Evaluation Report

NOVALURON

December 24, 2003
Food Safety Commission
Pesticides Expert Committee

(Progress of Evaluation)

November 28, 2001	Registration application submitted
October 29, 2003	Minister of Health, Labor and Welfare requests health risk assessment in line with establishment of residue standard
November 6, 2003	18th meeting of Food Safety Commission (explanation of MHLW's request outline)
November 12, 2003	2nd meeting of Pesticides Expert Committee
November 20, 2003	20th meeting of Food Safety Commission (reporting of discussion results from the Pesticides Expert Committee meeting)
November 20 to December 17, 2003	Public comments
December 24, 2003	Finalized

(Food Safety Commission Members)
Dr. Masaaki Terada (Chairman), Dr. Tadao Terao (Deputy Chairman), Dr. Naoko Koizumi, Dr. Takeshi Mikami, Dr. Motoko Sakamoto, Yasuhiko Nakamura, Dr. Seiichi Honma

(Food Safety Commission Pesticides Expert Committee Members)
Dr. Katsushi Suzuki (Chairman), Dr. Masao Hirose (Deputy Chairman), Dr. Makoto Ema, Dr. Yasuo Ishii, Dr. Toshihiro Ohta, Dr. Shogo Ozawa, Dr. Atsuya Takagi, Dr. Mitsuharu Takeda, Dr. Hiroyuki Tsuda, Dr. Masakuni Degawa, Dr. Makoto Hayashi, Dr. Akira Hiratsuka, Dr. Midori Yoshida

I Outline of Pesticide to be Evaluated

1. **Usage**

   Insecticide

2. **Common name** (ISO name)

   NOVALURON

3. **Chemical name**

   IUPAC name:

   (*RS*)-1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea

   CAS chemical name (No.116714-46-6):

   N-[[[3-chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl]amino]carbonyl]-2,6-difluorobenzamide

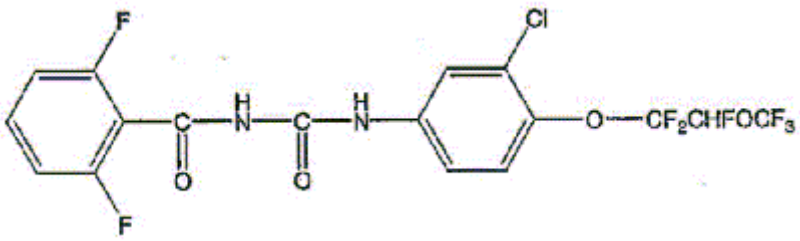
4. **Chemical formula**

   $C_{17}H_9ClF_8N_2O_4$

5. **Relative molecular mass**

   492.7

6. **Chemical structure**



7. Background of development

Novaluron is an insecticide of the class diflubenzoylureas. The compound shows the killing effect against various larvae of genus Lepidoptera, Coleoptera, Hemiptera, and Diptera.

Novaluron has been registered as an insecticide for food crop in foreign countries, including South Africa, Argentina, and Australia. United States authorities approved the compound's registration as a pest control drug for floriculture in September 2001.

The compound was applied for registration according to the Agricultural Chemicals Regulation Law by S.D.S. Biotec Inc. (hereinafter "The Applicant") in November 2001. (ref. 1)

II Summary of the Test Results

1. Absorption, distribution, metabolism and excretion

The study was conducted using [^{14}C-chlorophenyl ring] labeled NOVALURON (hereinafter “Labeled Substance A”) and [^{14}C-difluorophenyl ring] labeled NOVALURON (hereinafter “Labeled Substance B”).

The distribution was examined in male and female SD rats with a single oral dose of 2mg/kg bw (hereinafter “Single Low Dose”) or 1000 mg/kg bw (hereinafter ”Single High Dose”) of Labeled Substance A, and 2 mg/kg bw of Labeled Substance B, and with the repeated gavage administrations of low doses (hereinafter “Repeated Low Dose”) of Labeled Substance A.

The plasma concentrations of Labeled Substance A reached the maximum concentration (C_{max}) of 0.03 - 0.04 μg eq/g at 5 to 8 h after administration with a Single Low Dose, 1.86 - 3.01 μg eq/g at 2 to 5 h with a Single High Dose, and 0.04 - 0.05 μg eq/g at 2 to 8 h with Repeated Low Doses, respectively. The plasma levels with a single dose of Labeled Substance B reached the C_{max} of 0.04 - 0.05 μg eq/g 8 h after the treatment. Radioactivity was not detected after 96 h in all animals with a single dose, while it was detected up to 168 h and 120 h in the male and female animals with the Repeated Low Dose, respectively.

The highest level among the tissue concentrations was found in the fat, followed by liver, kidney, pancreas, and lymph node. Compared with the animals treated with a Single Low Dose, the tissue concentrations in the animals with a Single High Dose were about 50 to 90 times higher with a 500-fold increase in dosage. Concerning the tissue concentrations, they had a 3 to 5-fold increase in the animals with Repeated administrations, compared with those in the animals with a Single Low Dose. The half-lives (hereinafter “DT_{50}”) of the radioactivity in the fats after the treatment with the Repeated Low Dose were 52 h in males and 56 h in females. The facts that NOVALURON is not relatively metabolized easily, and that the unchanged substances mainly distribute in fat tissue because of its high LogPow (4.3), is retained there, and is eliminated only slowly from the tissues, are assumed to result in the high level of concentration in the fats. The amount of the substances bound to protein ranged from 1/5 to 1/10 of the residual amount in the fat. In treatment with a Single High Dose of Labeled Substance A, male and female rats eliminated 0.6% of dosage in the urine and from 93.8 to 95.4% in the feces by 168 h and the rats with a Single High Dose of Labeled Substance B excreted from 17.5 to 19.9% of the dosage in the urine and from 76.0 to 79.3% in the feces by 168 h, respectively. The above residual coefficients in the body were 0.1% and 0.7%, respectively. It was assumed that main elimination pathway was fecal excretion. Further, 20% of the dosage was absorbed. The amount of Labeled Substance B eliminated in urine and the excretory rate were higher as compared with those

for Labeled Substance A. The different urinary excretion between Labeled Substances A and B was assumed to be caused by the differences in the metabolic fates of the difluorophenyl and the chlorophenyl moieties after cleavage of the parent compound. .

Out of 14 radioactive components including the unchanged substance detected in urine, 12 components have not been identified and one component was identified as the metabolite, 3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) aniline. This metabolite was an electrophilic intermediate, and assumed to be related to the occurrence of hematotoxicity. The radioactivity derived from metabolites in urine after treatment with a Single Low Dose or a Single High Dose was 1.0% or less of the dose, and the amount of the unchanged substances was 0.1% or less of the dosage. The same metabolites were detected in the male and female urine after treatment with the Repeated Low Doses. Each component was 2.5% or less of the dosage, 0.3% or less for the unchanged substance. Eight radioactive components were detected in the urine samples after treatment with a Single Low Dose of Labeled Substance B. Out of the radioactivity (15.7 - 18.0% of the dosage), 2,6-difluorobenzoic acid (10.6 - 12.0% of the dosage) was identified as the main metabolite, but 6 components were unidentifiable. Also, the main component detected in the feces was the unchanged substance. After administering Labeled Substance A, 11 components were detected in the bile, including 0.1% based on the administrated dose, 0.2% of 3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) aniline and 0.1 - 0.2% of the other metabolites remainders. After administering Labeled Substance B, 15 components including the unchanged substance were detected in the bile, each in a small amount of 0.1% or less based on the administered dose.

It is assumed that the main metabolic pathway of the compound orally administered to rats is the hydrolysis of the amide (allophanoyl) bond between the chlorophenyl ring site and the difluorophenyl ring site. (ref. 2)

2. Plant metabolism

(1) Cabbage

Thirty to 45 g/ha of Labeled Substance A and Labeled Substance B were applied to cabbage twice (i.e., 8 weeks and 6 weeks before the harvesting or 5 weeks and 2 weeks before the harvesting). Then cabbage stems and leaves were collected as samples to conduct a metabolic test of the compound in cabbage. At the harvesting time, the radioactive residue levels (in terms of NOVALURON, the same will apply hereinafter) ranged from 0.234 to 0.448 mg/kg. The radioactivity was mostly washed off with acetonitrile from the plant surface. The ratios of radioactive substances extracted from the inner and outer leaves to the total residual radioactivity (TRR) were 8.0 and15.3%, respectively. Throughout the test period, other water-soluble residues were extracted at a ratio of 1.0% or

less of TRR and the un-extracted radioactive residue level was 2.8% or less of TRR. The unchanged substance amounted to nearly all (95.6 - 99.9%) of the extracted radioactive substances.

The major part of the compound applied to the cabbage was detected from the outer leaves and the unchanged substance alone was detected as the main radioactive component. Thus, it is considered that the compound is scarcely metabolized in cabbage. (ref. 3)

(2) Potato

Ninety-one to 100 g/ha of Labeled Substance A and Labeled Substance B were applied to potato twice (i.e., 43 days and 29 days before the harvesting). Then potato leaves and tubers were collected as samples for a metabolic test of the compound in potato.

Although the radioactive residue levels were lowered 10 days prior to the harvesting after the second treatment, the levels were elevated from 5.89 to 9.87 mg/kg at the harvesting time since the leaves had withered. The radioactivity was mostly washed off with acetonitrile from the leaf surface. The radioactive substances extracted from the leaves were from 15.5 -18.7% of TRR. Throughout the test period, water-soluble residues were extracted at a ratio of 0.6% or less of TRR and the un-extracted radioactive residue level was 1.2% or less of TRR. The unchanged substance amounted to nearly all (96.4 - 99.6%) of the extracted radioactive substances. Since radioactive residues were detected from the tubers only at an extremely low level (<0.01 mg/kg), TRR alone was examined in the tubers.

The major part of the compound applied to the potato remained in the leaves and no remarkable radioactivity was detected from the tubers. Based on these results, it is assumed that the compound applied to the leaves would not migrate into tubers. It can also be considered that the compound is scarcely metabolized in potato. (ref. 4)

(3) Apple

Twenty-five g/ha of Labeled Substance A and Labeled Substance B were applied to apple twice (i.e., 110 and 90 days before the harvesting), or three times (i.e., 110, 90 and 60 days before the harvesting). Then apple leaves and fruits were collected as samples for a metabolic test of the compound in apple.

The test revealed 0.02 mg/kg radioactive residues on fruits applied twice and 0.03 - 0.04 mg/kg on those applied three times. It also showed 0.6 - 1.1 mg/kg radioactive residues on leaves sprayed twice and 0.9 - 2.9 mg/kg on those sprayed three times. The radioactive substances washed off by acetonitrile were 47 - 57% of TRR. The extracts from fruits indicated 41 - 50% of TRR, most of which were obtained from the peels. The

un-extractable radioactive residues were only 3 - 5% of TRR. The radioactivity in washing fluid were 72 - 82% of TRR. The extract off radioactive residues from leaves indicated 18 - 26%of TRR. The level of un-extractable radioactive residues was below 3% of TRR. These radioactive substances, which were mostly unmetabolised, were detected at over 88.9% of TRR in fruits (washing fluids and extracts) and at over 92.6% of TRR in leaves. Other substances were detected at below 1.3% (0.001 mg/kg) in fruits and below 1.7% (0.024 mg/kg) in leaves. But the radioactivity was not remarkably found (>0.01 mg/kg) from fruits that were protected by protect-sacking and applied three times.

The radioactive residue was mostly found in the peels. The unchanged substance alone was detected as radioactive residual substances, it is considered that the compound is rarely metabolized by apple trees. It is also thought that the compound did not transfer inside apples from other part of tree, based on the results of the tests on fruits protected by the bag. (ref. 5)

3. Fate in soil

(1) Test of fate in aerobic soil (degradation pathway)

Labeled Substance A and Labeled Substance B were applied to sandy loam soil, Arrow, at 0.13 mg/kg. After 181 days incubation, a fate test of this compound in the soil was made.

As a result, extracted radioactivity gradually diminished. In 181 days, the radioactivities in Labeled Substance A and Labeled Substance B reduced to 64.0% and to 61.7%, respectively. In Labeled Substance A, residues in soil were over 10% after 14 days. Approximately 66% of the residue found in soil was associated with humine fraction and only 5% with fulvic fraction. The remainder was associated with the humic acid. In Labeled Substance B, the residues in soil were always below 10%. A major degradate of Labeled Substance A was identified as 1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) phenyl] urea, which was increased up to 18.1% up to Day 7 and then decreased to 4.9% by Day 120. Other degradate was 3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) aniline, which was found at about 5% after 14 days. A major degradate of Labeled Substance B was $^{14}CO_2$ which showed 26.5% at the maximum. Volatile radioactivity was not remarkably produced, 4.3% (up to Day 120) at most in Labeled Substance A. CO_2, volatile radioactivity increased to about 20% up to Day 59, and after remaining at this level, accumulated to 26.5% in 181 days. Other identified degradate was 2,6-difluoro benzoic acid, which was very low, and 6 non-identified degradates were detected at below 3.6%. DT_{50} and 90% degradation period (DT_{90}) of the compound in soil were 9.9 days and over test period 181days, respectively. The values of DT_{50} and DT_{90} for

major degradate, 1-[3-chloro-4-(1,1,2-trifluoro-2- trifluoromethoxyethoxy) phenyl] urea were 23.7 days and over test period 181 days, respectively. (ref. 6)

(2) Test of fate aerobic soil

Labeled Substance A was applied to clay, sandy loam and silty clayey loam, at 0.13 mg/kg. After the incubation of these soils up to 120 days (at 20°C, clay at both 10°C and 20°C), the fate test of the compound in soil was made. The DT_{50} value of the compound in clay (at 20°C), clay (at 10°C), sandy loam (at 20°C) and silty clayey loam (at 20°C) were 12 days, 20 days,10 days, and 5 days, respectively. The DT_{50} value of major degradate, 1-[3-chloro-4-(1,1,2-trifluoro-2- trifluoromethoxyethoxy) phenyl] urea, in clay (at 20°C), clay (at 10°C), sandy loam (at 20°C) and silty clayey loam (at 20°C) were 50 days, 110 days, 46 days, and 64 days, respectively. (ref. 7)

(3) Absorption test in soil

An absorption test in soils was attempted using Sand-dune Regosol (Sand), Light-colored Andosol (Loam), Gray Lowland soil (Light clay) A, and Gray Lowland soil (Light clay) B. However, since the aqueous solubility of NOVALURON was too little, it was not obtained and measured from any types of soils, and the soil adsorption coefficient was not acquired in a preliminary test. (ref. 8)

4. Hydrolytic fate test in water

Labeled Substance A and Labeled Substances B were dissolved in each buffer solutions pH 5.0 (0.01 mol/L sodium acetate buffer solution), pH 7.0 (0.01 mol/L sodium phosphate buffer solution), and pH 9.0 (0.01 mol/L sodium borate buffer solution) to prepare NOVALURON solution of 1.5 μg/L. These three solutions were incubated at 25°C, 50°C, and 70°C for 30 days. A hydrolytic fate test of NOVALURON in water was made. The disappearance rate of the pesticide in the solutions was calculated by assuming to follow pseudo-first-order reaction. DT_{50} in pH 9.0 at 25°C, 50°C, and 70°C were 101, 1.2, and 0.09 days, respectively. No changes were observed in the pH 5.0 and pH 7.0 solutions at 25°C.

In the pH 9.0 solution, 2,6-difluorobenzoic acid, 2,6-difluorobenzamide, 1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) phenyl] urea, and 3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) aniline were identified. (ref. 9)

5. Photolytic fate in water

(1) Photolytic fate in water (distilled and natural water)

NOVALURON was dissolved in distilled water (sterilized in an autoclave) or natural

water (filtrated to remove bacteria) to prepare the NOVALURON solution of about 1.99 μg/L. Then the solution was exposed to Xenon light (56.7～62.2 W/m² in the range of 280 - 800nm) at 25.0 - 25.5°C for 7 days to study photolytic degradation. After 7 days, the remaining ratio of NOVALURON was 56.4% in the distilled water and 76.5% in the natural water. The DT_{50} values were presumed to be 7.5 and 15.1 days, respectively. The remaining ratio of NOVALURON in the control dark solutions was 102.4% in the distilled water and 93.2% in the natural water. It is suggested that the major pathway for the degradation of NOVALURON in water is photolysis. (ref. 10)

(2) Photolytic fate in water (buffer solution)

Labeled Substance A and Labeled Substance B were dissolved in a sterilized buffer solution (Sodium acetate) of pH 5.0 to prepare NOVALURON solution of 1.5 μg/L. Then the solution was exposed to Xenon light (42.8 - 49.2 W/m^2 in the range of 290 - 400 nm) at 25°C for 15 days to study photolytic degradation. The DT_{50} of NOVALRON in the exposed solution was equivalent to the exposure level of 139 days of summer sunlight at latitude 40°N. This period was about double the duration of the actual irradiation period (irradiation for 15 days is equivalent to 67 days exposure under the sunlight at a location at latitude 40°N). Photolytic degradation products of NOVALURON included products that containing both phenyl rings, and other, cleared products that contained either the chlorophenyl rings or difluorophenyl ring only. The one that occupied 23% of all treated radioactivity by 23.6% was identified as 2,6-difluorobenzamide. The others were small quantity substances (less than 10% of applied radioactivity). It was found that the degradation of NOVALURON had gradually progressed under the control dark solution and the degraded products occupied up to around 85% of all treated radioactivity after incubation for 15 days. (ref. 11)

(3) Photolytic fate in water (natural water)

Labeled Substance A and Labeled Substance B were dissolved in natural sterilized water of pH 8.25 to make NOVALURON solution of 1.5 μg/L. Then the solution was exposed to Xenon light (average 39.1 W/m^2 in the range of 300 - 400 nm) at 25°C for 7 days to study photolytic degradation. DT_{50} of NOVALRON under the treated solution was equivalent to 31.3 days exposure of the spring sunlight at Tokyo, which is located at latitude 35°N. The analysis of the degraded products detected two kinds of products (i.e., one was with two phenyl rings and the other was the product derived from the cleavage of the ring with either a chlorophenyl ring or difluorophenyl ring). One of these products that occupied up to 19.4% of all treated radioactivity was identified as 2,6-difluorobenzamid and the others were small quantity substances (less than 10% of all recovered radioactivity). The degradation of NOVALURON gradually progressed even in a control dark solution and

NOVALURON decreased by around 73% after incubating for 7 days.

It is presumed that hydrolysis of the amide (Allophanoyl) bond between the chlorophenyl ring and difluorophenyl ring, and a radical replacement of the chlorophenyl ring and difluorophenyl ring could be a pathway for NOVALURON degradation. (ref. 12)

6. Residual in crops

Tomatoes, eggplants, cabbages, and Chinese cabbages were prescribed for NOVALURON (parent compound) residual tests in crops. As indicated in the results shown in Table 1, the highest residue detected was 0.41 mg/kg from Chinese cabbage, which were collected after 7 days from the last application. They were sprayed at 85 g ai/ha three times. No crop was found showing specifically high values. (refs. 13-14)

Table 1 Results of Residue Tests in Crops

Crops	Number of fields	Formulations	Amount used (g ai/ha)	Applications (times)	PHI (days)	Residues (mg/kg)	
						Highest	Average
Tomato (green house)	2	EC	85～137	4	1	0.32	0.21
				4	3	0.33	0.21
				4	7	0.32	0.23
Eggplant (green house)	2	EC	78～89	4	1	0.15	0.10
				4	3	0.17	0.08
				4	7	0.07	0.04
Cabbage (field)	2	EC	85	3	7	0.33	0.17
				3	14	0.27	0.11
				3	21	0.21	0.08
Chinese cabbage (field)	2	EC	85	3	7	0.41	0.25
				3	14	0.36	0.20
				3	21	0.36	0.14

PHI： Pre-harvest interval

ai： Active ingredient

EC： Emulsion Concentrate

7. Residual in soil

Residues of NOVALURON and two degradates in soils were studied (both in the vessel and the field) using volcanic ash light clay and alluvium clay loam. As the results shown in the Table 2 indicate, the assumed DT_{50} of those products were 6 - 34 days as NOVALURON and 6 - 43 days as the total amount of NOVALURON and two degradates. (ref. 15)

Table 2. Results of Residues in Soils (DT_{50})

Type of tests	Kinds of soil	Parents compound	Total amount of NOVALURON+two degradates*[1,2]
Field	Volcanic ash light clay	6 days	6 days
	Alluvium clay loam	25 days	29 days
Vessel	Volcanic ash light clay	34 days	43 days
	Alluvium clay loam	25 days	38 days

*1: 1-[3-chloro-4-(1,1,2- trifuoloromethoxyethoxy) phenyl]urea

*2: 2,6-difluorobenzamide

8. Acute toxicity

The acute toxicity through oral, dermal, and inhalation administrations were studied using SD rats. LD_{50} was determined as >5000 mg/kg bw for oral dose acute toxicity to male and female rats, >2000 mg/mg bw for dermal treatment acute toxicity to male and female rats, and >5150 mg/m^3 for inhalation acute toxicity to male and female rats. (refs. 16-18)

9. Skin and eye irritation and skin sensitization

The study of skin and eye mucous membranes on a group of New Zealand white rabbits revealed no sign of irritation. Skin sensitization potential was assessed in guinea pigs by the Maximization method. No skin sensitization was observed. (refs. 19-21)

10. Subchronic toxicity

(1) Subchronic toxicity test for 90 days in rats

SD rats (main groups of 10 males and 10 females, groups of 5 males and 5 females for recovery) were administered diets containing NOVALURON, technical grade at 0, 50, 100, 10000, or 20000 ppm for 90 days. The absolute weight of the spleen increased in males and urine volume increased in females at the dose of 20000 ppm. Acceleration of extrameduallary hematopoiesis in the spleen, a decrease in hemoglobin, an increase in the concentration of metohemoglobin, and the number of reticulocyte were observed both in males and females at the doses of 10000 ppm and higher. In these groups, a decrease in the number of red blood cell (hereinafter “RBC”) and hemosiderin deposition in the spleen was observed in males, and the relative spleen weight against body weight (relative weight against body weight will be described as relative weight hereinafter) increased in females. Extrameduallary hematopoiesis in the liver and pigmentation in the Kupffer cell in females

were also found at the doses of 10000 ppm and higher. Hemoglobin and hematocrit decreased in females at the doses of 100 ppm and higher. Bilirubin increased in males, RBC decreased, and hemosiderin deposition in the spleen increased in females at doses of 50 ppm and higher. Based on these findings, NOAEL was considered to be less than 50 ppm (<4.2 mg/kg bw/day for males, <4.7 mg/kg bw/day for females). (ref. 22)

(2) Subchronic toxicity test for 90 days in mice

ICR mice (main groups of 12 males and 12 females, groups of 6 males and 6 females for recovery) were administered diets containing NOVALURON, technical grade at 0, 30, 100, 1000, or 10000 ppm for 90 days. Centrilobular hypertrophy of hepatocytes was observed in males at the dose of 10000 ppm. An increase of the relative spleen weight both in males and females, the decrease in RBC and hematocrit in males, and the increase in the number of reticulocyte in females were observed at the doses of 1000 ppm and higher. An increase in total bilirubin concentration both in males and females, a decrease in methemoglobin concentration, and high values in sulfhemoglobin in males, and decreases in RBC and hematocrit in females were recognized at the doses of 100ppm and higher. NOAEL was considered as 30ppm (male:4.2 mg/kg bw/day, female: 4.7 mg/kg bw/day). (ref. 23)

(3) Subchronic toxicity test for 90 days in dogs (higher dosage)

Beagle dogs (main groups of 4 males and 4 females, groups of 2 males and 2 females for recovery) were administered NOVALURON, technical grade at 0, 100, 300, or 1000 mg/kg bw/day for 90 days by gavage. The number of reticulocyte and the relative spleen weight increased in females at 1000 mg/kg bw/day. Methemoglobin concentration, the average volume of red cells and pigmentation in the Kupffer cell in the liver increased both in males and females, and the hemoglobin and RBC decreased in females at the doses of 300 mg/kg bw/day and higher. A decrease of the mean corpuscular hemoglobin concentration (hereinafter ”MCHC”) Heinz body in reticulocytes both in males and females and an increase in RBC in males were recognized at doses of 100 mg/kg bw/day and higher. NOAEL was considered to be less than 100 mg/kg bw/day. (ref. 24)

(4) Subchronic toxicity test for 90 days in dogs (lower dosage)

Beagle dogs (groups of 4 males and 4 females) were administered NOVALURON, technical grade at 0 or 10 mg/kg bw/day for 90 days by gavage (as for the control group in this test, although the control dogs’ age differed by weeks, the 0 mg/kg group in the chronic toxicity test for 52 weeks running at the same time in the same laboratory was used). Interstitial pneumonia, craniopharyngeal duct cyst and erythrophagia in lymph nodes were

observed in both males and females. The number of white blood cells (hereinafter “WBC”), serum alanintransferase activity, and glucose levels increased in males, while inorganic phosphorus decreased and the number of reticulocytes increased in females.

The number of reticulocytes observed in females fell within the normal range (0.1 - 3.2%). An apparent increase in WBC in males would be incidental because there was no significant increase in WBC in the males 1000 mg/kg bw/day group in the test of 10(3). Also significant changes in alanintransferase and glucose in males and inorganic phosphorus in females would be incidental and not treatment-related because those values were comparable to those determined two weeks before administration started. The histopathological changes noted above could not be due to NOVALURON treatment, since the lesions were frequently observed at a comparable age of the same breed.

NOAEL was considered as 10 mg/kg bw/day. (ref. 25)

11. Chronic toxicity/carcinogenicity study

(1) Chronic toxicity study for 52 weeks in dogs

Beagle dogs (groups of 4 males and 4 females) were administered NOVALURON, technical grade at 0, 10, 100, or 1000 mg/kg bw/day for 52 weeks by gavage. Asignificant increase in hematocrit, RBC, and total bilirubin, a decrease in hemoglobin as compared to respective controls, and an aggregation of brown pigment in the liver cells (mainly hemosiderin deposition in Kupffer cells) were noted both in males and females andsignificant increases in mean corpuscular volume (hereinafter ”MCV”) and methohemoglobin contents were observed in males at the dose of 1000 mg/kg bw/day. A significant decrease in MCHC and having the appearance of Howell-Jolly bodies and Heinz bodies and histologically a congestion of splenic sinus were observed in both males and females and significant increases in reticulocyte and relative spleen weight were observed in males at the doses 100 mg/kg bw/day and higher. An increased hematopoiesis in the bone marrow of the sternum and femur was observed both in males and females at the doses of 10 mg/kg bw/day and higher, which would suggest that the RBC may somehow be affected by NOVALURON. Increased hematopoiesis observed in 10 mg/kg bw/day was less severe and would reflect the compensatory reaction of the bone marrow to the anemia. This idea was further supported by the fact that there was neither related influences of NOVALURON on RBC-related parameters such as hematocrit, nor histological appearances of hemosiderin deposition/pigmentation in the liver and spleen at 10 mg/kg. Therefore, the changes in RBC-related parameters observed in the 10 mg/kg group would be due to NOVALURON but would not be so adverse as to disturb the homeostasis of the RBC metabolism.

NOAEL was considered as 10 mg/kg bw/day. (ref. 26)

(2) Combined study of chronic toxicity for 52 weeks and carcinogenicity for 24 months in rats

SD rats (20 males and 20 females) were administered diets containing NOVALURON, technical grade at 0, 25, 700, or 20000ppm for 24 months. An increase in MCHC and the appearance of Heinz bodies and Howell-Jolly bodies were observed both in males and females at the dose of 20000 ppm. The incidence and extent of centrilobular hypertrophy of hepatocytes increased in males in the chronic toxicity study at the highest group at 52 weeks alone, although the lesions were not detected in the carcinogenicity study. In these groups, an increase in MCV, decreases in the hemoglobin content and RBC, and increase in reticulocyte were also noted in males in the highest dose group and increased pigmentation in hepatic Kupffer cell was noted in females. An increase in methohemoglobin concentration was found both in males and females at the dose of 700ppm and higher. In these groups, a decrease in MCHC and increased hemodiderin deposition in the spleen were noted in male and increases in MCV, thrombocyte, reticulocyte and the frequency of pigmentation in tubules of the renal cortex (significantly different at only 2000 ppm), decreases in hematocrit, hemoglobin and RBC, increases in the relative spleen weight and increased extramedullary hematopoiesis in the liver were observed in females. The administration of NOVALURON did not have an effect on the occurrence of the specific neoplasms.

NOAEL was considered as 25 ppm (male: 1.1 mg/kg bw/day, female: 1.4 mg/kg bw/day), and NOVALURON did not show any carcinogenicity in rats. (ref. 27)

(3) Carcinogenicity test for 18 monthes in mice

ICR mice (main groups of 51 males and 51 females, satellite groups of 15 males and 15 females) were administered diets containing NOVALURON, technical grade at 0, 30, 450, or 7000 ppm for 18 months. Increases in reticulocyte and augmented pigmentation in Kupffer cells were observed both in males and females at the dose of 7000 ppm. In these groups, increases in MCH, incidences and an extent of pigmentation in tubules of the renal cortex and relative liver weight, decreased ceroid pigmentation in the cortico-medullay zone of the adrenal gland, and congestion in the spleen were observed in females. Decreases in hematocrit, hemoglobin and RBC, increases in reticulocyte, the appearance of inclusion bodies in the blood (Heinz bodies, extrusion bodies, anaclasis bodies), spleen-swelling, acceleration of extramedullary erythropoiesis, and increased hemosiderin pigmentation in the spleen were observed both in males and females at the doses of 450 ppm and higher. In these groups, congestion in the spleen was observed in males and increases in the relative spleen weight and extramedullary erythropoiesis in the liver were observed in

females. There was no statistically significant difference in the incidence of neoplastic lesions in the treated group as compared to those in the control group.

NOAEL was considered as 30 ppm (male: 3.6 mg/kg bw/day, female: 4.3 mg/kg bw/day). NOVALURON did not show any carcinogenicicity in mice. (ref. 28)

12. Reproductive/developmental toxicity

(1) Two generation reproductive toxicity study in rats

SD rats (groups of 28 males and 28 females) were administered diets containing NOVALURON, technical grade at 0, 1000, 4000, or 12000ppm for two generations. In the parent animals, an increased deposition of the hemosiderin in the spleen both in males and females, an increased weight of the kidney (F_0), an accentuated hepatic lobular pattern (F_0) and swelling of the kidney (F_0), decreased relative weights of the epididymis and seminal vesicle (F_0), and centrilobular hypertrophy of the hepatic cells (F_1) in males, and an increased deposition of the hemosiderin in the uterine broad ligament (F_0) and peripheral fatty degeneration of the hepatic cells (F_1) in females were observed at the dose of 12000ppm. An increased relative kidney weight was found in males (F_1) at the doses of 4000ppm and higher. An increased relative spleen weight was noted both in males and females (F_0, F_1) at the doses of 1000ppm and higher.

A decreasing tendency of epididymal sperm was observed in F_1 males at the dose of 4000ppm. However, this change was not thought to be attributable to the administration of NOVALURON because this change in sperm was within the range of the background control data in this strain of rats and was unaccompanied by changes in the parameters of the testis and epidydimis, and no changes were detected in the reproductive outcome.

A decreased viability of F_1 offspring on postnatal days 14 and 21 and an increased relative spleen weight were found at the dose of 12000 ppm. An increased relative liver weight was observed both in males and females at the dose of 1000 ppm and higher, except for F_1 females at 1000 and 4000 ppm.

The NOAELs in this study for dams and offspring were considered to be less than 1000 ppm (F_0 male: <74.2 mg/kg bw/day, F_0 female: <90.7 mg/kg bw/day, F_1 male: <97.8 mg/kg bw/day, F_1 female: <106.0 mg/kg bw/day) in both sexes of rats. No adverse effects on the reproductive outcome were observed. (ref. 29)

(2) Developmental toxicity study in rats

A developmental toxicity study was conduced in SD rats (groups of 22 females) were administered NOVALURON on days 6 - 15 of pregnancy at 0, 250, 500, or 1000 mg/kg bw/day by gavage. No adverse effect was found in autopsy data and number of

implantations in dams at the highest dose and fetuses in all treated groups, although there were increases in body weight gain and food consumption in dams at all dosage groups.

The NOAELs in this study was considered as 1000 mg/kg bw/day for dams and fetuses. No teratogenicity of NOVALURON was evident. (ref. 30)

(3) Developmental toxicity study in rabbits

New Zealand white rabbits (groups of 22 females) were administered NOVALURON on days 6 - 19 of pregnancy at 0, 100, 300, or 1000 mg/kg bw/day by gavage. A suppression of body weight gain in dams was found at the dose of 1000 mg/kg bw/day. The incidence of unossified 5th sternebra in fetuses was increased at the dose of 300 mg/kg bw/day and higher. (There was a little controversy in the explanation of the results between the prosecutor of the experiments and the applicant; the former claimed insignificance of the incidences of the 5th sternebrar unossification at the dose of 300 mg/kg bw/day and higher based on the statistical comparison between treated and control groups, although the latter took the view that the effect might be due to NOBALURON because these incidences at 300 and 1000 mg/kg bw/day fell around the upper margin of the background incidence data.)

The NOAELs in this study were considered as 300 and 100 mg/kg bw/day for dams and fetuses, respectively, in rabbits. No teratogeniticity of NOVALURON was evident. (ref. 31)

13. Genotoxicity

NOVALURON has been tested in a range of *in vitro* and *in vivo* following standard protocols (Table 3). It was not mutagenic in bacteria with and without an exogenous metabolic activation system. NOVALURONs did not induce chromosomal aberrations in human primary lymphocyte culture. It also did not induce micronuclei in mouse bone marrow cells. NOVALURON was not genotoxic (refs. 32-34).

Table 3 Summary of Genotoxicity Test Results

Test systems		Cells/animals	Dose	Results
In vitro	Microbial reverse mutation (±S9 mix)	*S. typhimurium* TA100,TA98,TA1535 TA1537, *E. coli* WP2*uvrA*	0-5000 µg/plate	Negative
	Mammalian cell chromosome aberration (±S9 mix)	Cultured human lymphocyte	0-5000 µg/mL	Negative
In vivo	Rodent	ICR mice 5 males/5	1250, 2500, 5000	Negative

	micronucleus	females	mg/kg bwsingle oral treatment	

±S9 mix: with/without exogenous metabolic activation.

III. Evaluation

Absorption, distribution, metabolism and excretion of NOVALURON were studied using ^{14}C labeled chemical at the chlorophenyl ring and at the difuluorophenyl rings. In rats, 0.6 - 19.9% of NOVALURON was excreted in the urine, 76.0 - 95.4% in the feces after 168 h oral administration, and 0.1 - 4.3% of NOVALURON administered remained in the body. The distribution of NOVALURON was highest in fat tissue followed by the liver, kidney, spleen, and lymph node. Main metabolites detected in the urine were 3-chloro-4-(1,1,-2-trifluoro-2-trifluoromethoxyethoxy) aniline and 2,6-difluorobenzoic acid, although unchanged NOVALURON was the major component. The main metabolic pathway of NOVALURON was the hydrolysis of the amido bond between the chlorophenyl and difluorophenyl rings.

NOVALURON was stable in plants, i.e., cabbages, potatoes and apples and no transfer was observed in apples covered with protect-sacking.

DT_{50} of NOVALURON in three kinds of soils was 5 - 20 days at 20°C when 0.13 mg/kg of the compound had been applied. The main degradate was 1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) phenyl]urea, and its DT_{50} was 46 - 64 days at 20°C. The other main degradate of the Compound was $^{14}CO_2$, and all the degradates were converted to inorganic form finally.

NOVALURON was degraded by light in aqueous solution. DT_{50} of NOVALURON in natural water was 31.1 days under the light equivalent to the sunlight at Tokyo (latitude 35°N) in spring time, and the main degradate was 2,6-difluorobensamido.

The residual levels of NOVALURON were examined in tomatoes, eggplants, cabbages, and Chinese cabbages. The highest residual value of the compound (0.41 mg/kg) was observed in Chinese cabbage cropped 7 days after the 3rd spraying with 85 ai/ha. Although the size of experiment might not be big enough, no extreme residual value was observed in any vegetables examined.

DT_{50} of NOVALURON in soil was 6-34days, and the sum of NOVALURON and its two degragates was 6-43 days in volcanic light clayey soil and alluvium clayey loam soil (both in a flowerpot and on the field).

LD_{50} of NOVALURON was more than 5000 mg/kg bw/day in rats by oral gavage and NOAEL of NOVALURON was 4.2 mg/kg bw/day in mice and 10 mg/kg bw/day in dogs in a subchronic toxicity test.

NOAEL was also determined as 1.1 mg/kg bw/day in rats, 10 mg/kg bw/day in dogs, and 3.6 mg/kg bw/day in mice in a chronic toxicity test and carcinogenicity test. The main adverse effects of this chemical were observed on the RBC-related parameters (hematocrit, RBC, MCHC, etc) in rats, dogs, and mice. The mechanism of the effect was considered to the formation of methemoglobin. NOVALRON was not carcinogenic in rats, mice, and dogs.

In the two-generation reproductive toxicity study in rats, general toxicological symptoms were similar to those observed in the rat chronic toxicity/carcinogenicity study but no adverse effect on the reproductive outcome was observed.

The developmental toxicity studies were performed in rats and rabbits and the NOAEL was 1000 mg/kg bw/day for both dams and fetuses. The NOAELs in rabbits for dams and fetuses were 300 and 1000 mg/kg bw/day, respectively. NOVALURON has no teratogenic activity in either rats or rabbits.

It was not genotoxic in an adequate range of studies *in vitro* and *in vivo.* The Committee concluded that NOVALURON is not genotoxic.

NOAELs determined from the results of several evaluation tests are showed in Table 4.

Table 4. NOAELs Determined in Several Toxicity Evaluating Tests

Species	Evaluating test	NOAEL	Note
Mouse	90 days subchronic toxicity	Male: 4.2 mg/kg bw/day Female: 4.7 mg /kg bw/day	
	18 months carcinogenicity	Male: 3.6 mg/kg bw/day Female: 4.3 mg/kg bw/day	No carcinogenicity
Rat	90 days subchronic toxicity	Male: < 4.2 mg/kg bw/day Female: < 4.7 mg/kg bw/day	
	Combined study of chronic toxicity (52 weeks) and carcinogenicity (24 months)	Male: 1.1 mg/kg bw/day Female: 1.4 mg/kg bw/day	No carcinogenicity
	Two-generation reproductive toxicity study	F_0 male: <74.2 mg/kg bw/day F_0 female: <90.7 mg/kg bw/day F_1 male: <97.8 mg/kg bw/day F_1 female: <106.0 mg/kg bw/day	No reproductive Toxicity

	Developmental toxicity study	Dam and fetus : 1000 mg/kg bw/day	No teratogenicity
Rabbit	Developmental toxicity study	Dam: 300 mg/kg bw/day Fatus: 100 mg/kg bw/day	No teratogenicity
Dog	90 days subchronic toxicity (High dose level)	Male and female: <100 mg/kg bw/day	
	90 days subchronic toxicity (Low dose level)	Male and female: 10 mg/kg bw/day	
	52 weeks chronic toxicity	Male and female: 10 mg/kg bw/day	

Based on the evaluation, the Pesticides Expert Committee of the Food Safety Commission settled ADI value of NOVALURON as:

ADI: 0.011 mg/kg bw/day
(referred data for ADI): Combined study of chronic toxicity and carcinogenicity
Laboratory animal tested: Rat
Duration: 24 months
Administration route: mixed feeds
NOAEL: 1.1 mg/kg bw/day
Safety factor: 100
Residue definition for exposure assessment:
NOVALURON (parent chemical only)

(Appendix: List of References)

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21. “RIMON” Technical: Skin sensitization in the guinea pig (incorporating appositive control using hexyl cinnamic aldehyde) (GLP study): Huntingdon Life Sciences Ltd. (U.K.), 1997, unpublished.
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weeks followed by a 4-week reversibility period (GLP study): Huntingdon Life Sciences Ltd. (U.K.), 1998, unpublished.
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