in males at higher doses correlates with lower body-weight gain, which is not necessarily a treatment-related response.

Haematology revealed higher concentrations of methaemoglobin in both sexes at ≥ 400 mg/kg bw per day (Table 18). Additionally, in females at 400 mg/kg bw per day and above, EVF and Hb were slightly reduced, but in the absence of a dose–response relationship, the changes were considered to be of limited toxicological significance. The remaining haematology and blood chemistry parameters did not show any treatment-related intergroup differences. There were no significant changes in urine composition attributable to treatment. Organ weights, macroscopic and microscopic examinations of organs and tissues did not reveal any treatment-related changes.

The NOAEL was 75 mg/kg bw per day on the basis of increased methaemoglobin concentrations in both sexes at doses of 400 mg/kg bw per day and above (Rees, 1998b).

Table 17. Summary of selected findings from a 14-day study of dermal toxicity in rats

Parameter	Dose (mg/kg bw per day)							
	0	10	30	100	0	10	30	100
	Male				Female			
Body weight (g), at start	203	215	206	204	209	210	209	203
Body-weight change (g), days 0–14	70	68	68	81	16	21	23	22
Feed intake (g/animal), weeks 1–2	379	372	367	399	295	309	307	301
Haematology								
Met-Hb (%), week 2, before dosing	0.93	0.83	0.77	1.00	0.83	0.93	0.77	0.93
Met-Hb (%), week 2, 7 h after dosing	0.97	0.93	0.83	0.83	0.87	0.90	0.80	0.80

From Rees (1998a)

Met-Hb, methaemoglobin

Table 18. Summary of selected findings from a 28-day study of dermal toxicity in rats

Finding	Dose (mg/kg bw per day)							
	0	75	400	1000	0	75	400	1000
	Male				Female			
Body weight at start of dosing	204	201	205	200	202	196	197	199
Body-weight change (g), days 0–28	120	102	96	89	35	31	31	40
Feed consumption (g/animal), weeks 1–4	797	759	767	702	646	648	645	695
Feed conversion efficiency (%), weeks 1–4	15.1	13.4	12.5	12.7	5.4	4.8	4.8	5.8
Haematology								
EVF (l/l)	0.46	0.47	0.44	0.45	0.43	0.42	0.40*	0.41
Hb (g/dl)	15.5	15.8	14.9	15.1	14.9	14.8	14.0	14.2
EC (1012/l)	8.18	8.53	8.04	8.25	7.90	7.95	7.58	7.71
MCV (fl), week 4	55.9	54.9	54.4	53.9*	54.7	53.4	53.0	53.2
Met-Hb (%), week 4	0.80	0.95	1.02	1.08*	0.86	0.92	1.10**	1.28***

From Rees (1998b)

EC, erythrocyte count; EVF, erythrocyte volume fraction; Hb, haemoglobin; MCV, mean corpuscular volume; Met-Hb, methaemoglobin.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Dogs

In a preliminary study of toxicity, one male and one female beagle dog were given capsules containing novaluron (purity, 96.2%) at a dose of 50, 100, 200, 500 or 1000 mg/kg bw per day for consecutive periods of 3 days, 4 days, 3 days, 4 days and 5 days, respectively. On the basis of the reactions seen and results obtained from this group, one male and one female beagle dog were given a dose of 1000 mg/kg bw per day for 14 days for the fixed phase of the study. Animals were assessed for signs of toxicity, physical and behavioural effects, haematology and blood chemistry parameters (Table 19). At termination, organ weights and gross examinations of organs and tissues were assessed. The study was certified to comply with GLP and was intended to determine the maximum tolerated dose for a 13-week study of toxicity in dogs.

There were no signs of treatment-related toxicity or effects on feed consumption and body-weight gain in the animals participating in the incremental phase or fixed phase during the study. Limited haematology investigations after 16 days for the incremental-phase animals and after 12 days for the fixed-phase animals showed cellularity and coagulation response of the blood to be within normal ranges (Table 19). Gross examination and organ weight measurements did not reveal any abnormalities (Thirlwell, 1997).

In another preliminary study, groups of four male beagle dogs were given capsules containing novaluron (purity, 98.7–99.3%), at a dose of 0, 0.5, 2 or 10 mg/kg bw per day for 8 weeks. Animals were observed for signs of toxic effects, which included cage-side observations after test material administration. Feed consumption and body weight were measured weekly.

Table 19. Summary of selected findings in a range-finding study in dogs given capsules containing novaluron

Finding	Dose (mg/kg bw per day)			
	50–1000	1000	50–1000	1000
	Males		Females	
EVF (l/l)				
Pre-treatment	0.35	0.40	0.43	0.42
After treatment [a]	0.33	0.45	0.45	0.41
Hb (g/dl)				
Pre-treatment	11.5	13.6	15.1	13.9
After treatment [a]	10.5	14.9	14.8	13.9
EC (10^{12}/l)				
Pre-treatment	5.28	6.16	6.43	6.44
After treatment [a]	4.78	6.69	6.30	6.32
MCV (fl)				
Pre-treatment	66.4	65.7	67.5	65.8
After treatment [a]	68.8	71.0	71.0	65.1
Reticulocytes (%)				
Pre-treatment	3	< 2	3	< 2
After treatment [a]	2	2	2	2
Met-Hb (%)				
Pre-treatment	NT	0	NT	0
After treatment [a]	NT	0	NT	0.4

From Thirlwell (1997)

EC, erythrocyte count; EVF, erythrocyte volume fraction; Hb, haemoglobin; MCV, mean corpuscular volume; NT, not tested.

[a] After 16 days or 12 days of treatment for incremental or fixed dose, respectively.

Ophthalmological examination was not performed. Blood samples were obtained after overnight fasting before the start of the study and at one and 23 h after dosing during week 6 for detailed investigation of haematology (including methaemoglobin and microscopic assessments of blood for abnormalities) and blood chemistry parameters. Composition and cellularity of bone marrow samples obtained by biopsy were determined. Urine analysis parameters were measured for all animals before the start of treatment. Complete necropsy examinations were performed on all animals at the termination of the study and organ weights were recorded. Microscopic investigations were not undertaken. The study was certified to comply with GLP and conceived as a preliminary study for the 52-week dietary study in dogs.

There were no deaths or signs of toxicity during the treatment period. Feed consumption and body-weight gain were not affected by the treatment. Haematology and blood chemistry did not show any treatment-related changes (Table 20). The results of urine analysis before the start of the study were normal. Necropsy did not reveal any abnormalities and organ weights were within the normal range (Thirlwell, 1998b).

Groups of four male and four female beagle dogs were given gelatine capsules containing novaluron (purity, 99.3%) at a concentration of 0, 100, 300, or 1000 mg/kg bw per day for 13 weeks. Additional recovery groups of two male and two female dogs were treated at a dose of 0, 100 or 1000 mg/kg bw per day for 13 weeks and then were maintained for 4 weeks without treatment. Doses were based on a preliminary study. All animals were observed daily for signs of toxicity and physical examinations. Body weights and feed consumption were recorded weekly. Ophthalmoscopy was performed on all animals before treatment, during week 12 and week 4 of the reversibility phase. Peripheral blood was obtained for haematology and clinical chemistry assessments from fasted animals, before the start of treatment and during weeks 6, 14 and week 4 of the reversibility phase. Haematological parameters included methaemoglobin and sulfhaemoglobin investigations. Biopsies were performed for bone marrow samples. Urine analysis was performed before the start of treatment and during weeks 5, 12 and week 4 of the reversibility phase. All animals were killed at the termination of treatment in week 13 or reversibility phase and organs and tissues were examined macroscopically and microscopically. Organ weight measurements were performed during necropsy. The study was certified to comply with GLP and designed to meet the requirements of OECD test guideline 408.

There were no deaths or treatment-related signs of toxicity. Physical examinations and ophthalmoscopy did not reveal any treatment-related findings. There were no significant intergroup differences in feed consumption.

Table 20. Summary of selected parameters in an 8-week study in male dogs given capsules containing novaluron

	Dose (mg/kg bw per day)			
	0	0.5	2	10
EVF (l/l)	0.363	0.348	0.383	0.368
Hb (g/dl)	11.9	11.4	12.7	12.2
EC (10^{12}/l)	5.76	5.41	5.85	5.74
MCV (fl)	63.3	64.6	65.8	64.1
Reticulocytes (%)	< 2	< 2	< 2	< 2
Met-Hb (%)	0	0	0	0

From Thirlwell (1998b)

EC, erythrocyte count; EVF, erythrocyte volume fraction; Hb, haemoglobin; MCV, mean corpuscular volume.

Overall body-weight gains for males at 300 or 1000 mg/kg bw per day and females at 300 mg/kg bw per day were marginally higher than controls, but there was no clear evidence of a dose–response relationship and it is considered that the intergroup differences were most likely attributable to individual variation. During the reversibility period, body-weight gain for treated males and females was comparable to that of controls.

Haematology during week 6 of treatment showed a decrease in erythrocyte count, Hb and EVF in all animals at all doses. There was treatment-related increase in reticulocyte count, MCV, platelet count and methaemoglobin concentration in all treated animals. Heinz bodies were seen in the reticulocyte smear of two males at 100 mg/kg bw per day and in all animals at 300 mg/kg bw per day and above. The changes in erythrocyte count, Hb, reticulocyte count and methaemoglobin concentration were more marked in females than in males. Isolated incidences (one out of eight) of normoblasts were observed in smears at 300 and 1000 mg/kg bw per day (Table 21).

Similar findings to those in week 6 were observed during week 13, with higher methaemoglobin concentrations, MCV and lower erythrocyte counts in all treated animals. Lower EVF, platelet counts and Hb were observed at doses of 300 mg/kg bw per day and above. Sulfhaemoglobin was found to be increased in two males and one female at 1000 mg/kg bw per day. Heinz bodies were found in the reticulocytes of both sexes of all treated animals. At the end of the recovery period, haematological parameters including methaemoglobin and sulfhaemoglobin were noted to be similar to pre-treatment values and no Heinz bodies were seen in smears. Myeloid : erythroid ratios were not affected and bone marrow cells were not affected by the treatment.

Blood chemistry tests showed that concentrations of bilirubin were increased in all treated animals; however, by the end of the recovery period, concentrations of bilirubin were comparable to those before treatment (Table 21). Urine analysis did not reveal any treatment-related abnormalities.

Organ weights showed higher absolute and relative spleen weights at all doses, statistically significant at 1000 mg/kg bw per day. Liver weights were generally increased in all treated animals, but the increase in liver weights was more evident in both sexes at 300 mg/kg bw per day and above. At the end of the recovery period, only the livers of females at 1000 mg/kg bw per day remained distinctly larger than those of the controls. Histopathology of the liver revealed pigmentation of varying severity of Kupffer cells in the liver in some animals at 100 mg/kg bw per day and in all animals at 300 mg/kg bw per day and above (Table 21).

In conclusion, a NOAEL could not be identified in the 90-day study in dogs because of adverse haematological and related findings at doses of 100 mg/kg bw per day, the lowest dose tested, and above. Four weeks after termination of treatment, there was resolution of haematological, blood chemistry and histopathological findings (Thirlwell, 1998a).

In a supplementary study to facilitate the determination of a NOAEL, groups of four male and four female beagle dogs were given gelatin capsules containing novaluron (purity, 99.3–99.5%) orally at a concentration of 10 mg/kg bw per day for 13 weeks. Concurrent control data was obtained from a 52-week study in this species. All animals were assessed for clinical signs, body-weight gains, feed consumption, veterinary examination, ophthalmoscopy, haematology, blood chemistry, urine analysis, organ weights, macroscopic pathology and histopathology at various time points throughout the study. The study was certified to comply with GLP and satisfied the essential criteria of OECD guideline 408.

There were no deaths or signs of toxicity in animals. Ophthalmoscopy findings were normal. Feed intake and body weight development were not affected by treatment. Haematology investigations during week 13 revealed that findings in treated animals were generally similar to those in controls from the 52-week study and to pre-treatment values (Table 22).

Table 21. Summary of selected findings in a 13-week study in dogs given capsules containing novaluron

Finding	Time-point	Dose (mg/kg bw per day)							
		0	100	300	1000	0	100	300	1000
		Male				Female			
Haematology									
EC (1012/l)	Pre-treatment	6.01	6.36	6.32	6.15	6.05	6.04	6.01	6.19
	Week 6	6.67	5.81*	6.01	5.58**	6.46	5.95*	5.28***	5.34***
	Week 13	6.63	6.00	6.00	5.86*	6.47	6.00	5.05***	5.74*
EVF (l/l)	Pre-treatment	0.40	0.44	0.43	0.42	0.42	0.42	0.42	0.43
	Week 6	0.45	0.43	0.46	0.42	0.45	0.44	0.41	0.42
	Week 13	0.45	0.43	0.44	0.43	0.45	0.43	0.38*	0.43
Hb (g/dl)	Pre-treatment	13.6	14.5	14.4	13.8	13.8	13.9	13.8	14.5
	Week 6	14.8	13.4	14.0	12.8*	14.9	13.8*	12.6***	12.9***
	Week 13	14.6	13.6	13.6	13.2*	14.9	13.7	11.8***	13.3*
MCV (fl)	Pre-treatment	67.2	68.7	68.2	68.0	69.1	69.0	69.1	69.9
	Week 6	68.0	73.3*	75.8**	75.7***	69.6	73.4*	78.5***	78.7***
	Week 13	67.8	71.5	72.7*	73.6**	68.9	71.8*	75.4***	75.6***
Met-Hb (%)	Pre-treatment	0.02	0.00	0.00	0.03	0.02	0.03	0.03	0.07
	Week 6	0.07	0.12	0.73	0.63*	0.03	0.10	0.68*	0.88*
	Week 13	0.00	0.10	0.60*	0.93**	0.07	0.13	0.85**	0.98***
Reticulocytes (%)	Week 6	0.77	2.05	3.80***	3.45***	0.78	1.58	2.55**	3.33***
	Week 13	0.67	1.45*	1.55*	2.00***	0.87	1.25	1.60	2.58**
Reticulocyte inclusions	Week 4 reversibility	0	0	NT	1/2	0	0	NT	1/2
Platelets (109/l)	Pre-treatment	313	323	316	281	318	272	264	280
	Week 6	303	376	491	420	292	375	352	404
	Week 13	312	378	423	417	312	395	359	389
Sulfhaemoglobin, 'not high/high'	Pre-treatment	n 6/6	n 6/6	n 4/4	n 6/6	n 6/6	n 6/6	n 4/4	n 6/6
	Week 6	n 6/6	n 6/6	n 4/4	n 6/6	n 6/6	n 6/6	n 4/4	n 6/6
	Week 13	n 6/6	n 5/6 h 1/6	n 4/4	n 4/6 h 2/6	n 6/6	n 6/6	n 4/4	n 5/6 h 1/6
Heinz bodies	Pre-treatment	0/6	0/6	0/4	0/6	0/6	0/6	0/4	0/6
	Week 6	0/6	2/6	3/4	5/6	0/6	0/6	1/4	6/6
	Week 13	0/6	6/6	4/4	6/6	0/6	6/6	4/4	6/6
Howell-Jolly bodies	Pre-treatment	0	0	0	0	0	0	0	0
	Week 6	0	0	0	0	0	0	0	0
	Week 13	0	1/6	3/4	5/6	0	0	0	3/6
Normoblasts	Pre-treatment	0	0	0	0	0	0	0	0
	Week 6	0	0	0	1/6	0	0	1/4	0
	Week 13	0	0	0	0	0	0	0	0
Blood chemistry									

Finding	Time-point	Dose (mg/kg bw per day)							
		0	100	300	1000	0	100	300	1000
		Male				Female			
Bilirubin, total (μmol/l)	Pre-treatment	1	1	1	1	1	1	0	1
	Week 6	0	2***	3***	2***	1	3*	4*	4**
	Week 13	1	2	3*	3**	1	3**	4***	6***
	Week 4 reversibility	1	1	NT	1	1	1	NT	2
Organ weights									
Spleen:									
Absolute (g)	Week 13	75	90	111	128*	71	104	100	143*
	Week 4 recovery	111	48	NT	83	62	70	NT	64
Relative (%)	Week 13	0.6	0.7	0.8	0.9	0.5	0.9	0.8	1.1*
	Week 4 recovery	0.8	0.4	NT	0.7	0.5	0.6	NT	0.5
Liver:									
Absolute (g)	Week 13	447	462	532*	480	420	416	519	515
	Week 4 recovery	429	396	NT	405	370	372	NT	403
Relative (%)	Week 13	3.5	3.4	3.7	3.4	3.2	3.4	4.0	4.1
	Week 4 recovery	3.2	3.2	NT	3.2	2.9	3.0	NT	3.3
Histopathology									
Liver: pigmented Kupffer cells	Week 13	0/4	3/4	4/4*	4/4*	0/4	3/4	4/4*	4/4*

From Thirlwell (1998a)

EC, erythrocyte count; EVF, erythrocyte volume fraction; h, high; MCV, mean corpuscular volume; NT, not tested; n, not high.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Reticulocyte counts were significantly higher in females than in the controls during week 13; however, the study author stated that the values were within the anticipated normal range. One female animal had a high sulfhaemoglobin reading; in the absence of any other haematological changes, this finding is not considered to be toxicologically relevant. Clinical chemistry findings were normal. There were no treatment-related gross findings at necropsy, significant organ weight differences or microscopic findings when compared with historical data.

The NOAEL for novaluron in the 90-day study in dogs was considered to be 10 mg/kg bw per day (Thirlwell, 1998c).

Groups of four male and four female beagle dogs were given capsules containing novaluron (purity, 99.7%) at a dose of 0, 10, 100 or 1000 mg/kg bw per day for 52 weeks. The appropriate amount of the test material, based on the most recent body weight of each animal, was weighed into gelatin capsules. Purity and stability studies were conducted. Animals were observed daily for mortality and signs of toxicity. Feed consumption and body weight were measured weekly during the first 14 weeks and at 4-week intervals thereafter. An ophthalmological examination was performed on each animal before and during week 51. Blood samples were obtained via the jugular vein from overnight-fasted animals for haematology and blood chemistry assessments before the start of treatment and before dosing during weeks 13, 26 and 52. Urine analysis was performed before the start of dosing and during weeks 12, 25 and 51. Detailed necropsy examinations were performed on all animals at the end of the treatment, and organ weights were recorded. Histopathological examinations were performed on a range of organs and tissues.

Table 22. Summary of selected findings from a 13-week study in dogs given capsules containing novaluron

Parameter	Time-point	Dose (mg/kg bw per day)			
		0[a]	10	0[a]	10
		Male		Female	
EVF (l/l)	Pre-treatment	0.41	0.38	0.38	0.40
	Week 13	0.40	0.40	0.40	0.40
Hb (g/dl)	Pre-treatment	13.7	12.3*	12.2	12.8
	Week 13	13.4	13.1	13.2	13.3
EC (1012/l)	Pre-treatment	6.28	5.78	5.61	6.18
	Week 13	6.03	5.91	5.86	6.15
MCV (fl)	Pre-treatment	66.2	65.7	66.9	64.7
	Week 13	67.0	67.6	67.5	65.6
Reticulocytes (%)	Pre-treatment	1.2	0.6	0.7	0.8
	Week 13	1.1	1.0	0.8	1.7**
Met-Hb (%)	Pre-treatment	0	0	0	0
	Week 13	0	0	0.1	0

From Thirlwell (1998c)

EC, erythrocyte count; EVF, erythrocyte volume fraction; MCV, mean corpuscular volume; Met-Hb, methaemoglobin.

[a] Control data was obtained from 52-week study in dogs

* $p < 0.05$; ** $p < 0.01$

The study was certified to comply with GLP and designed to meet the requirements of OECD test guideline 408.

Two females at 1000 mg/kg bw per day were killed on humane grounds during weeks 15 and 23. Macroscopic and histopathological findings for both animals indicate that the deaths were not treatment-related. There were no treatment-related clinical signs. Ophthalmoscopy at week 51 did not reveal any treatment-related ocular effects. Feed consumption was not affected by treatment. Body-weight gain was increased in the both sexes at 1000 mg/kg bw per day.

Haematology revealed changes associated with erythrocyte status that ranged from minimal at 10 mg/kg bw per day to marked changes at 1000 mg/kg bw per day (Table 23). At week 13, slightly low EVFs, high Hb and erythrocyte counts were observed in males at 100 mg/kg bw per day and in both sexes at 1000 mg/kg bw per day. Also, high MCVs, reticulocyte counts and methaemoglobin concentrations were observed in both sexes at 100 mg/kg bw per day and above, when compared with values for controls. Howell-Jolly and Heinz bodies were observed in both sexes at 100 mg/kg bw per day and above. Low MCHCs were observed in males, while the females had slightly low MCHs at 1000 mg/kg bw per day. Sulfhaemoglobin concentration was high in one female at 100 mg/kg bw per day and in both sexes at 1000 mg/kg bw per day. During weeks 26 and 52, the changes observed at week 13 in animals at the highest dose and in males at 100 mg/kg bw per day were still evident. Males and females at 100 mg/kg bw per day still showed reticulocytosis and the presence of Howell-Jolly and Heinz bodies, but in females, the other erythrocyte findings were not so clearly apparent.

At 10 mg/kg bw per day, Howell-Jolly bodies were seen in all males in week 26 and in one male in week 52, and Heinz bodies in one male in week 26 (Table 23). Of these, Howell-Jolly bodies in all dogs at 10 mg/kg bw per day at week 26 was not a consistent and repeatable observation. Howell-Jolly bodies in a single male at 10 mg/kg bw per day at week 52 were not part of a clear treatment-related trend, despite the next dose being ten times, and Heinz bodies in a

single male at week 26 lacked both repeatability and a clear treatment-related trend. These findings are sufficiently inconsistent in nature that, in the absence of any corroborative changes at this dose, they are not considered to be representative of an adverse effect.

Biochemical investigations on the blood plasma in weeks 13, 26 and 52 showed a slight increase in total bilirubin concentration in females receiving a dose of 1000 mg/kg bw per day, but no clear and consistent effects were seen in males (Table 23). A number of other intergroup differences attained statistical significance when compared with the controls ($p < 0.05$), but they were minor, lacked a dose–response relationship or were inconsistent between examinations and were therefore attributed to normal biological variation.

Absolute and relative liver weights were increased in males at 100 mg/kg bw per day and above, and in two males at 10 mg/kg bw per day. In several females, absolute spleen weights were higher than those of controls , but relative spleen weights were greater than those of the controls in occasional animals receiving a dose of 100 or 1000 mg/kg bw per day (Table 24).

There were no macroscopic observations considered to be attributable to treatment. Microscopic changes considered to be attributable to treatment were seen in the liver, spleen and bone marrow (Table 24). The livers of treated animals showed occasional cellular aggregates containing brown pigment of varying severity. In view of the presence of this finding in one male and two females of the control group, additional staining of the liver sections was performed to ascertain the nature of the brown pigment. Liver sections stained with Schmorl stain for lipofuscin and Perl stain for haemosiderin demonstrated that the pigment containing cellular aggregates that stained positively for haemosiderin and variable numbers of Kupffer cells also contained haemosiderin pigment of increased incidence and severity, when compared with controls. The amount of lipofuscin pigmentation was generally similar to that in controls. There was increased haemosiderin pigment in the Kupffer cells and cellular aggregates of the liver were seen in all animals, but more markedly in animals at 1000 mg/kg bw per day. Engorged vascular sinusoids and increased severity of red pulp congestion were seen in the spleens of animals at 100 or 1000 mg/kg bw per day. In the bone marrow, there was a dosage-related increased incidence and severity of haematopoiesis in the femoral and sternal bone marrow.

The NOAEL was 10 mg/kg bw per day on the basis of haematological changes, elevated bilirubin concentrations, increased organ weight of the spleen and histopathological changes in the spleen and liver at doses of 100 mg/kg bw per day and above. Occasional findings (Howell-Jolly bodies, Heinz bodies) in erythrocytes in males and the slightly increased haematopoiesis in sternum and/or femur in both sexes at 10 mg/kg bw per day were not considered to be adverse effects (Thirlwell, 1999).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 66 male and 66 female Crl:CD-1(ICR)BR mice were given diets containing novaluron (purity, 98.7–100.1%) at a concentration of 0, 30, 450, or 7000 ppm (corresponding to mean achieved compound intakes of 0, 3.6, 53.4 and 800.0 mg/kg bw per day for males and 0, 4.3, 63.3 and 913.4 mg/kg bw per day for females) for 18 consecutive months. Of the 66 animals of each sex per dose, 15 of each sex were designated specifically to provide blood samples for haematological investigations only. All animals were assessed for clinical signs, body weight, feed consumption and palpable swellings, organ weights were measured, and macro- and microscopic (only the control group and group receiving the highest dose) pathology investigations were carried out. Selected tissues including kidneys, liver, lungs, spleen and grossly abnormal tissues for animals at the lowest and intermediate doses, which were killed on completion of treatment were examined microscopically. Homogeneity, stability and distribution of test material in diets were determined and diets were analysed for achieved concentrations. The study was certified to comply with GLP and designed to meet the requirements of the OECD test guidelines.

Table 23. Summary of body weight and haematology findings from a 52-week study in dogs given capsules containing novaluron

Finding	Time-point	Dose (mg/kg bw per day)							
		0	10	100	1000	0	10	100	1000
		Males				Females			
Body-weight gain (kg)	Weeks 0–52	4.4	3.8	4.5	5.2	4.0	3.5	4.3	5.1
Haematology									
EVF (l/l)	Week 13	0.40	0.42	0.39	0.37	0.40	0.41	0.41	0.36
	Week 26	0.42	0.44	0.43	0.40	0.43	0.43	0.43	0.41
	Week 52	0.48	0.46	0.45	0.45	0.48	0.47	0.47	0.45
Hb (g/dl)	Week 13	13.4	13.5	12.2	11.7*	13.2	13.4	12.8	11.1
	Week 26	13.9	13.6	13.2	12.2	14.0	13.8	13.4	12.3
	Week 52	15.5	14.7	13.8	13.4	15.7	15.1	14.2	13.3
EC (1012/l)	Week 13	6.03	6.17	5.54	5.17	5.86	5.89	5.83	5.09
	Week 26	6.27	6.33	5.97	5.43	6.19	6.08	6.10	5.68
	Week 52	6.97	6.65	6.19	6.01	6.88	6.59	6.41	6.09
MCH (pg)	Week 13	22.2	21.9	22.0	22.6	22.5	22.7	22.2	21.9
	Week 26	22.1	21.7	22.1	22.5	22.6	22.7	22.0	21.6
	Week 52	22.3	22.2	22.2	22.5	22.8	22.9	22.1	21.8
MCHC (g/dl)	Week 13	33.1	32.2*	31.5***	31.2***	33.3	32.7*	31.3***	30.8***
	Week 26	32.9	31.1***	30.8***	30.5***	32.6	32.0	30.8**	30.0***
	Week 52	32.2	31.7	30.8**	29.7***	32.4	31.8	30.3**	29.8**
MCV (fl)	Week 13	67.0	67.9	70.0	72.6*	67.5	69.6	70.4	70.9
	Week 26	67.2	69.8	71.8	73.9**	69.4	70.9	71.5	71.8
	Week 52	69.1	69.9	72.0	75.5	70.3	72.0	72.9	73.3
Reticulocytes (%)	Week 13	1.13	1.20	2.55	3.73	0.75	1.10	2.08	3.46
	Week 26	0.69	1.27	2.74***	4.34***	0.63	1.13	1.61	3.75
	Week 52	0.79	0.95	2.24*	3.55***	0.52	0.95	1.74	4.15
Met-Hb (%)	Week 13	0.00	0.05	0.20	0.75#	0.08	0.00	0.13	1.15
	Week 26	0.00	0.00	0.10	0.80#	0.10	0.03	0.50	1.65
	Week 52	0.00	0.00	0.18	0.38	0.03	0.00	0.20	0.85
Sulf-Hb (“high”)	Week 13	0/4	1/4	0/4	2/4	0/4	0/4	1/4	3/4
	Week 26	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/2
	Week 52	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/2
Howell-Jolly bodies	Week 13	0/4	0/4	1/4	3/4	0/4	0/4	3/4	3/4
	Week 26	0/4	4/4	4/4	4/4	0/4	0/4	4/4	2/2
	Week 52	0/4	1/4	1/4	4/4	0/4	0/4	2/4	2/2
Heinz bodies	Week 13	0/4	0/4	2/4	4/4	0/4	0/4	3/4	4/4

	Week 26	0/4	1/4	3/4	4/4	0/4	0/4	3/4	2/2
	Week 52	0/4	0/4	3/4	4/4	0/4	0/4	4/4	2/2
Clinical chemistry									
Bilirubin (µmol/l)	Week 13	1	1	2	2#	1	2	1	3**
	Week 26	1	1	1	2	0	1	2*	4***
	Week 52	1	2	2*	3*	1	2	2	5***

From Thirlwell (1999)

EC, erythrocyte count; EVF, erythrocyte volume fraction; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean corpuscular volume; Met-H, methaemoglobin; Sulf-Hb, sulfhaemoglobin..

* $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$ (Dunnett's t-test); # $p < 0.05$ (Wilcoxon rank sum test with Bonferroni adjustment).

There were no treatment-related deaths or signs of toxicity during the study. Statistical analysis of differential mortality for males and females, whether humane kills were included or not, did not indicate any significant difference (Table 25). The group distribution, incidence and onset of palpable swellings did not show any relationship to treatment. Feed consumption was not affected by treatment, but body-weight development showed a treatment-related increase during the first 4 weeks for both sexes at 450 pm and above. Body-weight gain was increased in females only for up 26 weeks at 450 ppm and above and for 52 weeks at 20 000 ppm (Table 25).

Haematology investigations showed treatment-related changes in erythrocyte-related parameters in both sexes at doses of 450 ppm and above. The effects tended to be more severe in females. The observed changes at 450 ppm and above were reduced EVF, Hb and erythrocyte count and increased MCV and reticulocyte count (Table 26). Changes in MCH and MCHC were slight in both sexes. Methaemoglobin could not be measured because of turbidity, but sulfhaemoglobin (a derivative) was found to be present at doses of 450 ppm and above in both sexes. Heinz bodies, and variants including refractile bodies and extruded bodies were found at doses of 450 ppm and above, but during week 13, Heinz bodies were also observed to be present in males and females at 30 ppm (Table 25). The occurrence of Heinz bodies at 30 ppm during week 13 appeared to be an isolated haematological change at that dose and is considered to be of limited toxicological significance. Sulfhaemoglobin was present throughout the study in some animals at 7000 ppm and, on isolated occasions, in a few animals receiving 450 ppm.

Necropsy examination revealed increased incidence of swollen spleen in both sexes at doses of 450 ppm and above. The absolute and relative spleen weights of females were significantly increased at 450 ppm and above compared with controls. Absolute and relative liver weight was also significantly increased in females at 7000 ppm (Table 26).

There was no treatment-related change in the incidence of neoplastic findings. Statistically non-significant but slightly higher incidences of benign hepatocellular adenomas and malignant hepatocellular carcinomas were seen in the livers of treated males when compared with controls, benign adenomas of the adrenal cortex in treated males and two benign papillomas of the gall bladder in males at 7000 ppm, with none being found in the controls. These findings were considered to be incidental and not related to treatment. There were higher incidences of malignant lymphoma in treated females compared with the controls, but this finding was considered to be caused by an unusually low incidence of this tumour in the controls and not related to treatment.

The main treatment-related histopathological findings were non-neoplastic and included increased extramedullary haematopoiesis and haemosiderosis in the spleens of males and females at doses of 450 ppm and above; splenic congestion in males at 450 ppm and above; increased incidence of cortical tubular pigment in the kidneys of females at 450 ppm and above; and reduced incidence of cortico-medullary ceroid pigment in the adrenals of females at 7000 ppm (Table 25).

Table 24. Summary of organ weight and histopathology findings from a 52-week study in dogs given capsules containing novaluron

Parameter	Dose (mg/kg bw per day)							
	0	10	100	1000	0	10	100	1000
	Males				Females			
Organ weights								
Spleen:								
Absolute (g)	66	105	149*	168**	77	91	107	121*
Relative (%)	0.48	0.80	1.13**	1.21**	0.60	0.71	0.89	0.91
Histopathology								
Liver:	4	4	4	4	4	4	4	4
Cellular aggregates containing brown pigment:								
Minimal	1/4	0/4	3/4	0/4	1/4	1/4	1/4	1/4
Slight	0/4	1/4	0/4	1/4	1/4	0/4	2/4	0/4
Moderate	0/4	1/4	0/4	3/4	0/4	0/4	1/4	2/4
Marked	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Kupffer cell: Perl stain-positive pigment:								
Minimal	1/4	2/4	1/4	0/4	0/4	3/4	0/4	0/4
Slight	2/4	1/4	2/4	0/4	1/4	1/4	2/4	1/4
Moderate	1/4	1/4	1/4	1/4	1/4	0/4	1/4	1/4
Marked	0/4	0/4	0/4	2/4	0/4	0/4	1/4	2/4
Severe	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Spleen:								
Red pulp congestion:								
Minimal	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Slight	0/4	1/4	0/4	0/4	1/4	2/4	0/4	1/4
Moderate	2/4	1/4	1/4	0/4	3/4	2/4	2/4	0/4
Marked	1/4	2/4	3/4	4/4	0/4	0/4	2/4	3/4
Engorged sinusoids	0/4	0/4	1/4	4*/4	0/4	0/4	2/4	2/4
Sternum:								
Increased haematopoiesis								
Slight	0/4	3/4	3/4	0/4	1/4	1/4	2/4	0/4
Moderate	0/4	0/4	1/4	4/4	0/4	0/4	2/4	4/4
Femur:								
Increased haematopoiesis								
Slight	1/4	2/4	3/4	0/4	0/4	3/4	0/4	0/4
Moderate	0/4	0/4	1/4	4/4	0/4	0/4	4/4	4/4

From Thirlwell (1999)

* $p < 0.05$; ** $p < 0.01$ (Dunnett's t-test)

Table 25. Summary of selected findings from a 78-week study in mice given diets containing novaluron

Parameter	Dietary concentration (ppm)							
	0	30	450	7000	0	30	450	7000
	Males				Females			
Mortality up to week 78								
No. of decedents (n = 51)[a]	21	16	12	16	11	12	14	14
Survival (%)	59	69	76	69	78	76	73	73
Body-weight gain (g), group means								
Weeks 0–4	9.4	9.9	11.8**	11.9**	4.1	4.5	6.8**	7.1**
Weeks 0–26	21.3	19.9	22.1	22.6	12.8	13.2	16.1*	16.6*
Weeks 0–50	25.1	23.7	26.4	26.1	17.8	18.8	20.0	22.5*
Organ weights, week 78								
Spleen:								
Absolute weight (g)	0.171	0.167	0.189	0.201	0.138	0.181	0.220**	0.333*
Relative weight (% of bw)	0.3190	0.3219	0.3329	0.3779	0.3281	0.4201	0.4602*	0.7717*
Liver:								
Absolute weight (g)	2.93	2.77	3.13	2.88	1.95	2.03	2.23*	2.34**
Relative weight (% of bw)	5.367	5.375	5.506	5.306	4.464	4.567	4.588	5.011*
Macroscopic pathology								
Spleen, swollen (n = 51)[b]	15	16	26*	36***	12	13	35***	39***
Microscopic pathology								
Liver, pigment-laden Kupffer cells (n = 51)[b]	6	4	14	25***	11	8	10	29***
Spleen (n = 51)[b]:								
Increased extramedullary haematopoiesis	19	25	32*	41***	22	25	42***	35*
Increased haemosiderosis	0	1	22***	29***	5	5	27***	41***
Congestion	1	2	13***	14***	2	0	3	9
Kidneys, cortical tubular pigment (n = 51)[b]	1	3	3	2	2	1	7	13**
Adrenals, cortico-medullary ceroid pigment[c]	0/51	2/21	1/14	0/51	12/51	2/12	1/15	2**/51

From Thirlwell (2000b)

[a]Total No. of animals

[b] No. of animals examined

[c]No.of animals showing condition/No. of animals examined

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Dunnett's t-test)

The NOAEL in the 18-month dietary study in mice was 30 ppm (equal to 3.6 mg/kg bw per day) on the basis of changes in haematological parameters, changes in organ weights of the spleen and liver (females only) and histopathological changes in the spleen at doses of 450 ppm, equal to 53.4 mg/kg bw per day, and above (Thirlwell, 2000b).

Table 26. Summary of selected haematology findings from a 78-week study in mice given diets containing novaluron

Parameter	Time-point	Dietary concentration (ppm)							
		0	30	450	7000	0	30	450	7000
		Males				Females			
EVF (l/l)	Week 13	0.40	0.40	0.39	0.38**	0.41	0.40	0.39*	0.38***
	Week 26	0.38	0.38	0.37	0.36	0.42	0.40	0.37***	0.37***
	Week 52	0.40	0.41	0.37*	0.36***	0.41	0.40	0.38	0.36**
	Week 78	0.41	0.40	0.39	0.35	0.37	0.37	0.35	0.34
Hb (g/dl)	Week 13	13.2	13.0	12.4*	12.3*	13.6	13.3	12.9*	12.6**
	Week 26	12.4	12.6	12.3	12.0	13.7	13.2	12.4***	13.0
	Week 52	12.6	12.5	11.3***	11.3**	12.7	12.3	11.6**	11.5**
	Week 78	12.8	12.9	12.4	11.6	11.7	11.5	11.0	10.8
EC (10^{12}/l)	Week 13	8.60	8.52	7.90**	7.85**	8.69	8.50	8.05**	7.72***
	Week 26	8.12	8.25	7.90	7.72	8.87	8.46	7.76***	7.76***
	Week 52	8.24	8.32	7.18***	7.12***	7.89	7.69	7.17*	6.78***
	Week 78	8.29	8.54	7.93	7.08	7.42	7.55	6.85	6.56*
MCH (pg)	Week 13	15.3	15.3	15.7	15.7	15.7	15.6	16.0	16.4**
	Week 26	15.3	15.3	15.5	15.6	15.5	15.6	16.0	16.8***
	Week 52	15.3	15.1	15.7	15.9	16.1	16.0	16.3	17.1**
	Week 78	15.6	15.1	15.7	16.4*	15.8	15.2	15.9	16.5
MCHC (g/dl)	Week 13	32.7	32.2	32.2	32.8	33.0	32.8	32.9	33.6
	Week 26	33.0	32.9	33.1	33.7	33.1	32.7	33.2	34.7***
	Week 52	31.2	30.8	30.3**	31.7	31.1	30.8	30.8	32.1
	Week 78	31.6	32.0	31.6	32.8	31.8	31.4	31.6	32.1
MCV (fl)	Week 13	46.9	47.5	48.8*	47.8	47.6	47.5	48.6	48.8
	Week 26	46.4	46.5	46.9	46.3	46.9	47.7	48.1	48.3
	Week 52	49.0	49.0	51.9*	50.3	51.7	51.8	52.7	53.2
	Week 78	49.2	47.1	49.5	50.1	49.5	48.5	50.5	51.6
Reticulocytes (%)	Week 13	2.2	2.7	3.5**	4.5***	2.1	2.4	3.4	5.4***
	Week 26	0.7	0.6	1.3#	2.5#	0.6	0.7	2.8###	5.5###
	Week 52	0.7	1.4	1.5	3.3##	0.7	1.0	1.6	3.2#
	Week 78	1.2	1.1	1.9	2.4	1.3	1.3	2.4	3.5**
Met-Hb (%)	Week 13	0.82	0.77	0.83	0.72[b]	0.91	0.92	0.96	[a]
	Week 26	0.90	0.94	0.94	[a]	0.99	0.96	0.92	[a]
	Week 52	0.79	0.70	0.75	[a]	0.78	0.67	0.93	[a]
	Week 78	0.64	0.55	0.61	[a]	0.63	0.72	0.73	[a]
Sulf-Hb (“high”)	Week 13	0/10	0/10	0/10	3/10	0/10	0/10	0/10	1/10
	Week 26	0/10	0/10	1/10	5/10	0/10	0/10	0/10	1/10
	Week 52	0/10	0/10	2/10	5/10	0/10	0/10	1/10	1/10
	Week 78	0/10	0/10	3/10	5/10	0/10	0/10	3/10	0/10
Heinz bodies	Week 13	0/10	8/10	10/10	10/10	0/10	4/10	9/10	10/10

Parameter	Time-point	Dietary concentration (ppm)							
		0	30	450	7000	0	30	450	7000
		Males				Females			
	Week 26	0/10	0/10	8/10	7/10	0/10	0/10	7/10	6/10
	Week 52	1/10	1/10	10/10	10/10	0/10	1/10	10/10	10/10
	Week 78	3/10	0/10	8/10	10/10	0/10	0/10	8/10	10/10
Refractile bodies	Week 13	0/10	0/10	0/10	3/10	0/10	0/10	0/10	0/10
	Week 26	0/10	0/10	10/10	9/10	0/10	0/10	10/10	10/10
	Week 52	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	Week 78	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Extruded bodies	Week 13	0/10	0/10	2/10	8/10	0/10	0/10	7/10	10/10
	Week 26	0/10	0/10	10/10	9/10	0/10	0/10	10/10	10/10
	Week 52	0/10	0/10	2/10	8/10	0/10	0/10	5/10	10/10
	Week 78	0/10	0/10	1/10	10/10	0/10	0/10	6/10	10/10

From Thirlwell (2000b)

EVF, erythrocyte volume fraction; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean corpuscular volume; Met-Hb, methaemoglobin; Sulf-Hb, sulfhaemoglobin.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Dunnett's t-test)

# $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ (Wilcoxon rank sum test)

[a] Insufficient data—turbidity.

[b] Results only taken from five animals—high turbidity.

Rats

Groups of 72 (52 for main group and 20 for satellite toxicity phase group) male and 72 female Fischer (F344/DuCrj) rats were given diets containing novaluron (purity, 99.3%) at a concentration of 0, 25, 700 or 20 000 (females only) ppm (corresponding to achieved daily intakes of 0, 1.1, 30.6, and 884.2 mg/kg bw per day for males and 0, 1.4, 39.5, and 1113.5 mg/kg bw per day for females in the main group, and 0, 1.3, 36.0, and 1029.9 mg/kg bw per day for males and 0, 1.6, 45.4, and 1305.8 mg/kg bw per day for females in the satellite group) for up to 2 years. Access to feed and water were provided ad libitum. Animals were assessed for clinical signs, feed consumption, body-weight changes, ophthalmoscopy, haematology, clinical chemistry, and urine analysis. All surviving animals were killed after their scheduled treatment period and organs and tissues were examined macroscopically. Organ weights were recorded at necropsy. Histopathology was performed on the animals subjected to scheduled sacrifices after 12 months and 24 months of treatment and animals found dead or killed in extremis during the treatment period. Homogeneity, stability and distribution of active substance was investigated and acceptability of test diets confirmed. The study was certified to comply with GLP and designed to meet the requirements of the OECD and the USEPA test guidelines.

There were treatment-related trends in mortality during the study (Table 27). For the animals in the toxicity phase, six animals (one male at 25 ppm, one male and one female at 700 ppm, and two females at 20 000 ppm) died or were killed prematurely during the 52-week treatment period. There were no treatment-related signs of toxicity. The incidence and mean time of onset of palpable swellings did not show any significant intergroup differences.

Table 27. Summary of selected findings in a 2 year study in rats given diets containing novaluron

Finding	Dietary concentration (ppm)							
	0	25	700	20 000	0	25	700	20 000
	Males				Females			
Achieved dosage (mg/kg bw per day)								
Toxicity phase	0.0	1.3	36.0	1029.9	0.0	1.6	45.4	1305.8
Oncogenicity phase	0.0	1.1	30.6	884.2	0.0	1.4	39.5	1113.5
Mortality								
Toxicity phase (n = 20)[a]:								
Decedents up to week 52	0	1	1	0	1	0	1	2
Oncogenicity phase (n = 20)[a]								
Decedents up to week 104	32	27	26	31	39	38	36	38
Survival (%) to week 104	38	48	50	40	25	27	31	27
Body-weight gain (g)								
Toxicity phase:								
Weeks 0–4	187	195	206**	221**	96	96	102	111**
Weeks 0–14	339	356	372	390	178	174	177	185
Oncogenicity phase:								
Weeks 0–4	196	194	201	212**	97	99	103*	109**
Weeks 0–14	363	363	360	385	176	182	183	185
Weeks 0–52	580	587	572	592	304	328	314	310

From Thirlwell (2000a)

* $p < 0.05$; ** $p < 0.01$ (Dunnett's t-test)

[a] Total No.of animals

Feed consumption was marginally higher in males than in controls during the early weeks of the study. There was a transient dose-related increase in body-weight gain compared with the controls, mainly during the first 4 weeks of treatment in males and females. This trend was evident in both toxicity and oncogenicity phase animals (Table 27).

Haematology showed treatment-related changes in erythrocyte-related parameters in both sexes at doses of 700 ppm and above (Table 28). The effects were more severe in females, but did not increase in severity with time. The observed changes at 700 ppm and above were reductions in EVF, Hb, erythrocyte count, MCHC and increased MCV and reticulocyte count. Other findings at 700 ppm and above were increased methaemoglobin in both sexes and increased platelet counts mainly in females. Howell-Jolly bodies and Heinz bodies were observed primarily in both sexes at 20 000 ppm, and occasionally at 25 and 700 ppm.

Clinical chemistry and urine analysis did not provide any treatment-related findings. Gross examinations at necropsy did not reveal any treatment-related abnormalities. Organ weights showed a dose-related increase in absolute and relative spleen weights in males at 20 000 ppm, and in females at 700 ppm and above. The organ-weight changes in the spleen were greater in females at 25 ppm in the animals in the toxicity phase, but not in the main study, and can be attributed to the smaller population size.

Table 28. Summary of selected haematology findings from a 2 year study in rats given diets containing novaluron

Parameter	Time-point	Dietary concentration (ppm)							
		0	25	700	20 000	0	25	700	20 000
		Males				Female			
EVF (l/l)	Week 13	0.43	0.43	0.42	0.41**	0.42	0.42	0.39***	0.38***
	Week 26	0.44	0.44	0.44	0.42*	0.42	0.42	0.39***	0.39***
	Week 52	0.44	0.45	0.44	0.43	0.41	0.41	0.38***	0.38***
	Week 78	0.44	0.44	0.43	0.43	0.42	0.43	0.41	0.39**
	Week 101/104	0.47	0.43*	0.45	0.42***	0.41	0.42	0.41	0.38
Hb (g/dl)	Week 13	14.8	14.9	14.5	14.0***	14.8	14.8	13.8***	13.3***
	Week 26	14.9	14.9	14.7	14.1***	14.6	14.3	13.4***	13.2***
	Week 52	15.0	15.1	14.6	14.3***	14.3	14.4	13.5***	13.0***
	Week 78	14.7	14.7	14.6	14.1	14.0	14.2	13.6	12.9***
	Week 101/104	15.5	14.4*	14.8	13.5***	13.9	14.5	14.1	13.5
EC (1012/l)	Week 13	8.63	8.57	8.39	7.89***	8.01	8.01	7.33***	6.87***
	Week 26	8.88	8.73	8.69	8.13***	7.94	7.79	7.28***	6.89***
	Week 52	8.73	8.69	8.34**	8.06***	7.58	7.57	7.00***	6.59***
	Week 78	8.22	8.29	8.25	7.86	7.43	7.54	7.13	6.54***
	Week 101/104	8.33	7.80	8.42	7.34***	7.03	7.59	7.18	6.58
MCH (pg)	Week 13	17.2	17.3	17.3	17.7*	18.5	18.5	18.8	19.3***
	Week 26	16.8	17.1	17.0	17.4*	18.3	18.4	18.5	19.2***
	Week 52	17.2	17.4	17.5	17.8*	18.9	19.1	19.3	19.7***
	Week 78	17.9	17.7	17.8	18.0	18.9	18.9	19.1	19.8***
	Week 101/104	18.6	18.5	17.6	18.4	19.9	19.1	19.6	20.5
MCHC (g/dl)	Week 13	34.7	34.5	34.2**	34.2***	35.3	35.3	34.8	34.5***
	Week 26	33.9	34.0	33.5**	33.3***	34.5	34.4	34.1*	33.9***
	Week 52	33.9	33.5**	33.3***	33.3***	34.6	35.2***	35.1**	34.3
	Week 78	33.2	33.3	33.7	32.8	33.6	33.2	33.4	33.2
	Week 101/104	33.0	33.1	33.1	32.2*	33.7	34.3*	34.3*	33.9
MCV (fl):	Week 13	49.6	50.2	50.5	51.9***	52.3	52.4	53.8**	56.0***
	Week 26	49.6	50.3	50.7	52.3***	53.2	53.4	54.1	56.6***
	Week 52	50.8	51.9	52.7*	53.6***	54.6	54.3	54.9	57.5***
	Week 78	53.9	53.2	52.7	54.9	56.2	56.7	57.1	59.5***
	Week 101/104	56.3	55.7	53.3*	57.1	59.0	55.8*	57.1	60.4
Platelets (109/l)	Week 13	1052	1027	1047	1099	978	1010	1089**	1166***
	Week 26	1011	1026	1036	1068	948	961	1009	1086***
	Week 52	1012	983	1013	1064	822	914#	963##	1011##
	Week 78	963	1021	971	1058	885	883	917	985
	Week 101/104	944	973	1040	1177**	980	984	902	997
Reticulocytes (%)	Week 13	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
	Week 26	3.96	3.96	4.13	5.11***	2.22	2.49	3.37###	4.90###

Parameter	Time-point	Dietary concentration (ppm)							
		0	25	700	20 000	0	25	700	20 000
		Males				Female			
	Week 52	2.45	2.34	2.65	3.64***	1.98	2.22#	3.03###	4.22###
	Week 78	2.63	2.93	2.89	3.76###	2.62	2.41	3.12#	5.29###
	Week 101/104	2.93	3.15	3.10	4.56***	4.55	3.69	3.27	5.48
Met-Hb (%)	Week 13	0.44	0.48	0.75**	1.24***	0.63	0.68	1.34***	2.12***
	Week 26	0.57	0.74	0.93###	1.33###	0.93	0.98	1.43***	2.13***
	Week 52	0.77	0.65*	0.95***	1.41***	0.76	0.60#	1.44###	1.91###
	Week 78	0.6	0.52	0.92***	1.21***	0.58	0.71	1.09***	1.70***
	Week 101/104	0.57	0.64	0.97***	1.39***	0.79	0.82	1.19###	1.83###
Heinz bodies	Week 13	0	0	0	4/20	0	0	0	8/20
	Week 26	0	1/20	0	4/20	0	0	0	6/20
	Week 52	0	0	0	4/20	0	0	0	5/19
	Week 78	0	1/20	2/20	5/20	0	0	0	7/20
	Week 101/104	0	0	0	4/10	0	0	0	2/10
Howell-Jolly bodies	Week 13	0	0	0	4/20	0	0	01/20	5/20
	Week 26	0	0	0	6/20	0	0	0	6/20
	Week 52	0	0	0	3/20	0	1/20	2/20	2/19
	Week 78	0	0	0	1/20	0	0	0	1/20
	Week 101	0	0	0	0	0	0	0	2/10

From Thirlwell (2000a)

EVF, erythrocyte volume fraction; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean corpuscular volume; Met-Hb, methaemoglobin.

[a] Manual examination; mean value not appropriate

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Dunnett's t-test);

# $p < 0.05$; ### $p < 0.01$; ### $p < 0.001$ (Wilcoxon rank sum test)

Histopathological findings were increased incidence and severity of periacinar hepatocyte hypertrophy in the males at 700 ppm and above, and increased incidence and severity of haemosiderosis in the spleen of both males and females (Table 29). The background incidence of haemosiderosis in the spleen was noted to be high in females, but the treatment-related increase in females was apparent at all doses for both the toxicity phase and main study. A marked increase in the incidence of pigment-laden Kupffer cells was observed in females at the highest dose. There was no increase in the incidence of neoplastic findings.

An increased incidence of progressive senile nephropathy was seen in the kidneys of animals at 20 000 ppm, and a statistically significant reduced incidence of hyperplasia of the white pulp of the spleen was seen in males given diets containing novaluron at 20 000 ppm.

The NOAEL was 25 ppm (equal to 1.1 mg/kg bw per day) on the basis of changes in haematological parameters, increased spleen weights and an increased incidence and severity of haemosiderosis in the spleen at doses of 700 ppm, equal to 36 mg/kg bw per day, and above (Thirlwell, 2000a).

Table 29. Summary of selected postmortem findings from a 2-year study in rats given diets containing novaluron

Finding	Dietary concentration (ppm)							
	0	25	700	20 000	0	25	700	20 000
	Males				Females			
Organ weights								
Spleen:								
Absolute weight (g), week 52	0.957	0.965	1.047	1.114*	0.598	0.676*	0.788**	0.881**
Relative (% of bw), week 52	0.143	0.137	0.146	0.151	0.145	0.159	0.191**	0.199**
Absolute weight (g), week 104	1.149	1.264	1.183	1.476**	0.782	0.777	0.928	1.164**
Relative (% of bw), week 104	0.155	0.159	0.154	0.187**	0.143	0.152	0.186*	0.226**
Microscopic pathology								
Liver, Periacinar hypertrophy, week 52								
No. examined	20	19	19	20	19	20	19	18
Slight	1	3	6	10	1	1	0	1
Moderate	0	0	0	5	0	0	0	0
Total	1	3	6*	15***	1	1	0	1
Spleen, Haemosiderosis:								
Week 52								
No. examined	20	19	19	20	19	20	19	18
Slight	1	5	6	7	4	9	6	1
Moderate	0	0	1	11	9	7	10	9
Marked	0	0	0	0	0	2	2	8
Total	1	5	7*	18***	13	18	18	18*
Week 104:								
No. examined	51	52	51	52	52	52	52	52
Light	24	23	23	14	19	17	6	1
Moderate	7	7	20	30	23	24	34	13
Marked	0	1	3	6	5	10	12	38
Total	31	31	46**	50***	47	51	52	52
Kidneys, week 104:								
No. examined	52	52	52	52	52	52	52	52
Cortical tubular pigment	0	0	3	3	4	4	10	20***
Liver, week 104:								
No. examined	52	52	52	52	52	52	52	52
Pigment laden Kupffer cells	4	0	4	6	6	4	6	17*

From Thirlwell (2000a)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Dunnett's t-test)

2.4 Genotoxicity

The results of studies of genotoxicity with novaluron are summarized in Table 30.

(a) In vitro

Novaluron (purity not stated) in DMSO was investigated for its potential to induce mutations in an assay for gene mutation in *Salmonella* in the presence and absence of metabolic activation. The study was certified to comply with GLP and satisfied the essential criteria for OECD guidelines 471 and 472. According to the results obtained, novaluron showed no mutagenic activity or toxicity in any of the strains used. Very slight precipitation occurred at the highest concentration of 3333 μg/plate (McGregor & Reynolds, 1986).

Novaluron (purity, 99.3%) was investigated for its potential to induce mutations in an assay for gene mutation in *S. typhimurium* and *E. coli*. The study was certified to comply with GLP and satisfied the essential criteria of current OECD guidelines 471 and 472. There was no substantial increase in the number of revertants in any of the test strains used in the assay. Positive controls provided the expected results, thus confirming the sensitivity of the assays. Novaluron was not genotoxic in bacteria under the conditions of this test (Gant, 1997).

Two strains *of Bacillus subtilis,* H17 *rec*+, a repair-proficient strain and M45 *rec*⁻, a repair-deficient strain, were exposed to novaluron (purity, 99.3%) in DMSO. DMSO was also used as a solvent control, with and without metabolic activation (S9 mix from rats induced with Aroclor 1254). The study was certified to comply with GLP and was conducted in accordance with USEPA guidelines.

There was a substantial difference in the relative toxicity of the two strains in all three differential killing assays in the absence of metabolic activation at the two higher concentrations. Comparable differences were also observed at lower concentrations in the second and third assays, and the patterns of toxicity seen indicated no evidence of a dose–response relationship. Thus, while these results might indicate some potential to damage bacterial DNA in the absence of metabolic activation, the findings were inconsistent and equivocal. No substantial differences in toxicity between the two strains were observed in the presence of metabolic activation. It was concluded that novaluron showed no potential to damage bacterial DNA in the presence of metabolic activation. Evidence that might indicate some potential to damage bacterial DNA was observed in the absence of metabolic activation in this test system, but the results were inconsistent and equivocal (Gant & Anderson, 1998).

Novaluron (purity, 97.5%) was investigated for clastogenic potential in an assay for chromosomal aberration in a human lymphocyte cell line. The solvent used was DMSO. In an initial assay, a precipitate was observed in the cultures treated at 1000 and 5000 μg/ml, which affected cell staining; at 5000 μg/ml, the metaphases could not be scored. A second assay was performed (in triplicate) with novaluron at lower concentrations. Concentrations were limited at approximately 1000 μg/ml by the precipitate that affected the staining of the cells, and the majority of the total metaphase cell population at this concentration could not be scored; concentrations of 200 μg/ml and lower were acceptable. Novaluron was not clastogenic in the chromosomal aberration assay in human lymphocyte cell line (Edwards, 1992).

Table 30. Results of studies of genotoxicity with novaluron

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100)	Tests 1 & 2: ±S9: 10, 33, 100, 333, 1000, 3333 µg/plate	Not stated	Negative	McGregor & Reynolds (1986)
Reverse mutation	*S. typhimurium* (TA1535, TA1537, TA98 and TA100) *E. coli* (WP2 *uvrA*)	Tests 1 & 2: ±S9: 312.5, 625, 1250, 2500, 5000 µg/plate	99.3	Negative	Gant (1997)
DNA repair	*Bacillus subtilis* H17 (rec^+) and M45 (rec^-)	One spot test: 50, 150, 500, 1500, 5000 µg/disk Three differential killing assays: 50, 150, 500, 1500, 5000 µg/ml All ±S9	99.3	Equivocal (–S9) Negative (+S9)	Gant & Anderson (1998)
Gene mutation	Mouse lymphoma L5178Y cells (*Tk* locus)	Test 1 & 2: ± S9 50, 100, 125, 150, 175, 200 µg/ml	94.3	Negative	Adams (1989)
Chromosomal aberration	Human lymphocytes	Test 1: ±S9, 5000, 1000, 200, 5000 µg/ml Test 2: ±S9 1000, 200, 40 µg/ml +S9, exposure time: 3 h; cell harvest 21 h later. –S9, exposure time: 24 h	97.5	Negative	Edwards (1992)
Unscheduled DNA synthesis	Cultured human epithelioid HeLaS3 cells	Tests 1 & 2: ±S9 0.125–256 µg/ml	94.3	Negative	Proudlock (1989)
In vivo					
Micronucleus formation	Male and female Swiss mice, bone-marrow cells	0, 1250, 2500, 5000 mg/kg bw	94.3	Negative	Henderson et al. (1989)

S9, 9000 × *g* supernatant of rat liver cells

Cultured mouse lymphoma L5178Y cells were exposed to novaluron (purity, 94.3%) in DMSO in two independent tests in the absence and presence of metabolic activation (S9 from rat liver preparations). The study was certified to comply with GLP. In the test without metabolic activation, there was no significant increase in mutant frequency after treatment with novaluron in test 1. In test 2, a twofold increase in mutant frequency was observed at 100 µg/ml. However, this increase was not reproducible at the higher concentrations tested and therefore did not fulfil the criteria required for a positive response. In both tests, in the presence of S9 there was no significant increase in mean mutant frequency after treatment with novaluron. Novaluron was not mutagenic in cultured mouse lymphoma L5178Y cells (Adams, 1989).

Cultured human epithelioid HeLaS3 cells were exposed to novaluron (purity, 94.3%) in DMSO in two tests with or without metabolic activation (S9). The study was certified to comply with GLP and was conducted in accordance with USEPA guidelines. Occasional statistically significant increases in the nuclear grain count were noted to be small, sporadic and not reproducible between tests, and do not satisfy the assessment criteria for a positive response. In

conclusion, novaluron did not show a genotoxic potential in the assay for unscheduled DNA repair synthesis in cultured human epitheloid HeLaS3 cells (Proudlock, 1989).

(b) In vivo

Groups of 15 male and 15 female Swiss mice were given novaluron (purity, 94.3%) as a single oral dose at 0, 1250, 2500 or 5000 mg/kg bw suspended in 1% aqueous methylcellulose by gavage to in order to assess the potential for induction of micronuclei in bone-marrow cells. The study was certified to comply with GLP and satisfied the essential requirements of OECD guideline 474.

Novaluron did not cause any substantial increase in the incidences of micronucleated normochromatic erythrocytes at any of the three kill times. The ratio of polychromatic to monochromatic erythrocytes at 48 h showed a small but statistically significant decrease ($p < 5\%$) at 2500 mg/kg bw. However, no other such decreases were observed at any other dose or sampling time. In addition, there was no decreasing trend in these ratios with increasing doses with time. The report concluded this decrease is not considered sufficient evidence to indicate bone-marrow toxicity. However, from other investigations of toxicity caused by novaluron, it is clear that exposure would be sufficient for the results of the assay to be valid (Henderson et al., 1989).

2.5 Reproductive toxicity

(a) Multigeneration studies

In a dietary range-finding study to support a multigeneration study of reproductive toxicity, groups of six male and six female CD rats were given diets containing novaluron (purity, 98.7–99.3%) at a concentration of 0, 5000, 10 000 or 20 000 ppm. Treatment continued throughout mating, gestation and lactation for females and until termination after 6 weeks of treatment for males. Selected F_1 offspring were treated from weaning until age approximately 6 weeks. Mean compound intakes before pairing were 458.6, 901.0 and 1844.8 mg/kg bw per day for males at 5000, 10 000 and 20 000 ppm, and 476.0, 989.5 and 2059.3 mg/kg bw per day for females, respectively, at the same doses. Achieved intakes increased between the end of gestation and the first 2 weeks of lactation, which was considered to reflect the additional feed intake required to sustain the offspring after birth.

There were no deaths among parents during the study. The general condition of parental males and females was essentially similar to that of the controls. Feed intake for all treated groups during days 8 to 10 of the pre-pairing period was higher than that of the controls. During gestation, there was a trend towards marginally higher feed consumption for females at 10 000 and 20 000 ppm during days 13 to 19 of gestation. Body-weight gain in males of all treated groups was slightly higher than that of the controls during the first 2 weeks of treatment. The overall body-weight gain of females before pairing was in all the treated groups slightly higher than that of the controls. Body-weight gains during gestation were unaffected by treatment, but body-weight gain during lactation was lower than expected for females at 20 000 ppm. Estrous cycles, mating performance and fertility, duration of gestation and the gestation index were unaffected by treatment. A loss of one litter at 10 000 ppm (day 21 after birth) and two litters at 20 000 ppm (days 3 and 15 after birth) resulted in a reduction in lactation and weaning indices at these doses.

Body weights of male and female offspring on day 1 after birth were similar for all groups. The rate of weight gain thereafter was slightly lower at 10 000 and 20 000 ppm up to days 18 and 14 of age respectively, as the mean weights were affected by those litters that died before day 21 of age. Body-weight gain at 10 000 ppm was also affected by one litter with poor growth up to weaning. The sex ratio was not affected by treatment. The body-weight gain of selected males and

females up to 6 weeks of age did not reveal any effects considered to be related to treatment with novaluron. Feed consumption in F_1 males and females was not affected. Mean achieved intakes for males and females during weeks 5 and 6 of the F_1 generation were 708 and 739 mg/kg bw per day at 5000 ppm, 1521 and 1640 mg/kg bw per day at 10 000 ppm and 3208 and 3275 ppm at 20 000 ppm, respectively. Macroscopic examination of F_0 and F_1 animals at necropsy did not reveal any treatment-related abnormalities.

The NOAEL was 5000 ppm on the basis of reduced lactation and weaning indices at 10 000 ppm and above. Target doses of 1000, 4000 and 12 000 ppm were considered for the main multigeneration study (Reynolds, 1998a).

In a two-generation study of reproductive toxicity, with one mating per generation, groups of 28 male and 28 female Crl:CD(SD)BR rats were give diets containing novaluron (purity, 99.3–99.7%.) at a concentration of 0, 1000, 4000 or 12 000 ppm continuously throughout the growth, mating, gestation and lactation phases of the study, and the offspring were exposed to the test diets throughout lactation. The growth period for the F_0 and F_1 parental animals was 10 weeks. The animals were then mated for 2 weeks to produce the F_1 and F_2 litter. Selection for the F_1 generation was made from the weaned F_1 litters. The growth period for the F_1 generation was 10 weeks. The F_1 parental animals then went through a similar reproduction sequence as the F_0 animals to produce the F_2 litters. On day 4 of lactation, the size of litters with nine or more pups was reduced to eight pups.

Parental animals were observed for clinical signs, mortality, body weight, feed consumption, vaginal smears for 14 days before mating, mating procedure, pre-coital interval (females only) parturition and duration of gestation. Litters were investigated for: observations at day 1 of age (including the number of live and dead offspring, birth weights, sex distribution and individual observations of offspring), clinical signs, mortality and litter size, sex ratio, body weight. Terminal studies on parental animals included gross findings at necropsy and histopathological examinations (for the control group and the groups at 12 000 ppm only). F_0 and F_1 males were investigated for seminology (including assessments of sperm motility, sperm count, sperm morphology and counts of homogenization resistant spermatids). F_1 and F_2 offspring, which were not selected for further development were subjected to necropsy, histopathology and organ weight examinations. The study was certified to comply with GLP and designed to meet the requirements of OECD test guideline 416. The achieved compound intakes during the study are shown in Table 31.

There was no treatment related effect on the incidence of deaths or clinical signs during the study. Two deaths occurred in the F_0 generation, one control male and a female at 4000 ppm. In the F_2 generation two females died, both at 12 000 ppm. On the basis of the findings for individual animals, it was considered that there was no clear relationship with treatment.

Minor differences in feed consumption were not considered to be treatment-related. In each generation, the weight gain of males and females in all treated groups was increased for the first few weeks of treatment compared with that of controls. For females, weight gain during gestation was slightly increased in all treated groups in the F_0 generation and at 12 000 ppm in the F_2 generation.

No effects of treatment were apparent. Mating performance and fertility as assessed by pre-coital interval and the number of animals mating and achieving pregnancy, was not affected by treatment with novaluron. A slight increase in pre-coital interval for animals in the F_1 generation at 4000 and 12 000 ppm was considered to be incidental. Gestation durations were within the expected range of 22 to 23.125 days. Parturition was unaffected by treatment.

There was no effect on total litter size at birth in either generation. There were some minor differences in litter size for live pups at birth and during lactation, but the extent of the differences was too small to indicate a relationship with treatment. There was a low incidence of total litter losses in treated groups in both generations, which in some cases at 12 000 ppm was associated with poor maternal condition and humane sacrifice, but was considered not to be treatment related.

Table 31. Summary of selected findings in a multigeneration study in rats given diets containing novaluron

Parameter	Dietary concentration (ppm)							
	0	1000	4000	12 000	0	1000	4000	12 000
	Males				Females			
Substance intake (mg/kg bw per day)								
F0 —before pairing	0	74.2	297.5	894.9	0	84.0	336.7	1009.8
—during gestation	—	—	—	—	0	79.3	316.1	948.0
—during lactation	—	—	—	—	0	148.1	572.8	1689.6
F1 —before pairing	0	97.8	390.2	1182.6	0	108.5	432.5	1306.8
—during gestation	—	—	—	—	0	80.5	316.6	951.3
—during lactation	—	—	—	—	0	131.6	492.8	1428.0
F0 generation								
Body-weight gain (g):								
Weeks 0–17/0–10 (M/F)	407	430	428	438	153	158	154	162
Days 0–20 of gestation	—	—	—	—	140	152	145	152**
Days 1–21 of lactation	—	—	—	—	36	26	27	32
Body weight (g), terminal	615.3	638.4	629.5	643.5	348.8	354.0	357.7	368.0*
Organ weights:								
Spleen —absolute (g)	0.824	0.955**	0.972**	1.042**	0.610	0.766**	0.768**	0.852**
—relative (% of bw)	0.134	0.150**	0.155**	0.163**	0.175	0.217**	0.214**	0.231**
Liver —absolute (g)	22.8	24.2	23.9	24.3	21.3	20.6	22.2	22.0
—relative (% of bw)	3.70	3.80	3.80	3.78	6.10	5.85	6.20	5.98
Histopathology								
Spleen —haemosiderosis	5/26	4/4	1/1	28/28	3/28	3/4	2/3	22***/28
Uterus —haemosiderosis	—	—	—	—	0/28	—	—	9**/28
F1 generation								
Body weight (g), at day 35 of age[a]	141.4	145.9	140.3	147.3	122.2	128.5	121.9	131.1*
Organ weights, at day 35 of age[a]								
Spleen —absolute (g)	0.526	0.598**	0.563	0.617***	0.435	0.497**	0.465	0.520***
—relative (% of bw)	0.370	0.409*	0.408*	0.420***	0.352	0.389*	0.381	0.398**
Liver —absolute (g)	8.436	9.258	8.774	9.460*	7.577	8.051	7.916	8.712**
—relative (% of bw)	5.997	6.354*	6.439*	6.408*	6.217	6.310	6.562	6.631*
Body-weight gain (g):								
Weeks 0–17/0–10 (M/F)	541	554	557	559	216	225	227	241**
Days 0–20 gestation	—	—	—	—	139	141	141	154**
Days 1–21 lactation	—	—	—	—	30	19	23	19
Body weight (g), terminal	626.6	637.1	640.1	645.2	336.8	347.9	361.8**	370.8**
Organ weights, terminal								
Spleen —absolute (g)	0.887	1.017**	1.064**	1.079**	0.654	0.803**	0.781**	0.896**
—relative (% of bw)	0.142	0.160**	0.167**	0.168**	0.195	0.232**	0.216*	0.243**
Liver —absolute (g)	22.5	23.7	24.7	24.1	19.9	19.9	20.8	21.1

Parameter	Dietary concentration (ppm)							
	0	1000	4000	12 000	0	1000	4000	12 000
	Males				Females			
Histopathology								
Spleen —haemosiderosis	0/28	0/1	0/0	6*/27	0/28	0/0	0/0	20***/26
Liver —periacinar hypertrophy	0/28	0/0	0/2	14***/28	0/28	0/0	0/1	0/26
—centriacinar fatty vacoulation	0/28	0/0	0/2	0/28	1/28	0/0	0/1	6*/26
F2 generation								
Body weight (g), at day 35 of age	143.4	152.3	146.7	145.0	123.8	128.9	127.7	125.3
Organ weights								
Spleen —absolute (g)	0.524	0.569	0.553	0.581*	0.418	0.461*	0.456	0.474**
—relative (% of bw)	0.365	0.376	0.378	0.402**	0.336	0.360	0.358	0.381***
Liver —absolute (g)	8.641	9.604**	9.494*	9.266	7.620	8.349*	8.494**	8.326*
—relative (% of bw)	6.014	6.306*	6.474***	6.389**	6.143	6.471*	6.648***	6.631***

From Blee (1999)

F, female; M, male

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

[a] Body weights and organ weights from F_1 offspring not selected for continuation in the study.

There was no clear effect on the body weight of offspring in either generation. Sexual maturation of the F_1 generation as assessed by the mean age of attainment of vaginal opening and balano-preputial separation in males was unaffected by treatment.

Macroscopic examination of offspring did not reveal any treatment-related changes, but there was a tendency for slightly higher spleen and liver weights for the offspring from treated groups in both generations (Table 31). Variation in other organ weights was minor and inconsistent.

Slightly higher spleen weights were recorded for the adults in both generations in all the treatment groups and histopathological examination (performed for animals at 12 000ppm) showed haemosiderosis of the spleen (Table 31). There was also an increase in liver weight for males of all treatment groups, but in the F_1 generation statistical significance occurred only at 12 000 ppm. Histopathology showed periacinar hepatocyte hypertrophy in the F_1 males at 12 000 ppm. There was an increase in the incidence of macroscopic changes for the spleen and liver associated with these differences. Haemosiderosis of the broad ligament of the uterus was observed at 12 000 ppm in some females of both generations.

Sperm analysis did not reveal any differences in sperm count, motility or morphology that were considered to be related to treatment. A reduction in epididymal sperm count among treated males of the F_1 generation was considered to be incidental.

In the multigeneration study, there was no evidence of effects on fertility or pregnancy in rats treated with novaluron at up to the highest dose tested (12 000 ppm). A NOAEL for systemic toxicity was not identified because there were secondary changes in the spleen and liver that related to increased erythrocyte damage at all doses tested. The NOAEL for systemic toxicity in parental animals and offspring was < 1000 ppm (equal to 74.2 mg/kg bw per day) on the basis of increased spleen weights in adults and increased spleen and liver weights in offspring (Blee, 1999).

(b) Developmental toxicity

Rats

In a preliminary study of prenatal developmental toxicity, groups of six mated CD rats were given novaluron (purity, 99.5–99.8%) at a dose of 0, 250, 500 or 1000 mg/kg bw per day in 1% aqueous methyl cellulose. All dams were killed on day 20 of gestation and the uterine contents were examined. The study was certified to comply with GLP. Homogeneity and stability of the test material was confirmed by analysis. There was no evidence of maternal or fetal toxicity. The main study was therefore conducted using the same doses (Reynolds, 1997c).

In a study of prenatal developmental toxicity, groups of 22 mated female Cr.CD BR rats were given novaluron (purity, 98.7–99.3%) at a dose of 0, 250, 500 or 1000 mg/kg bw per day, suspended in 1% w/v aqueous methylcellulose, by oral gavage, from day 6 to day 15 (inclusive) of gestation. Dams were investigated for mortality, signs of toxicity, body-weight change and feed consumption and gross examination at necropsy. Terminal studies on day 20 included a detailed examination of the reproductive tract during necropsy for numbers of corpora lutea in ovaries, implantations, number and distribution of fetuses, resorptions and extent of pre- and postimplantation losses. Fetuses were examined for external, visceral and skeletal abnormalities. Homogeneity and stability of the test material was investigated and found to be acceptable. The study was certified to comply with GLP and designed to meet the requirements of OECD test guideline 414.

There were no deaths or treatment-related signs of toxicity in the dams. Feed consumption was statistically significantly greater in treated groups than in controls. Body-weight gain was marginally but statistically significantly greater in all treated groups (11–16% of that of the controls), with no dose–response relationship (body-weight gain, day 6 to 16: 64, 71, 74 and 72 g for the controls, and at the lowest, intermediate and highest dose, respectively). There were no other treatment-related effects on dams or fetuses.

The NOAEL for both maternal and developmental toxicity was > 1000 mg/kg bw per day, the highest dose tested. The slight increase in body-weight gain of dams at all doses was not considered to be of toxicological relevance (Reynolds, 1997b).

Rabbits

In a range-finding study of toxicity, which was certified to comply with GLP, two non-pregnant female New Zealand White rabbits were given novaluron (purity, 99.5–99.8%) at a dose of 100, 200, 400, 800 or 1000 mg/kg bw per day, in 1% aqueous methyl cellulose, by gavage stepwise for 2 days per dose during 10 days. The formulations of test compound were demonstrated to have acceptable homogeneity and stability. Treatment did not result in deaths, signs of toxicity or gross abnormalities at necropsy. Two mated rabbits given novaluron at 1000 mg/kg bw per day from day 6 to day 12 of gestation and necropsied on day 13 did not show any signs of toxicity during the study. Body-weight gain was reduced in one animal after the onset of dosing, but improved towards the end of the treatment period. Necropsy did not reveal any abnormalities in females and there was evidence of implantations in each female (Reynolds, 1997a).

In a range-finding study of prenatal developmental toxicity, groups of four mated New Zealand White rabbits were given novaluron (purity, 99.8%) at a dose of 0, 250, 500 or 1000 mg/kg bw per day, in aqueous 1% methylcellulose, by gavage from day 6 to 19 of gestation. On day 29 of gestation, the animals were killed to allow examination of their uterine contents. Detailed examination of the reproductive tract included determination of the number of corpora lutea in ovaries, implantations, number and distribution of fetuses, resorptions and extent of pre and postimplantation losses, placental and fetal weights. Fetuses were examined for external and visceral abnormalities. The study was certified to comply with GLP and satisfies the essential

criteria of OECD guideline 414 with the exception of numbers of animals. Homogeneity and stability of the test material was confirmed by analysis.

There were no treatment-related deaths or signs of toxicity. At a dose of 1000 mg/kg bw per day, there were slightly lower mean number of implantations and a higher preimplantation loss when compared with those of the controls (Table 32). There were no other dose-related findings in dams or fetuses. The main study was therefore conducted with a highest dose of 1000 mg/kg bw per day (Reynolds, 1998b).

In the main study of prenatal developmental toxicity, groups of 22 mated female New Zealand White rabbits were given novaluron (purity, 99.3%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day, suspended in 1% w/v aqueous methylcellulose, by oral gavage from days 6 to 19 (inclusive) of gestation. Homogeneity and stability of the test material was confirmed by analysis. On day 29 of gestation, females were killed to allow examination of their uterine contents. Dams were investigated for mortality, signs of toxicity, body-weight change and feed consumption and gross examination at necropsy. Terminal studies on day 29 included a detailed examination of the reproductive tract for numbers of corpora lutea in ovaries, implantations, number and distribution of fetuses, resorptions and extent of pre- and postimplantation losses. Fetuses were examined for external, visceral and skeletal abnormalities. The study was certified to comply with GLP and designed to meet the requirements of OECD test guideline 414.

There were no treatment-related deaths or differences in general condition between treated females and controls. One control female was killed in extremis on day 8 of gestation because of a dosing accident. A female at 100 mg/kg bw per day was found dead on day 20 of gestation after exhibiting reduced faecal output and reduced feed intake from day 9 and emaciation from day 13. Three abortions occurred, two in the control group on days 17 and 29 of gestation respectively and one at 100 mg/kg bw per day on day 28 of gestation. Feed consumption was not affected by treatment. Body-weight gain of females in all treated groups showed no clear evidence of a treatment-related effect. After cessation of treatment, the body-weight gain of females that had received novaluron at a dose of 1000 mg/kg bw per day was 56% that of the controls, possibly a result of prior treatment with novaluron (Table 33).

Gross examination of females at necropsy on day 29 after mating did not reveal any treatment-related abnormalities. In females with viable young on day 29 of gestation, litter responses were considered to be unaffected by treatment. The mean placental weight for females at the highest dose was marginally higher than that for the controls, but this was influenced by one litter where only one pup was present, and was not considered to be an effect of treatment (Table 33).

One female at 1000 mg/kg bw per day had corpora lutea, but no evidence of any implantation at termination. A further female at this dose exhibited a preimplantation loss of 91%. These findings were considered to be incidental and not related to treatment.

Table 32. Summary of findings from a range-finding study of prenatal developmental toxicity in rabbits given novaluron by gavage

Parameter	Dose (mg/kg bw per day)			
	0[a]	250[a]	500	1000
Implantations (No.)	11; 11; 11	13; 11	12; 13; 12; 14	4; 15; 12; 3
Preimplantation loss (%)	21.4; 0; 15.4	18.8; 15.4	7.7; 0; 7.7; 6.7	50; 11.8; 7.7; 72.7
Postimplantation loss (%)	9.1; 81.8; 0	30.8; 27.3	8.3; 7.7; 0; 0	25; 26.7; 25; 0

From Reynolds (1998b)

[a] Two animals from the group at 250 mg/kg bw per day were not pregnant and one control animal died. The figures in each cell are the values for individual rabbits.

Table 33. Summary of main findings from a study of prenatal developmental toxicity in rabbits given novaluron by gavage

Parameter	Dose (mg/kg bw per day)			
	0	100	300	1000
Body-weight gain (kg):				
Days 6–20	0.18	0.24	0.20	0.22
Days 20–28	0.16	0.14	0.15	0.08*
Animals pregnant at term with viable young	19	17	19	17
Pregnant at term—resorbing material only	0	0	0	1
Corpora lutea present, no implantation	0	0	0	1
No. of corpora lutea	12.7	12.1	12.3	11.8
No. of implantations	10.8	10.4	11.6	9.6
No. of viable young	10.1	9.6	10.6	8.8
Resorptions, total	0.7	0.8	1.0	0.9
Preimplantation loss	15.3	14.1	6.8	18.4
Postimplantation loss	6.8	7.3	8.6	9.1
Placental weight (g)	5.6	5.7	5.6	6.2
Fetal weights (g), males/females	40.7/39.7	42.1/41.1	39.9/38.6	41.3/38.3
Fetuses/litters examined	189/19	162/17	199/19	149/17
Incomplete ossification, fifth sternebrae[a]	7/4	9/6	19/8	24/11
Other sternebrae[a]	10/6	1/1	6/2	7/4
Sternebrae, total[a]	17/10	10/7	25/9	30/12

From Reynolds (1998c)

[a] No. of fetuses/litters

* $p < 0.05$.

Fetal examination revealed a slight, not statistically significantly increased incidence of incompletely ossified fifth sternebrae at 300 and 1000 mg/kg bw per day (Table 33). In the absence of any other evidence of an effect on fetal development, the slight increase in incomplete ossification of the fifth sternebra of fetuses from groups receiving the intermediate and highest doses was not considered to be adverse. With this exception, the incidence of fetal malformations, skeletal and visceral anomalies and skeletal variants for treated groups were considered to be comparable with those of the controls.

The NOAEL for both maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Reynolds, 1998c).

2.6 Special studies

(a) Neurotoxicity

In a study of acute neurotoxicity, groups of 10 male and 10 female fasted Crl:CD BR rats were given novaluron (purity, 99.3%) as a single oral dose at 0, 200, 650 or 2000 mg/kg bw by gavage suspended in 1% w/v methylcellulose. The study was certified to comply with GLP and designed to the requirements of the guidelines for the USEPA (Pesticide Assessment Guidelines, Subdivision F, Series 81-8). All animals were subjected to assessments for mortality, clinical signs, body-weight gain and feed consumption. A functional observation battery was carried out,

before treatment, and at 1 h (day 1) 7 days (day 8) and 14 days (day 15) after dosing, to investigate any neurotoxicity effects. After 14 days (day 15) animals were killed and subject to a detailed necropsy, including brain weight and measurements of the brain. Tissues of the nervous system (animals from the control group and the group at 2000 mg/kg bw) were examined microscopically.

There were no deaths during the study. Observations at the time of peak effect on the day of administration (1 h after dosing) were performed as part of the functional observation battery. A dose-related increase in the incidence of fast respiration and piloerection was observed in both sexes at all doses and a dose–response relationship was evident for males. Recovery occurred by day 5 in males and by day 4 in females. In females, isolated incidents of vocalization, irritable behaviour and generalized brown staining was observed (Table 34).

Feed consumption and body weight development was comparable between treated and control animals. In the functional observation battery of tests; home-cage observations, in-the-hand observations, behavioural responses in the arena, manipulation observations including grip strength and motor activity showed no adverse reaction attributable to treatment.

Organ weight and measurements were limited to the brain in which both absolute and relative brain weights and brain measurements were unaffected by treatment. There were no treatment-related macroscopic or microscopic findings.

In this study of acute neurotoxicity in rats, non-specific clinical signs (fast respiration, piloerection) of minor toxicological relevance were seen in all groups treated at doses of 200 mg/kg bw and above. The NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested (Harvey, 1999).

Table 34. Summary of selected findings from a study of acute neurotoxicity in rats given novaluron by gavage

Parameter	Dose (mg/kg bw)							
	0	200	650	2000	0	200	650	2000
	Males				Females			
Piloerection:								
Day 1	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Day 2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Day 3	0/10	0/10	0/10	0/10	0/10	5/10	4/10	5/10
Day 4	0/10	2/10	5/10	5/10	0/10	1/10	1/10	3/10
Day 5	0/10	2/10	2/10	6/10	0/10	0/10	1/10	1/10
Day 6	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
Fast respiration:								
Day 1	—	—	0/10	0/10	—	0/10	0/10	0/10
Day 2	—	—	0/10	0/10	—	0/10	0/10	0/10
Day 3	—	—	0/10	0/10	—	4/10	1/10	5/10
Day 4	—	—	3/10	4/10	—	1/10	1/10	2/10
Day 5	—	—	0/10	3/10	—	0/10	1/10	1/10
Day 6	—	—	0/10	0/10	—	0/10	1/10	0/10
Functional battery observation	No treatment-related effects							

From Harvey (1999)

(b) Toxicity of impurities

Acute toxicity and genotoxicity of the manufacturing impurity, MCW RI 458 and the genotoxicity of the manufacturing intermediate MCW I were investigated.

(i) MCW RI 458

Five male and five female fasted Hsd:Sprague-Dawley rats were given MCW RI 458 (purity, 98.3%) by gavage in 1.0% w/v aqueous methylcellulose as a limit dose at 5000 mg/kg bw (10 ml/kg). The study was certified to comply with GLP and is considered to satisfy the essential requirements of OECD 401.

There were no mortalities during the study. Clinical signs of reaction to treatment were confined to piloerection, hunched posture and faecal disturbances, seen in all rats with recovery complete in all instances by day 5. All rats were considered to have achieved satisfactory body-weight gains during the study. Gross examination at necropsy did not reveal any abnormalities. The acute oral LD_{50} for MCW RI 458 in rats was > 5000 mg/kg bw (McRae, 1998a).

A single dose of 2000 mg/kg bw (2.7 ml/kg) MCW RI 458 (purity, 98.3%) formulated at a maximum practical concentration of 74.1% w/v in 1% w/v aqueous methylcellulose was administered under an occlusive dressing to the clipped dorso–lumbar skin of five male and five female Hsd:Sprague-Dawley (CD) rats for 24 h. The study was certified to comply with GLP and was conducted in accordance with and satisfied the essential criteria of OECD guideline 402.

There were no deaths or treatment-related signs of toxicity during the 14-day observation period. Body-weight gain was not affected. The treated skin site showed signs of transient very slight erythema with or without oedema in three males and one female, which was completely resolved by day 4. Localized spots and/or scabbing was observed in one male; one male and four females had no dermal reactions. Necropsy revealed no gross changes in organs and tissues. The acute dermal LD_{50} for MCW RI 458 in male and female rats was > 2000 mg/kg bw (McRae, 1998b).

MCW RI 458 (purity, 98.3%) in DMSO was investigated for its potential to induce mutations in an assay for gene mutation in *S. typhimurium* (and *E. coli* strain CM891) in the presence and absence of metabolic activation.. The study was certified to comply with GLP and satisfied the essential criteria for current OECD guidelines 471 and 472. There were no substantial increases in numbers of revertant colonies of any of the tester strains after treatment with MCW RI 458 at any dose, in the presence or absence of metabolic activation (S9), in either test. No toxicity was observed at any dose; the higher doses showed precipitation without adversely affecting evaluation. The concurrent positive controls all showed substantial increases in numbers of revertant colonies, confirming the sensitivity of the assay. MCW RI 458 was not genotoxic in the assay for bacterial gene mutation with or without metabolic activation (Kitching, 1998b).

(ii) MCW I

MCW I (purity, 98.3%) in DMSO was investigated for its potential to induce mutations in an assay for gene mutation in *S. typhimurium* and *E. coli* (strain CM891). The study was certified to comply with GLP and satisfied the essential criteria for OECD guideline 471 and 472. There were no substantial increases in numbers of revertant colonies of any of the tester strains after treatment with MCW I at any dose, in the presence or absence of metabolic activation (S9), in either test. MCW I was not genotoxic in the assay for bacterial gene mutation with or without metabolic activation (Kitching, 1998a).

3. Observations in humans

No reports of adverse effects were identified during routine monitoring of production plant workers and among personnel involved in the experimental biological testing or field trials. There is no evidence or data available to support any findings in relation to poisoning with novaluron.

Comments

Biochemical aspects

After oral administration in rats, [chlorophenyl-^{14}C (U)]novaluron was poorly absorbed (≤ 7%) after a single low dose (2 mg/kg bw) and about tenfold less well-absorbed after a single high dose (1000 mg/kg bw), with maximum plasma concentrations occurring at 5–8 h or 2–5 h, respectively. Novaluron was widely distributed. The tissue concentrations of radioactivity were highest in fat, liver and kidneys and were about three- to fivefold higher after 14 repeated daily doses than after a single dose, with a terminal half-life of 52–56 h in fat after the final dose. Excretion was rapid, primarily via the faeces (> 94%; via bile ≤ 1%) and to a lesser extent via urine (about 5%), with most of the administered dose being excreted within 48 h.

Absorbed novaluron was extensively metabolized, mainly by cleavage of the urea bridge between the chlorophenyl and difluorophenyl moieties. In urine and bile, up to 15 metabolites were detected, and individual metabolites accounted for ≤ 1% of a low dose of [chlorophenyl-^{14}C (U)]novaluron. Most of the faecal radioactivity consisted of unchanged novaluron, which was also the major component present in fat, liver and kidneys. The aniline metabolite of novaluron, 3-TFA, (3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)aniline) was identified at low levels (≤ 0.7%) in the urine, bile, liver and kidneys.

Toxicological data

Novaluron had low acute toxicity in rats, causing no mortality at limit doses after oral (LD_{50} > 5000 mg/kg bw), dermal (LD_{50} > 2000 mg/kg bw) or inhalation (LC_{50} > 5.15 mg/l air) exposure. Novaluron was not irritating to the skin and eyes of rabbits and not sensitizing to guinea-pig skin.

In short-term and long-term studies of toxicity, the erythrocyte was identified as the primary target of toxicity attributable to novaluron, with secondary effects apparent in the spleen and, less commonly, in the liver and kidneys. The spectrum of effects was essentially similar in mice, rats and dogs, and the underlying mechanism was considered to be the same. Although the mechanism of the effects on erythrocytes has not been elucidated, it was considered to be most likely that the aniline metabolite of novaluron, 3-TFA, caused oxidative damage to the mature erythrocyte, resulting in increased concentrations of methaemoglobin (caused by accelerated oxidation of haemoglobin from the ferrous to the ferric state) and increased numbers of erythrocytes containing Heinz bodies (which are formed when damaged haemoglobin precipitates onto the cell membrane). The presence of Heinz bodies led to early destruction of erythrocytes by the spleen, with the consequence of increased erythrocyte turnover, characterized by stimulated erythropoiesis in both normal sites (sternum, femur) and in functional reserve sites (spleen, liver) and increased deposition of the products of haemoglobin catabolism (haemosiderin) in the spleen, liver and kidneys. After cessation of treatment, the adverse effects regressed, although incompletely, over a 4-week period after treatment in rats and dogs, and completely within 8 weeks in mice.

In 28-day and 90-day studies of toxicity in mice treated orally, the overall NOAEL was 30 ppm (equal to 4.2 mg/kg bw per day) on the basis of haematological changes (decrease in EVF and erythrocyte counts, increase in Heinz bodies and sulfhaemoglobin) at dietary concentrations of 100 ppm (equal to 12.8 mg/kg bw per day) and above, while changes in the spleen (increased weight, increased haematopoiesis and haemosiderosis) were evident at 700 ppm (equal to 114.7 mg/kg bw per day) and above.

In 28-day and 90-day studies in rats treated orally, the overall NOAEL was 50 ppm (equal to 4.2 mg/kg bw per day) on the basis of haematological changes (decrease in Hb, EVF and erythrocyte counts) and histopathological changes in the spleen and liver (increased haematopoiesis and haemosiderosis) at dietary concentrations of 100 ppm (equal to 8.3 mg/kg bw per day) and above. By week 4 of the reversibility period, there was full recovery for most changes, except for increased concentrations of methaemoglobin, spleen weights and splenic haemosiderosis at dietary concentrations of 20 000 ppm (equal to 1667 mg/kg bw per day).

In 90-day and 1-year studies in dogs treated orally, the overall NOAEL was 10 mg/kg bw per day on the basis of haematological changes (decrease in Hb, EVF and erythrocyte counts; increase in reticulocytes, Heinz bodies and Howell-Jolly bodies), increased serum concentrations of bilirubin and changes in the spleen and liver (increased weight; increased red pulp congestion, increased haemosiderin in Kupffer cells) at doses of 100 mg/kg bw per day or greater, while increased concentrations of methaemoglobin were evident at doses of 300 mg/kg bw per day or greater. By week 4 of a reversibility period there was full recovery for most changes, except for increased liver weights in female dogs at 1000 mg/kg bw per day.

In a 28-day study in rats treated dermally, the NOAEL for systemic toxicity was 75 mg/kg bw per day on the basis of increased concentrations of methaemoglobin at doses of 400 mg/kg bw per day or greater.

Novaluron gave negative results in an adequate battery of studies of genotoxicity in vitro and in vivo.

The Meeting concluded that novaluron was unlikely to be genotoxic.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In the study of carcinogenicity in mice, the NOAEL was 30 ppm (equal to 3.6 mg/kg bw per day) on the basis of increased body-weight gain (in the first 4 or 26 weeks in males or females, respectively), haematological changes (decrease in Hb, EVF and erythrocyte counts; increase in reticulocytes, sulfhaemoglobin, and Heinz bodies) and changes in spleen (increased weight, increased incidence of extramedullary haematopoiesis, haemosiderosis and congestion) and kidneys (increase in cortical tubular pigment) at dietary concentrations of 450 ppm (equal to 53.4 mg/kg bw per day) and greater. There was no evidence of carcinogenicity in mice at dietary concentrations of up to 7000 ppm (equal to 800 mg/kg bw per day), the highest dose tested.

In the long-term study of toxicity and carcinogenicity in rats, the NOAEL was 25 ppm (equal to 1.1 mg/kg bw per day) on the basis of haematological changes (decreases in Hb, EVF and erythrocyte counts; increases in methaemoglobin formation and reticulocytes) and changes in the spleen (increase in weight, haemosiderosis) and kidneys (increase in cortical tubular pigment) at dietary concentrations of 700 ppm (equal to 30.6 mg/kg bw per day) and greater. There was no evidence of carcinogenicity in rats at dietary concentrations of up to 20 000 ppm (equal to 884.2 mg/kg bw per day), the highest dose tested.

In view of the absence of a carcinogenic potential in rodents and the lack of genotoxic potential in vitro and in vivo, the Meeting concluded that novaluron is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, the NOAEL for effects on fertility was 12 000 ppm (equal to 894.9 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity in parental animals and offspring could not be identified since there were secondary changes in spleen and liver relating to increased erythrocyte damage at all doses tested. The LOAEL for systemic toxicity was 1000 ppm (equal to 74.2 mg/kg bw per day) on the basis of increased spleen weights in adults and increased spleen and liver weights in offspring.

In a study of prenatal developmental toxicity in rats, the NOAEL for maternal and for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested. The increases in body-weight gain and food consumption in all treated groups were not considered to be adverse effects.

In a study of prenatal developmental toxicity in rabbits, the NOAEL for both maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested. In the absence of any

other evidence for an effect on fetal development, the slight increase in incidence of incompletely ossified fifth sternebrae at 300 mg/kg bw per day and 1000 mg/kg bw per day was not considered to be adverse. The finding of absent implantation or high rates of pre-implantation loss in two dams at 1000 mg/kg bw per day was considered to be incidental and not related to treatment.

The Meeting concluded that novaluron is not a developmental toxicant.

In a study of acute neurotoxicity in rats, non-specific clinical signs (fast respiration, piloerection) of minor toxicological relevance were seen in all groups treated at doses of 200 mg/kg bw and greater. The NOAEL for neurotoxic effects was 2000 mg/kg bw, the highest dose tested.

The manufacturing impurity MCW RI 458 had low acute oral and dermal toxicity in rats (LD_{50} > 5000 and > 2000 mg/kg bw, respectively) and was not mutagenic in an assay for gene mutation in bacteria. The manufacturing intermediate MCW I was not mutagenic in an assay for gene mutation in bacteria.

The Meeting concluded that the existing database on novaluron was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.01 mg/kg bw on the basis of the NOAEL of 1.1 mg/kg bw per day for erythrocyte damage and secondary splenic and liver changes in a 2-year dietary study in rats, and a safety factor of 100.

The Meeting concluded that it was not necessary to establish an ARfD for novaluron in view of its low acute toxicity, the absence of relevant developmental toxicity in rats and rabbits that could have occurred as a consequence of acute exposure, and the absence of any other toxicological effect that would be elicited by a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	3-month study of toxicity[a]	Toxicity	30 ppm, equal to 4.2 mg/kg bw per day	100 ppm, equal to 12.8 mg/kg bw per day
	78-week study of carcinogenicity[a]	Toxicity	30 ppm, equal to 3.6 mg/kg bw per day	450 ppm, equal to 53.4 mg/kg bw per day
		Carcinogenicity	7 000 ppm, equal to 800 mg/kg bw per day[d]	—
Rat	3-month study of toxicity[a]	Toxicity	50 ppm, equal to 4.2 mg/kg bw per day	100 ppm, equal to 8.3 mg/kg bw per day
	2-year study of toxicity and carcinogenicity[a]	Toxicity	25 ppm, equal to 1.1 mg/kg bw per day	700 ppm, equal to 30.6 mg/kg bw per day
		Carcinogenicity	20 000 ppm, equal to 884.2 mg/kg bw per day[d]	—
	Multigeneration study of reproductive toxicity[a]	Reproduction/fertility	12 000 ppm, equal to 894.9 mg/kg bw per day[d]	—
		Parental toxicity	—	1000 ppm, equal to 74.2 mg/kg bw per day[e]
		Offspring toxicity	—	1000 ppm, equal to 74.2 mg/kg bw per day[e]
	Developmental toxicity[b]	Maternal toxicity	1 000 mg/kg bw per day[d]	—
		Embryo- and fetotoxicity	1 000 mg/kg bw per day[d]	—

	Acute neurotoxicity[b]	Neurotoxicity	2 000 mg/kg bw per day[d]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	1 000 mg/kg bw per day[d]	—
		Embryo- and fetotoxicity	1 000 mg/kg bw per day[d]	—
Dog	3-month study of toxicity[c]	Toxicity	10 mg/kg bw per day	100 mg/kg bw per day
	1-year study of toxicity[c]	Toxicity	10 mg/kg bw per day	100 mg/kg bw per day

[a] Dietary administration

[b] Gavage administration

[c] Capsules

[d] Highest dose tested

[e] Lowest dose tested

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposures

Critical end-points for setting guidance values for exposure to novaluron

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; ≤ 7% at low dose
Distribution	Widely; highest concentrations in fat, liver, kidneys
Rate and extent of excretion	Largely complete within 48 h; primarily via faeces (> 94%) and to a lesser extent via urine (< 5%)
Potential for accumulation	Evidence of accumulation in fat after repeated doses
Metabolism in mammals	Extensive for absorbed material; cleavage of the urea bridge between the chlorophenyl and difluorophenyl moieties
Toxicologically significant compounds (animals, plants and the environment)	Parent compound and animal metabolite 3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) aniline
Acute toxicity	
Rat LD_{50} oral	> 5000 mg/kg bw
Rat LD_{50} dermal	> 2000 mg/kg bw
Rat LC_{50} inhalation	> 5.15 mg/l (4-h, nose-only exposure)
Rabbit, skin irritation	Non-irritant
Rabbit, eye irritation	Non-irritant
Skin sensitization (test method)	Not sensitizing (Magnusson & Kligman test, Buehler test)
Short-term studies of toxicity	
Target/critical effect	Erythrocytes (haemoglobin oxidation, resulting in

	methaemoglobinaemia and haemolysis), secondary changes in spleen, liver and kidneys
Lowest relevant oral NOAEL	4.2 mg/kg bw per day (90-day studies in rats and mice)
Lowest relevant dermal NOAEL	75 mg/kg bw per day (28-day study in rats)
Lowest relevant inhalation NOAEC	No data
Genotoxicity	
	Not genotoxic in vitro or in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Erythrocytes (haemoglobin oxidation, resulting in methaemoglobinaemia and haemolysis), secondary changes in spleen, liver and kidneys
Lowest relevant NOAEL	1.1 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic in rats or mice
Reproductive toxicity	
Reproduction target/critical effect	No effect on fertility at highest dose tested; splenic and liver changes in offspring at parentally toxic doses
Lowest relevant reproductive NOAEL	895 mg/kg bw per day for effects on fertility (two-generation study in rats) < 74.2 mg/kg bw per day for systemic toxicity in offspring and parents
Developmental target/critical effect	No developmental effect at highest dose tested
Lowest relevant developmental NOAEL	1000 mg/kg bw per day (rats and rabbits)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	No evidence for neurotoxicity at highest dose tested (2000 mg/kg bw)
Medical data	
	No data

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Rat, 2-year study	100
ARfD	Unnecessary	—	—

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