CHLORANTRANILIPROLE

First draft prepared by
G. Wolterink[1] and V. Dellarco[2]

[1]Centre for Substances and Integrated Risk Assessment,
National Institute for Public Health and the Environment, Bilthoven, The Netherlands; and
[2]United States Environmental Protection Agency, Office of Pesticide Programs
Health Effects Division, Washington DC, USA

Explanation

Chlorantraniliprole is the International Organization for Standardization (ISO) approved common name for 3-bromo-*N*-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carboxamide). Chlorantraniliprole (CAS No. 500008-45-7) is an insecticide that operates by a highly specific biochemical mode of action. It binds and activates ryanodine receptors, resulting in depletion of intracellular calcium stores and leading to muscle paralysis and death. Comparative studies have demonstrated that differential selectivity of chlorantraniliprole for insect receptors is more than 350-fold that for mammalian receptors.

Chlorantraniliprole is being evaluated for the first time by the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR). The present JMPR review was based on a global assessment of the substance, which was performed in 2007 by 10 countries under the auspices of the Organization for Economic Co-operation and Development (OECD).

All critical studies complied with good laboratory practice (GLP).

Evaluation for accetable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

As part of a preliminary study of oral toxicity, groups of at least five male and five female Crl:CD® (SD)IGS BR rats were given chlorantraniliprole (purity, approximately 100%) at a dose of 0, 25, 100 and 1000 mg/kg bw per day by gavage for 14 consecutive days. Blood was collected from three male rats in each of the groups at 25, 100, and 1000 mg/kg bw on test days 14 and 15 immediately before dosing, and then 30 and 60 min, 2, 4, 8, 12, and 24 h after dosing for determination of plasma concentrations of chlorantraniliprole. On day 14, liver tissue of five male and five female rats per group was processed for hepatic biochemical evaluations (beta-oxidation activity, total and specific cytochrome P450 content). Blood was separated into plasma and erythrocytes. In additional male rats assigned to the groups at 25, 100, and 1000 mg/kg bw per day, fat samples were collected for the purpose of assessing potential bioaccumulation of the test substance.

The area under the curve of concentration–time (AUC) for chlorantraniliprole was not proportional to dose, indicating that absorption was decreased at higher doses. Calculated half-lives for chlorantraniliprole in rats in the groups at 25, 100, and 1000 mg/kg bw per day were 3.4, 3.4, and 4.0 h, respectively). Peak plasma concentrations occurred at 0.25, 0.42 and 2.75 h in the groups at 25, 100, and 1000 mg/kg bw per day. The maximum plasma concentrations (up to 0.48 µg/ml at 25 mg/kg bw) were similar at all doses. The concentrations of the test substance in fat were below the limit of quantitation at 24 h after dosing, indicating no significant accumulation of the parent compound.

In females, chlorantraniliprole was a weak inducer of cytochrome P450 isozyme 3A. This enzyme induction was considered to be related to the administration of chlorantraniliprole, but not adverse, and is consistent with a pharmacological response to increased metabolism. Otherwise, chlorantraniliprole did not alter beta-oxidation activity or total and specific cytochrome P450 content (Munley, 2006a).

In a study performed in accordance with OECD guideline 417, a number of kinetic experiments were carried out with Sprague Dawley Crl:CD®(SD)IGS BR rats. All experiments, except a study to determine radioactive residues in the expired air, were performed with a 1 : 1 µCi/µCi mixture of [benzamide carbonyl ^{14}C]chlorantraniliprole (radiochemical purity, 97%) and [pyrazole-carbonyl ^{14}C]chlorantraniliprole (radiochemical purity, 99%), diluted with chlorantraniliprole technical (purity, 96.45%). In all experiments, the rats were dosed by gavage. The study design is presented in Table 1. Statements of adherence to quality assurance (QA) and GLP were included.

Chlorantraniliprole was readily absorbed after oral administration, although absorption was incomplete and dose-related, with T_{max} values of 5–9 h after the lower dose and 11–12 h after the higher dose. At a dose of 10 mg/kg bw, plasma concentrations peaked at 3.0 and 5.4 µg equivalents/g in males and females, respectively. After 24 h, plasma concentrations in males and females were about 1.4 and 3.6 µg equivalents/g. At 200 mg/kg bw, plasma concentrations peaked at 5.1 and 7.1 µg

Table 1. Design of a study of the absorption, distribution and excretion of radiolabelled chlorantraniliprole in rats treated by gavage

Experiment	Dose (mg/kg bw)	Label[a]	No. of rats		Time of sacrifice (h)	Samples
			Male	Female		
Pharmacokinetics	10	Mix[a]	4	4	120	Plasma, erythrocytes[b]
	200	Mix[a]	4	4	120	Plasma, erythrocytes[b]
Volatiles	10	BC[c]	1	1	48	Exhaled volatiles and CO_2, urine, faeces
	10	PC[c]	1	1	48	Exhaled volatiles and CO_2, urine, faeces
Material balance and tissue distribution (terminal)	0	PEG	1	1	168	Urine, faeces, tissues, carcass[d]
	10	Mix[a]	4	4	168	Urine, faeces, tissues, carcass, cage-wash and feed residue
						Metabolite profile in urine and faeces
	200	Mix[a]	4	4	168	Urine, faeces, tissues, carcass, cage-wash and feed residue
						Metabolite profile in urine and faeces
Tissue distribution						
(T_{max})[e]	10	Mix[a]	4	4	5, 9	Urine, faeces, tissues, carcass, cage-wash and feed residue
	200	Mix[a]	4	4	11, 12	Urine, faeces, tissues, carcass, cage-wash and feed residue
(T_{max}/2)[e]	10	Mix[a]	4	4	21, 41	Urine, faeces, tissues, carcass, cage-wash and feed residue
	200	Mix[a]	4	4	52, 64	Urine, faeces, tissues, carcass, cage-wash and feed residue
Biliary elimination	10	Mix[a]	5[f]	5[f]	48	Bile, urine, faeces, GIT, carcass and cage-wash
						Metabolite profile in bile
	200	Mix[a]	4[g]	4[g]	48	Bile, urine, faeces, GIT, carcass and cage-wash

From Himmelstein (2006a)

BC, [benzamide carbonyl-^{14}C]chlorantraniliprole]; GIT, gastrointestinal tract tissue and contents; PC, [pyrazole carbonyl-^{14}C]-chlorantraniliprole; T_{max}, time at maximum plasma concentration (C_{max}); T_{max}/2, time at half of plasma C_{max}.

[a] 'Mix' indicates that each rat was given a single oral dose containing a mixture of [benzamide carbonyl-^{14}C]chlorantraniliprole and [pyrazole carbonyl-^{14}C]chlorantraniliprole in a 1 : 1 ratio (μCi : μCi) at approximately 30 μCi.

[b] Whole blood was collected from the jugular vein at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 h, for measurement of concentrations of radioactivity in erythrocytes and plasma.

[c] Each rat was dosed with approximately 30 μCi of [benzamide carbonyl-^{14}C]chlorantraniliprole (BC) or [pyrazole carbonyl-^{14}C]chlorantraniliprole (PC) for determination of exhaled volatiles. Exhaled air was sampled 0–24 h and 24 h after dosing.

[d] Control samples were collected for blank analysis. Urine and faeces were collected at intervals of 0–6 h, 6–12 h, 12–24 h after dosing and every 24 h thereafter until termination on day 7. Urine and faeces were analysed for metabolites.

[e] T_{max} and T_{max}/2 were determined experimentally based on pharmacokinetic data. First and second values for time of sacrifice are for male and female rats, respectively. Urine and faeces were collected at intervals of 0–6 h, 6–12 h, and 12–24 h after dosing and every 24 h thereafter until termination at T_{max} or T_{max}/2. At termination, a range of tissues and organs were collected for analysis of content of radioactivity.

[f] Groups of eight male and eight female rats were given [^{14}C]chlorantraniliprole at a dose of 10 mg/kg bw. At this dose, data were reported for five males and five females that had functional bile-duct cannulae throughout the collection, i.e. 48 h. Urine, faeces and bile were collected at intervals of 0–6 h, 6–12 h, and 12–24 h.

[g] [^{14}C]chlorantraniliprole was administered to four rats per sex at the 200 mg/kg bw dose level all of which had functional cannulas. Urine, faeces and bile were collected at intervals of 0-6, 6-12, 12-24h.

equivalents/g in males and females, respectively. In the experiment with bile-duct cannulated rats, total absorption was 73–85% after a dose of 10 mg/kg bw and 12–13% after a dose of 200 mg/kg bw. At the lower dose, 48 h after dosing 18–30% and 49–53% of the absorbed radiolabel was excreted in urine and bile respectively, while 2–6% and 10–20% was found in tissue and faeces respectively. At the higher dose, 48 h after dosing 4% and 5–7% of the absorbed radiolabel was excreted in the urine and bile, respectively, while 3% and 55–71% was found in tissue and faeces, respectively. ^{14}C residues showed extensive distribution in the tissues. In the rats at the lower dose, 0.8% and 3.3% of the administered dose was recovered from the tissues of males and females, respectively, at 168 h after dosing. At this time-point, tissues of males and females at the higher dose contained 0.2% and 0.5%, respectively, of the administered dose. No significant radioactivity was exhaled as ^{14}C-labelled volatiles or $^{14}CO_2$. Concentrations of ^{14}C residues were lower in erythrocytes and tissues than in plasma. The mean plasma elimination half-lives were shorter in males (38–43 h) than in females (78–82 h) rats (Himmelstein, 2006a).

In a kinetic study that complied with OECD guideline 417, male and female Sprague-Dawley Crl:CD®(SD)IGS BR rats were given up to 14 daily doses of [^{14}C]chlorantraniliprole at 10 mg/kg bw per day by gavage. The experiments were performed with a 1 : 1 μCi/μCi mixture of [benzamide carbonyl ^{14}C]-chlorantraniliprole (radiochemical purity, 97%) and [pyrazole-carbonyl ^{14}C]chlorantraniliprole (radiochemical purity, 99%), diluted with chlorantraniliprole technical (purity, 96.45%). Rats were checked daily for clinical signs of toxicity. In three females per group, ^{14}C residues were quantified in whole blood, plasma, erythrocytes, fat, kidney, liver and muscle on days 5, 9, 12, 17, and 27. An evaluation of the distribution of ^{14}C residues in 21 tissues of three males and three females per group was performed on days 15 and 21. Material balance and rate and extent of urine and faecal excretion by male and female rats was quantified until day 21 (seven days after the last dose). Metabolites in urine and faeces (% of accumulating dose), collected for intervals of 24 h after the first, seventh, and last (fourteenth) day of dosing were profiled. Statements of adherence to QA and GLP were included.

More than 98.4% of the administered dose was recovered. Plasma and tissue concentrations indicated that steady-state kinetic behaviour was reached in male rats after 14 days of dosing. In female rats, concentrations of radiolabel in the plasma and tissue were near steady-state at the end of the 14-day dosing period. At day 15, plasma concentrations peaked at 4.6 and 32 μg equivalents/g in males and females, respectively, these concentrations being about two- and sevenfold higher than 24 h after a single dose at 10 mg/kg bw. The concentrations of ^{14}C residues in tissues were higher in females than in males (2.35% vs 0.35% of the administered dose) at 168 h after the last dose. After dosing, the concentration of ^{14}C residues in the selected tissues of female rats declined, with half-lives ranging from 3.9 to 7.7 days. The half-life in plasma ($T_{1/2}$ = 7.2 days) was approximately twofold that determined from plasma collected for up to 5 days after administration of a single dose ($T_{1/2}$ = 3.4 days; see Himmelstein 2006a). A more extensive evaluation of tissue residues in 21 different tissues produced profiles of concentration and percent of dose that were similar to those observed in the single-dose study. Ratios of concentrations in tissue and plasma were less than 1.

Most of the administered dose was excreted in the faeces (males, 72.9%; females, 81.6%). In the urine, 16.7% and 12.1% of the administered dose was excreted by males and females, respectively. The overall pattern of distribution and excretion for multiple dosing (10 mg/kg bw per day × 14 days) generally resided between the pattern observed for administration of a single low dose (10 mg/kg bw) and a single high dose (200 mg/kg bw) (Himmelstein, 2006b).

1.2 Biotransformation

The metabolism of chlorantraniliprole was investigated in two studies in Sprague-Dawley Crl:CD®(SD)IGS BR rats, performed in accordance with OECD guideline 417. The experiments

were performed with a 1 : 1 (μCi : μCi) mixture of [benzamide carbonyl ^{14}C]chlorantraniliprole (radiochemical purity, 97%) and [pyrazole-carbonyl ^{14}C]chlorantraniliprole (radiochemical purity, 99%), diluted with chlorantraniliprole technical (purity, 96.45%). The rats were given a single dose at 10 or 200 mg/kg bw (Himmelstein, 2006a) or daily doses of 10 mg/kg bw by gavage for 14 days (Himmelstein 2006b). Metabolites were identified and quantified by high-performance liquid chromatography (HPLC) and mass spectrometry (MS) or tandem mass spectrometry (MS/MS). Statements of adherence to QA and GLP were provided.

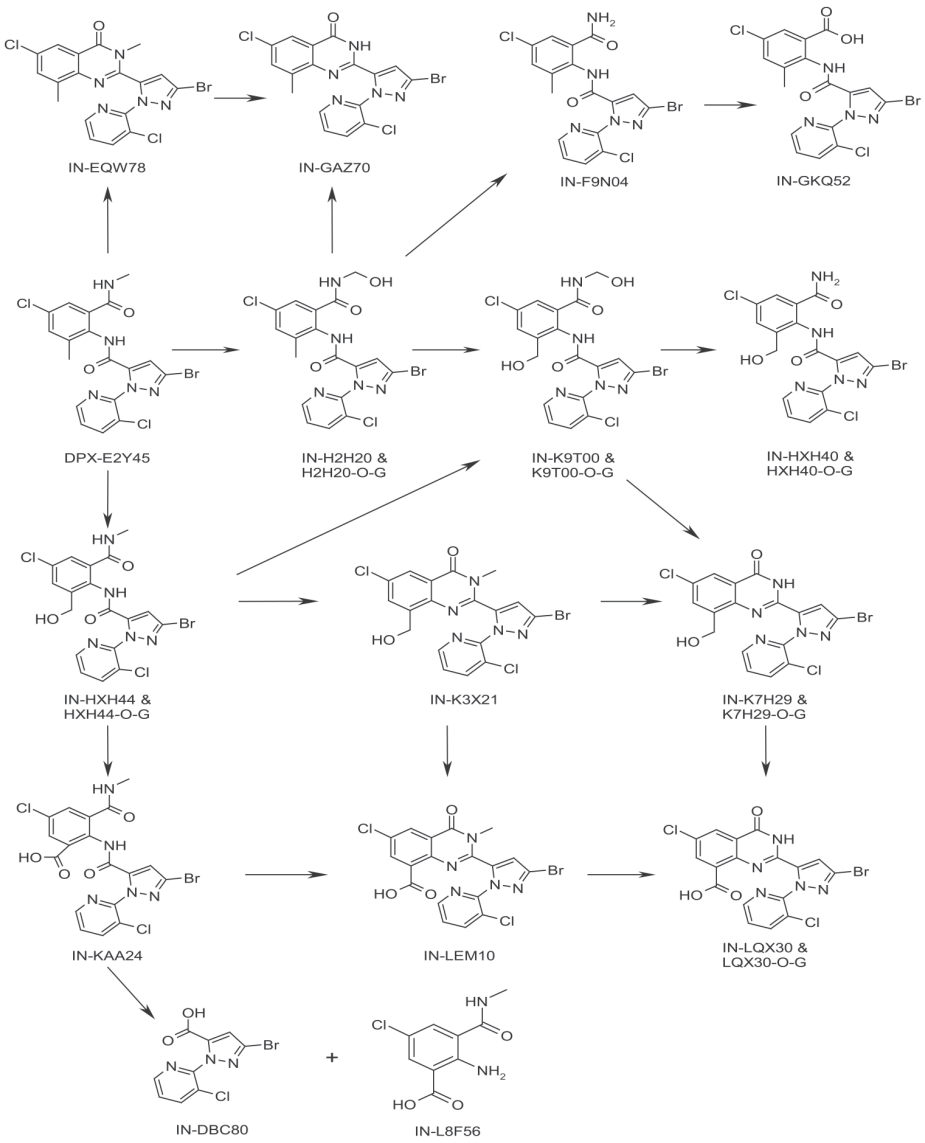
The metabolism of chlorantraniliprole was extensive and characterized by tolyl methyl and *N*-methyl carbon hydroxylation, followed by *N*-demethylation, nitrogen-to-carbon cyclization with loss of a water molecule resulting in the formation of the pyrimidone ring, oxidation of alcohols to carboxylic acids, amide-bridge cleavage, amine hydrolysis, and *O*-glucuronidation. At both doses, a significant difference between the sexes was apparent in the profile of metabolites in the urine and faeces, which indicated greater potential for hydroxylation of the tolyl methyl and *N*-methyl carbon groups in male rats than in female rats. For example, in rats at 10 mg/kg bw, the percentage of the administered dose represented by the di-hydroxylated metabolite IN-K9T00 was greater in males (urine, 7.4%; faeces, 10.4%) than in females (urine, 2.2%; faeces, 4.8%). Concentrations of the methylphenyl mono-hydroxylated metabolite IN-HXH44 were higher in the urine (4.6%) and faeces (7.4%) of males than urine (2.4%) and faeces (3.5%) of females. IN-KAA24, a carboxylic-acid metabolite of IN-HXH44, was a significant metabolite observed in the urine and faeces of males (10.6% combined), but not in females. Percentages of the *N*-methyl carbon hydroxylated metabolite IN-H2H20 were higher in females (urine, 3.4%; faeces, 15.0%) than in males (urine, 0.3%; faeces, 1.4%). At the higher dose, excretion of the parent compound in the urine and faeces (78.9–85.5%) was 12–16-fold that at the lower dose (4.9–7.3%). The profile of metabolites in rats at 200 mg/kg bw was similar to that in rats at 10 mg/kg bw.

The profile of metabolites in the urine and faeces of rats given repeated doses was similar to that observed for rats given single doses. Some minor differences included an apparent increase in the percentages of hydroxylated and polar metabolites such as IN-H2H20, IN-K7H29, and IN-KAA24 after repeated doses. IN-GAZ70 was observed in the faeces of female rats after 7 and 14 days of repeated doses, but not after a single dose. The proposed metabolic pathway is depicted in Figure 1 (Himmelstein, 2006a, 2006b).

As part of a 3-month feeding study in rats, performed in accordance with OECD guideline 408, concentrations of chlorantraniliprole and the two major metabolites, IN-GAZ70 and IN-H2H20 (for structures, see Figure 1) were measured in the plasma. Groups of 10 male and 10 female Crl:CD®(SD) IGS BR rats were given diets containing chlorantraniliprole (purity, 95.9%) at a concentration of 0, 600, 2000, 6000, or 20 000 ppm, equal to 0, 36.9, 120, 359, or 1188 mg/kg bw per day for males and 0, 47.0, 157, 460, or 1526 mg/kg bw per day for females. Concentrations of chlorantraniliprole, IN-GAZ70 and IN-H2H20 were determined by liquid chromatography (LC)/MS in plasma obtained on day 59. Statements of adherence to QA and GLP were provided.

Chlorantraniliprole, IN-GAZ70 and IN-H2H20 were present in the plasma at greater concentrations in female rats (up to 0.83, 112 and 0.54 μg/ml, respectively) than in male rats (up to 0.18, 3.7 and 0.08 μg/ml, respectively) with concentrations of IN-GAZ70 being highest. The plasma concentrations of all three analytes were similar at the three higher dietary concentrations in both sexes (MacKenzie, 2004; Gannon, 2005; Sykes, 2006a).

Figure 1 Proposed metabolic pathway of DPX-E2Y45 in the rat



From Himmelstein (2006a), Himmelstein (2006b)

DPX-E2Y45 (chlorantraniliprole):	3-Bromo-1-(3-chloro-2-pyridinyl)-*N*-[4-chloro-2-methyl-6-[(methylamino)carbonyl] phenyl]-1*H*-pyrazole-5-carboxamide
IN-EQW78:	2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3, 8-dimethyl-4-(3*H*)-quinazolinone
IN-GAZ70:	2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-8-methyl-4(3*H*)-quinazolinone
IN-F9N04:	*N*-[2-(Aminocarbonyl)-4-chloro-6-methylphenyl]-3-bromo-1-(3-chloro-2-pyridinyl)1*H*-pyrazole-5-carboxamide
IN-GKQ52:	2-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]carbonyl]amino]-5-chloro-3-methylbenzoic acid
IN-H2H20:	3-Bromo-*N*-[4-chloro-2-[[(hydroxymethyl)amino]carbonyl]-6-methylphenyl]-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide
IN-H2H20-O-glucuronide:	[[2-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl)carbonyl]amino]-5-chloro-3-methylbenzoyl]amino]methyl β-D-glucopyranosiduronic acid

IN-K9T00:	3-Bromo-*N*-[4-chloro-2-(hydroxymethyl)-6-[[(hydroxymethyl)amino)carbonyl] phenyl]-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide
IN-K9T00-O-glucuronide:	[[2-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]carbonyl]amino]-5-chloro-3-(hydroxymethyl)benzoyl]amino]methyl β-D-glucopyranosiduronic acid
IN-HXH40:	*N*-[2-Aminocarbonyl]-4-chloro-6-(hydroxymethyl)phenyl]-3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide
IN-HXH40-O-glucuronide:	[3-(Aminocarbonyl)-2-[[[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]carbonyl]amino]-5-chlorophenyl]methyl β-D-glucopyranosiduronic acid
IN-HXH44:	3-Bromo-*N*-[4-chloro-2-(hydroxymethyl)-6-[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide
IN-HXH44-O-glucuronide:	[2-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]carbonyl]amino]-5-chloro-3-[(methylamino)carbonyl]phenyl]methyl β-D-glucopyranosiduronic acid
IN-K3X21:	2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-8-(hydroxymethyl)-3-methyl-4(3*H*)-quinazolinone
IN-K7H29:	2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-8-(hydroxymethyl)-4(3*H*)-quinazolinone
IN-K7H29-O-glucuronide:	2-[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-1,4-dihydro-4-oxo-8-quinazolinyl]methyl β-D-glucopyranosiduronic acid
IN-KAA24:	2-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]carbonyl]amino]-5-chloro-3-[(methylamino)carbonyl]benzoic acid
IN-LEM10:	2-[5-Bromo-2-(3-chloro-pyridin-2-yl)-2*H* pyrazol-3-yl]-6-chloro-3,4-dihydro-3-methyl-4-oxo-8-quinazolinecarboxylic acid
IN-LQX30:	2-[3-Bromo-1-(3-chloro-2-pyridyl)-1*H*-pyrazol-5-yl]-6-chloro-1,4-dihydro-4-oxo-8-quinazolinecarboxylic acid
IN-LQX30-O- glucuronide:	β-D-Glucopyranuronic acid 1-[2-[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-1,4-dihydro-4-oxo-8-quinazolinecarboxylate
IN-DBC80:	3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxylic acid
IN-L8F56:	2-Amino-5-chloro-3-[(methylamino)carbonyl]benzoic acid

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with chlorantraniliprole are summarized in Table 2. No substance-related clinical signs were observed in studies of oral or dermal toxicity. In the study of toxicity after inhalation, ocular and nasal discharge was observed.

Table 2. Acute toxicity with chlorantraniliprole

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50}/LC_{50} (mg/kg bw; mg/l)	Reference
Rat	Crl:CD®(SD)IGS BR	Female	Oral	Aqueous methylcellulose	96.45	> 5000	Finlay (2004a)[a, b]
Rat	Crl:CD®(SD)IGS BR	Males and females	Dermal	Water	96.45	> 5000	Finlay (2004b)[a, c]
Rat	Crl:CD®(SD)IGS BR	Males and females	Inhalation	Air	96.45	> 5.1	Kegelman (2004)[a, d]

[a] Statements of adherence to good laboratory practice and quality assurance were provided.

[b] Performed according to the up-and-down procedure, OECD guideline 425.

[c] Performed according to OECD guideline 402. Observed clinical signs, i.e. red ocular and red nasal discharge, were attributed to the restraining procedure.

[d] Performed according to OECD guideline 403, nose-only exposure. Mass median aerodynamic diameter (MMAD) was 3.1 µm; geometric standard deviation (GSD) 1.8.

(a) Dermal irritation

In a study of dermal irritation, performed according to OECD guideline 404, three New Zealand White male rabbits were dermally exposed for 4 h to 0.5 g of chlorantraniliprole technical (purity, 96.45%; solid powder) moistened with deionized water. Dermal irritation was scored according to the Draize system at 1, 24, 48 and 72 h after patch removal. Statements of adherence to QA and GLP were included.

No dermal irritation was observed at any point during the study (Finlay, 2004c).

(b) Ocular irritation

In a study of ocular irritation, performed according to OECD guideline 405, 0.1 ml (approximately 72 mg) of chlorantraniliprole technical (purity, 96.45%; solid powder) was instilled into the conjunctival sac of the right eye of one young adult New Zealand White male rabbit. The untreated left eye served as a control. Since no severe irritation or corrosion was observed, two additional rabbits were treated in the same way. Ocular irritation was scored according to the Draize system at 1, 24, 48 and 72 h after instillation. Statements of adherence to QA and GLP were included.

No corneal opacity was noted. Iritis was noted in one eye after 1 h. Conjunctival redness (score, 1) was noted in two out of three eyes at 1 h, persisting in one eye at 24 h and 48 h. Chemosis (score, 1) was noted in two out of three eyes at 1 h. Discharge (scores, 2 and 3) was noted in one eye at 1 h and 24 h. All eyes were free of irritation by 72 h. The Meeting concluded that chloroantraniliprole is not an ocular irritant (Finlay, 2004d).

(c) Dermal sensitization

In a study of dermal sensitization using the local lymph node assay method according to OECD guideline 429, groups of five female CBA/JHsd mice were given chlorantraniliprole technical (purity, 96.45%; solid powder) at a concentration of 0%, 5%, 25%, 50% or 100% (prepared at 1 g/ml in dimethylformanimide) w/v in the vehicle N,N-dimethylformanimide (DMF). The substance used as a positive control was 25% alpha-hexylcinnamaldehyde (HCA). For three consecutive days, the mice in each group were treated with 25 µl of the respective solutions on the dorsal surface of each ear. One group of mice was dosed similarly with the positive control, HCA in 4 : 1 acetone : olive oil (AAO), and one group of mice was dosed similarly with the vehicle used for the positive control only, AAO. On study day 5, the tail vein of each mouse was injected with 20 µCi of [3H]methyl thymidine and the mice were killed 5 h later. The single-cell suspensions of the auricular lymph nodes of each ear were incubated overnight and [3H] activity was counted on day 6. A stimulation index was derived for each experimental group by comparison with the group receiving the vehicle control. A stimulation index of ≥ 3 and/or a statistically significant increased stimulation index were considered to be a positive response. Statements of adherence to QA and GLP were included.

No clinical signs of toxicity or statistically significant differences in body weights and body-weight gains were observed. Stimulation indexes were approximately 1 at all concentrations tested. No statistically significant increases in cell proliferation were observed. The test system was validated by the dermal-sensitization response of the positive control, HCA. In this study, chloroantraniliprole was not a dermal sensitizer (Hoban, 2006).

In a study of dermal sensitization using the Magnusson and Kligman maximization test, according to OECD guideline 406, 20 Dunkin Hartley guinea-pigs were exposed to chlorantraniliprole technical (purity, 96.45%; solid powder). During the preliminary testing phase, the concentrations of the test substance used for the intradermal induction, topical induction and topical challenge,

respectively, were 5% (w/w), 80% (w/w) and 20% (w/w), the highest non-irritating concentration. The vehicle was mineral oil. The control group consisted of 10 guinea-pigs.

The first induction phase involved three paired intradermal injections of 0.1 ml of test substance, 0.1 ml of 50% (v/v) mixture of complete Freund adjuvant in distilled water and 0.1 ml of complete Freund adjuvant with test substance. The guinea-pigs in the control group were treated similarly with vehicle only. The topical induction phase was carried out 1 week later: the guinea-pigs were pre-treated with sodium lauryl sulfate 24 h before topical applications of 0.5 g of chloroantraniliprole on a gauze pad for 48 h under occlusion. In the challenge phase, 21 days after study initiation, the guinea-pigs received topical applications of 0.5 ml of the challenge dose and 0.5 ml of a 33% dilution of the challenge dose for 24 h. At 24 h and 48 h after patch removal, the degree of dermal irritation was scored according to the Magnusson and Kligman grading scale. Data from appropriate historical controls exposed to α-hexylcinnamaldehyde technical (HCA) were used as the positive control. Statements of adherence to QA and GLP were included.

After the intradermal and topical induction phases, very faint to moderate erythema (scores, 0.5–2) was noted at the treatment site in most guinea-pigs receiving chlorantraniliprole and in the control group. At challenge, very faint erythema (score, 0.5) was noted in some guinea-pigs receiving chlorantraniliprole at the highest non-irritating concentration and in some guinea-pigs in the control group. At treatment sites to which the 33% dilution was applied, no dermal reactions were noted. The Meeting concluded that chlorantraniliprole is not a dermal sensitizer under the conditions of the maximization test (Moore, 2004).

2.2 Short-term studies of toxicity

Mice

In a 28-day feeding study, performed in accordance with OECD guideline 407, 10 male and 10 female Crl:CD-1®(ICR)BR mice were fed diets containing chlorantraniliprole (purity, 95.9%) at a concentration of 0, 300, 1000, 3000, or 7000 ppm, equal to 0, 52, 182, 538, and 1443 mg/kg bw per day for males and 0, 64, 206, 658, and 1524 mg/kg bw per day for females (corrected for purity). The mice were observed at least once per day for mortality and clinical signs of toxicity. A detailed clinical examination and body-weight and food-consumption measurements were performed weekly. For five males and five females per group, haematology and clinical chemistry (plasma total protein only) were performed at termination, 4 weeks after initiation of the study. At the same time, gross examinations were performed and selected organs were weighed. Organs and tissues of mice in the control group and in the group at 7000 ppm were examined histologically. On day 13 or 14, five males and five females per group were killed, and peroxisomal β-oxidation and total cytochrome P450 content in liver tissue were determined. Statements of adherence to QA and GLP were included.

At termination, reductions in mean body weight (92% of values for controls) and body-weight gain (43% of values for controls) were observed in males at the highest dose over the 28 days. It was noted that the body-weight gain of the mice in the control group varied greatly during this period, with an initial drop in weight during the first week, followed by a rapid recovery during the following 3 weeks. Weight gain in the groups receiving chlorantraniliprole was constant throughout the treatment period. No dose-dependent effect on body-weight gain was observed in the treatment groups. Although the effects on body weight and body-weight gain in males at the highest dose may have been treatment-related, in isolation, they were considered to be not adverse. The reduction in body-weight gain was accompanied by a reduction in food efficiency (up to 50%). No effect on body weight and food efficiency occurred in females. A slight increase in mean liver weight in 3000 and 7000 ppm females and a mild increase in cytochrome P450 content observed in males and females at 3000 or 7000 ppm were considered to be non-adverse pharmacological responses to metabolism of chlorantraniliprole. Decreased hepatic β-oxidation activity in males at the highest dose (79% of

values for the controls) and females (54% of values for the controls) was also considered to be not adverse, due to the magnitude of change (less than twofold). No histological evidence of organ toxicity was observed. No other parameters were affected.

The NOAEL was 7000 ppm, equal to 1443 mg/kg bw per day, the highest dose tested (Finlay, 2003).

In a 3-month feeding study, performed according to OECD guideline 408, groups of 15 male and 15 female Crl:CD-1®(ICR)BR mice were given diets containing chlorantraniliprole (purity, 95.9%) at a concentration of 0, 200, 700, 2000, or 7000 ppm, equal to 0, 32.6, 115, 345, or 1135 mg/kg bw per day for males and 0, 40.7, 158, 422, or 1539 mg/kg bw per day for females (corrected for purity). The mice were observed at least once per day for mortality and clinical signs of toxicity. A detailed clinical examination and body weight and food consumption measurements were performed weekly. Ophthalmoscopy was performed before treatment and before scheduled termination. At days 92–93 of treatment, blood was collected from all mice for haematology, clinical chemistry and determination of plasma concentrations of chloranatraniliprole and some metabolites. All mice were killed after 3 months. Ten males and ten females per group were examined grossly and selected organs were weighed. An extensive range of organs and tissues of mice from the control group and the group at 7000 ppm was histologically examined. Statements of adherence to QA and GLP were included.

No test substance-related effects were observed on survival, nutritional parameters, haematology, clinical chemistry, clinical or ophthalmological observations. Plasma concentrations of chlorantraniliprole were below the limit of detection in all groups. The plasma concentration of metabolite IN-GAZ70 in females was twofold that in males. In males at 2000 and 7000 ppm, statistically significant reductions in mean body weights (92% and 93% of values for controls, respectively) and mean body-weight gains (67 and 74% of values for controls, respectively) were observed. It was noted that these effects were not dose-dependent, often did not occur on consecutive weeks and were predominantly due to differences in body-weight gain during the last 2 weeks of treatment. Moreover, they were not found in an 18-month study in mice given chlorantraniliprole at similar doses. Therefore the Meeting considered that these effects were not adverse.

The reduction in body-weight gain was accompanied by a reduction in food efficiency. Slight increases in incidences of hyperactivity, hyper-reactivity and convulsions were not dose-dependent, were not confirmed in an 18-month study in mice and were therefore considered to be incidental. No adverse test substance-related effects were observed on organ weights, or any clinical pathology, gross or microscopic pathology endpoints. A slight increase in liver weight in the group at 7000 ppm (9–17%) was considered to be test substance-related but not adverse. The increased liver weights were not associated with any liver histopathology and were attributed to enzyme induction.

The NOAEL was 7000 ppm, equal to 1135 mg/kg bw per day, the highest dose tested (Finlay, 2006a; Gannon, 2006)

Rats

In a preliminary study of oral toxicity, groups of five male and five female Crl:CD®(SD)IGS BR rats were given chlorantraniliprole (purity, about 100%) at a dose of 0, 25, 100 and 1000 mg/kg bw per day by gavage for 14 consecutive days. Data on body weights and clinical observations were collected (at least once) daily. At termination, haematology, blood coagulation, clinical chemistry, and urine analysis were conducted and the rats were examined macroscopically, and selected organs were weighed and examined histopathologically. Bone-marrow smears were examined for the presence of micronuclei to assess potential genetic toxicity; an additional group of rats was given cyclophosphamide to provide a positive control for the presence of genetic toxicity.

There were no test substance-related effects on body weight, clinical observations, haematology, coagulation, clinical chemistry, urine analysis, gross and microscopic pathology, organ weights,

frequency of micronucleated polychromatic erythrocytes or in the ratio of polychromatic erythrocytes to normochromatic erythrocytes (PCEs/NCEs) in bone marrow at any dose tested. There was no microscopic evidence of increased adrenal cortical microvesiculation, which had been observed in some male rats in feeding studies of longer duration (Munley, 2006a).

In a preliminary 28-day feeding study conducted in accordance with OECD guideline 407, groups of five male and five female Crl:CD®(SD)IGS BR rats were given diets containing chlorantraniliprole (purity, 98.6%) at a concentration of 0, 300, 1500, or 8000 ppm, equal to 0, 20.7, 106, and 584 mg/kg bw per day for males and 0, 24, 128, and 675 mg/kg bw per day for females. the rats were observed at least once per day for mortality and morbidity. Clinical signs of toxicity, body weights and food consumption were recorded weekly. Ophthalmological examinations were performed before study start and again before termination. Haematology, clinical chemistry, coagulation tests, bone-marrow smears, urine analysis and measurement of UDP-glucuronyl transferase (UDP-GT) activity in liver tissue were performed at termination 4 weeks after initiation of the study. Also at termination, gross examinations were performed on all rats and selected organs were weighed. Organs and tissues of rats in the control group and in the group at 8000 ppm were examined histologically. In the groups receiving the lowest and intermediate dose, microscopic examinations were conducted on lung, liver, and kidneys. The adrenal glands of male and female rats in the control group and the group at the highest dose were examined microscopically for microvesiculation of the adrenal cortex.

No treatment-related adverse effects were observed. In females, statistically significant increases in relative liver weights (11–14%) and UDP-GT activity (37–51%) were observed in the groups at 1500 and 8000 ppm. Minimal centrilobular hepatocellular hypertrophy was observed in females at 8000 ppm. There was no evidence of hepatic cell damage. The liver effects in females were considered to be treatment-related but not adverse and were attributed to enzyme induction. There was no microscopic evidence of increased adrenal cortical microvesiculation (Donner, 2006a; Sykes, 2006a).

In a 3-month feeding study performed in accordance with OECD guideline 408, groups of 10 male and 10 female Crl:CD®(SD)IGS BR rats were given diets containing chlorantraniliprole (purity, 95.9%) at a concentration of 0, 600, 2000, 6000, or 20 000 ppm, equal to 0, 36.9, 120, 359, or 1188 mg/kg bw per day for males and 0, 47.0, 157, 460, or 1526 mg/kg bw per day for females. The rats were observed at least once per day for clinical signs of toxicity and mortality. Detailed clinical examinations were performed weekly. Body weight and food consumption were measured weekly. Ophthalmological examinations were performed before study start and again before termination. Haematology, clinical chemistry and urine analysis were performed mid-study (days 48–49) and at termination about 3 months after initiation of the study. In plasma obtained at day 59, concentrations of chlorantraniliprole and the two major metabolites, IN-GAZ70 and IN-H2H20 (for structure see Figure 1), were measured by LC/MS. At termination, blood coagulation was tested and bone-marrow smears were examined. Also at termination, gross examinations were performed on all rats and selected organs were weighed. Organs and tissues of rats in the control group and in the group at 20 000 ppm and male hearts and gross lesions observed in males and females at the lowest and intermediate dose were examined histologically. Statements of adherence to QA and GLP were included.

A slight increase in liver weight in the females at 20 000 ppm females (11–17%) and a reduction in bilirubin in females at 2000 ppm and above, not associated with any liver histopathology, were considered to be test substance-related but not adverse and were attributed to enzyme induction. A minimal to mild increase in microvesiculation in the zona fasciculata region of the adrenal cortex in some males at 2000 ppm was considered to be test substance-related but not adverse as the adrenal morphology was within the range for controls, was not associated with cytotoxicity of the adrenal gland and had no impact on adrenal function (assessed in separate studies). No other test substance-related effects were observed.

The NOAEL was 20 000 ppm, equal to 1188 mg/kg bw per day, the highest dose tested (MacKenzie, 2004; Gannon, 2005; Sykes, 2006b).

Dogs

In a 28-day dose range-finding study, groups of two male and two female beagle dogs were given gelatin capsules containing chlorantraniliprole (purity, 97.6%) at a dose of 0, 300 or 1000 mg/kg bw per day (corrected for purity). As part of a subsequent study, groups of four male were given chlorantraniliprole at a dose of 0 or 1000 mg/kg bw per day for 28 days. These additional dogs were randomized into dosing groups on the basis of pre-test testicular volume. All dogs were observed at least twice per day for mortality, morbidity and injury. Detailed clinical examinations and neurobehavioral observations were conducted before the study and then weekly thereafter. Body weight and food consumption were measured weekly. All dogs were examined ophthalmoscopically before the start of the study and again before scheduled termination. Blood and urine were sampled for haematology, clinical chemistry and urine analysis before the test and before termination. In addition, plasma was collected from all dogs on the day before dosing and at 1 h after dosing on test days 1, 2, and 3. Plasma was also collected before dosing and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 18, and 24 h after dosing on day 28. Plasma samples were analysed for concentration of chlorantraniliprole. A sample of liver was collected at necropsy and analysed for hepatic cytochrome P450 (total and isozyme profile: 1A1, 2B1/2, 2E1, 3A2, 4A1). At termination of the study, the dogs were killed and subjected to gross examinations, and selected organs were weighed. An extensive range of tissues was evaluated microscopically.

No treatment-related effects on survival, body weight or nutritional parameters, clinical or neurobehavioral findings, ophthalmology, clinical pathology, or anatomic pathology were observed in dogs exposed to chlorantraniliprole. In the preliminary study with two male and two female dogs per group, testes weights were decreased in a dose-dependent manner (38–49%) at 300 and 1000 mg/kg per day. In these dogs, microscopic changes comprising hypospermatogenesis in the testes, characterized by reduced proportions or total absence of germ cells, accompanied by tubular shrinkage, and Sertoli cell prominence, were observed. However, in the subsequent study in which dogs were randomized into dosing groups on the basis of pre-test testicular volume, no effects on testicular weight were observed. In these dogs, hypospermatogenesis was observed in one out of four dogs in the control group (moderate) and two out of four dogs at 1000 mg/kg per day (minimal). Therefore, given the relatively young age of the dogs, the Meeting concluded that the microscopic testicular observations in the main study were due to sexual immaturity, and not to exposure to chlorantraniliprole.

Oral exposure of male and female dogs to chlorantraniliprole induced hepatic cytochrome P450 enzymes. Although treatment-related, these increases in liver enzymes are not considered to be adverse. Pharmacokinetic parameters (T_{max}, C_{max}, half-life, and AUC) were similar in male and female dogs at 300 mg/kg bw per day. At 1000 mg/kg bw per day, the maximum plasma concentrations and half-lives were similar in males and females; however, the AUC for female dogs was approximately 1.75 fold that for male dogs (Serota, 2003).

In a 28-day feeding study, conducted to investigate potential palatability issues, two groups of two male and two female beagle dogs were given diets containing chlorantraniliprole (purity, 95.9%) at escalating dietary concentrations of 1000 ppm (week 1), 5000 ppm (week 2), and 10 000 ppm (weeks 3–4) for one group and 30 000 ppm (weeks 1–2) and 40 000 ppm (weeks 3–4) ppm for the other group. The mean daily intakes for the groups at 1000, 5000, 10 000, 30 000, and 40 000 ppm were 26, 138, 266, 797, and 1302 mg/kg bw per day in male dogs, and 28, 138, 298, 888, and 1240 mg/kg bw per day in female dogs. A control group of males and two females was fed untreated diet. All dogs were observed at least twice per day for mortality and morbidity. Detailed clinical examinations and neurobehavioral observations were conducted weekly. Body weight was measured

weekly, food consumption was measured daily. At termination of the study, the dogs were killed and subjected to gross examinations.

Palatability of the diet was not affected by inclusion of the test material. Throughout the study, no adverse, test substance-related effects were observed on survival, clinical or neurobehavioral findings, body weight or weight gain, food consumption, or food efficiency. No adverse, test substance-related effects on gross pathology were noted in any dog (Luckett, 2003).

In a 90-day feeding study, performed in accordance with Office of Prevention, Pesticides and Toxic Substances (OPPTS) guideline 870.3150 which resembles OECD guideline 408, groups of four male and four female beagle dogs were given diets containing chlorantraniliprole (purity, 95.9%) at a concentration of 0, 1000, 4000, 10 000, or 40 000 ppm, equal to 0, 32.2, 119, 303 and 1163 mg/kg bw per day for males and 0, 36.5, 133, 318, and 1220 mg/kg bw per day for females (corrected for purity). All dogs were observed at least twice per day for mortality, morbidity and injury. Detailed clinical examinations were conducted twice per day, neurobehavioral observations were conducted weekly. Body weight was measured weekly, food consumption was measured daily. Ophthalmoscopy was performed before start of the study and before termination. Blood and urine samples for haematology, coagulation, clinical chemistry and urine analysis were collected before the test, at week 6 and at week 12. At termination of the study, the dogs were killed and subjected to gross examinations. Selected organs were weighed. An extensive range of organs were examined histologically. Statements of adherence to QA and GLP were included.

No test substance-related effects were observed on mean body-weight gain and nutritional parameters, clinical, neurobehavioral, or ophthalmological signs during the 90 days. No test substance-related effects on clinical pathology, gross or microscopic pathology were observed in dogs exposed to chlorantraniliprole at any dietary concentration. Although not statistically significant, a 20% reduction in body weight and a decrease of approximately 25% in heart weight were reported for females in the group at 10 000 ppm. There were no body-weight reductions in the females at 40 000 ppm and no significant difference in total body-weight gain in females at 10 000 ppm. Furthermore, the 20% reduction in body weight was only experienced in study weeks 11 and 12. Similarly, the decrease in heart weight was not experienced by females at the highest dose and no corresponding microscopic changes were noted in the heart. On the basis of the lack of dose–response for both body weight and heart-weight effects, these effects were not considered to be treatment-related.

Increases in relative liver weights (up to 26%), not dose-dependent, were observed in male dogs in all groups receiving chlorantraniliprole. At 40 000 ppm, the increase (26%) was statistically significant. This finding was not associated with any liver histopathology and was attributed to enzyme induction and was not considered to be adverse.

The NOAEL was 40 000 ppm, equal to 1163 mg/kg bw per day, the highest dose tested (Luckett, 2004).

In a 1-year feeding study, performed in accordance with OECD guideline 452, groups of four male and four female beagle dogs were given diets containing chlorantraniliprole (purity, 96.45%) at a concentration of 0, 1000, 4000, 10 000, or 40 000 ppm, equal to 0, 32, 112, 317, and 1164 mg/kg bw per day for males and 0, 34, 113, 278, and 1233 mg/kg bw per day for females (corrected for purity). All dogs were observed daily for mortality, morbidity and injury. Detailed clinical examinations were conducted, neurobehavioral observations were conducted weekly. Body weight and food consumption were measured weekly. Ophthalmoscopy was performed before start of the study and before termination.

Blood and urine samples for haematology, coagulation, clinical chemistry and urine analysis were collected pre-test, at weeks 13, 26 and 52. At termination of the study, the dogs were killed

and examined grossly. Selected organs were weighed. An extensive range of organs was examined histologically. Statements of adherence to QA and GLP were included.

No test substance-related effects were observed on survival, clinical and neurobehavioral signs, ophthalmology, body weight and nutritional parameters, clinical pathology, or gross or microscopic pathology. Test substance-related increases in absolute and relative liver weights (25–40%) were observed in 40 000 ppm male and female dogs. In the absence of hepatic cell damage the changes in liver weight were considered to be not adverse and attributed to enzyme induction. One male dog in the group at 40 000 ppm demonstrated clinical signs of toxicity, clinical pathology, and anatomic pathology changes consistent with canine juvenile polyarteritis syndrome; these effects were not considered to be test substance-related.

The NOAEL was 40 000 ppm, equal to 1164 mg/kg bw per day, the highest dose tested (Luckett, 2006).

In a 28-day study of dermal administration, performed in accordance with OECD guideline 410, groups of 10 male and 10 female Crl:CD®(SD)IGS BR rats were given chlorantraniliprole (purity, 96.45 %) at a dose of 0, 100, 300, or 1000 mg/kg bw per day (corrected for purity), applied to the shaved, intact dorsal skin under semi-occlusion for 6 h per day for 29 consecutive days. Parameters evaluated included body weight, body-weight gain, food consumption, food efficiency, clinical signs, clinical pathology, organ weights, and gross and microscopic pathology.

All rats were observed daily for mortality, clinical signs and injury. Body weight was recorded twice per week and food consumption was measured weekly. Blood samples were collected on day 29 for haematology, coagulation, and clinical chemistry. At termination of the study, the rats were killed and subjected to gross examinations. Selected organs were weighed. An extensive range of organs of rats in the control group and rats in the group at 1000 mg/kg bw per day, and organs with gross lesions from rats in all groups were examined histologically. In addition, adrenal glands from all groups of males were evaluated histologically. Statements of adherence to QA and GLP were included.

No test substance-related effects were observed on survival, clinical observations or food consumption. No adverse test substance-related effects were observed on organ weights, any clinical pathology, gross or microscopic pathology end-point. Treatment-related reductions in mean body weight (6% and 5% in males and females, respectively) and body-weight gain (22% and 19% in males and females, respectively) and food efficiency were observed over the 28 days in males and females at the highest dose, but these effects were not considered to be adverse. Minimal increases in adrenal microvesiculation in some males at all doses were considered to be test substance-related, but not adverse, as adrenal morphology was within the normal range, was not accompanied by microscopic evidence of toxicity and had no impact on adrenal function (assessed in a separate study).

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Finlay, 2006b).

In a 28-day study of dermal administration, 10 male Crl:CD®(SD)IGS BR rats were given chlorantraniliprole (purity, 96.45%) at a dose of 1000 mg/kg bw per day applied to the shaved, intact dorsal skin for 6 h per day for 29 daily (consecutive) applications. A control group of 10 male rats was treated with deionized water in a similar manner. A control group of 10 male rats underwent no shaving, application, or wrapping. Rats were observed daily for clinical signs after removal of the test substance. Body weight and food consumption were measured weekly. On the day following the last dermal treatment, each rat received an intravenous injection of adrenal corticotropic hormone (ACTH) and blood was collected for determination of corticosterone concentration. After blood collection, the rats were killed and the adrenal glands were examined microscopically. Statements of adherence to QA and GLP were included.

Although a significant reduction in body-weight gain after the first week of dosing (a decrease of 75% compared with the control group receiving deionized water) was observed, absolute body

weights of the treated rats was never less than 95% of that of the control group receiving deionized water. Similar effects were noted on food efficiency. There was a greater incidence of increased adrenal cortical microvesiculation in rats given chlorantraniliprole and stimulated with ACTH when compared to both groups of in-study control rats (non-wrapped control group, 0 out of 10; control group receiving deionized water, 1 out of 10; group receiving chlorantraniliprole, 4 out of 10). There were no effects on ACTH-stimulated serum corticosterone concentrations in rats treated dermally with chlorantraniliprole compared with the concurrent control group receiving deionized water.

The study indicated that dermal exposure to chloranatraniliprole does not affect adrenal corticosterone function (synthesis and release) at a dose that results in an increased degree of adrenal cortical microvesiculation (Finlay, 2006c).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In an 18-month feeding study of carcinogenicity, performed in accordance with OECD guideline 451, groups of 70 male and 70 female Crl:CD-1®(ICR)BR mice were given diets containing chlorantraniliprole (purity, 96.45%) at a dose of 0, 20, 70, 200, 1200, or 7000 ppm, equal to 0, 2.60, 9.20, 26.1, 158 and 935 mg/kg bw per day in males and 0, 3.34, 11.6, 32.9, 196 and 1155 mg/kg bw per day in females (corrected for purity). Mice were checked daily for clinical signs, and a detailed clinical examination was performed weekly. Body weights and food consumption were recorded weekly for the first 13 weeks and every other week thereafter. Ten male and ten female mice per group were tested by functional operational battery (FOB) before the start of the treatment and on days 45, 90 and 180 (approximately). Ophthalmological examinations were performed before treatment and before termination. Leukocyte relative differential counts were performed on blood smears of mice killed in extremis and on mice in the control group and at the highest dose at 18 months. At termination, all mice were examined grossly. Selected organs were weighed. An extensive range of tissues from rats in the control group and at the highest dose and all decedent mice were examined microscopically. Gross observations, observed at necropsy and male livers (i.e. suspected target organ) were examined microscopically for all rats. Statements of adherence to QA and GLP were included.

Absolute and relative liver weights showed a dose-related increase at 1200 (6–11%) and 7000 ppm (15–19%). Statistically significant increased hepatocellular hypertrophy was observed in males at 1200 and 7000 ppm. These effects are consistent with pharmacological enzyme induction and in isolation they are not considered to be adverse. In males at 7000 ppm, an increased incidence (7.1%; historical control range, 0–1.9%) of eosinophilic foci of cellular alteration (slightly nodular, focal, cluster of enlarged "eosinophilic" hepatocytes within the hepatic parenchyma) was observed. No information on the chemical-specific mechanism of action was available to evaluate the relevance of liver foci to exposure of humans. However, since these eosinophilic foci may potentially be preneoplastic lesions and are likely to be test substance-related, they are considered to be an adverse effect. The incidence of bronchioloalveolar adenoma was slightly increased (not statistically significant) in males at 7000 ppm; however, the combined incidence of bronchioloalveolar adenoma and carcinoma was similar in males at 7000 ppm and in the controls. Although statistically significant increases of malignant lymphoma metastasis were observed in females at 7000 ppm, the incidence of primary malignant lymphoma was not statistically significantly increased. No dose–response relationship was observed in the incidence of malignant lymphoma in mice at the intermediate dose, although it should be noted that haemolymphatic tissue was evaluated in only about half the mice at the intermediate dose. Historical data on the incidence of lymphoma were not provided. No increase in the incidence of lymphoma was observed in males at any dose. The Meeting concluded that there was no test substance-related increase in tumour incidence.

The NOAEL was 1200 ppm, equal to 158 mg/kg bw per day, on the basis of the presence of eosinophilic foci accompanied by hepatocellular hypertrophy and increased liver weight in males at 7000 ppm (Finlay, 2006d)

Rats

In a 2-year combined study of toxicity and carcinogenicity, performed in accordance with OECD guideline 453, groups of 70 male and 70 female Crl:CD®(SD)IGS BR rats were given diets containing chlorantraniliprole (purity, 96.45%) at a concentration of 0, 200, 1000, 4000, or 20 000 ppm, equal to 0, 7.71, 39.0, 156, and 805 mg/kg bw per day in males and 0, 10.9, 51.0, 212, and 1076 mg/kg bw per day in females (corrected for purity), for approximately 23 months. An interim sacrifice of 10 male and 10 female rats per group was conducted after 1 year. The rats were observed daily for mortality, morbidity and clinical signs. Detailed clinical examinations were conducted weekly. Body weight and food consumption were measured weekly for the first 13 weeks, and once every 2 weeks thereafter. Ophthalmoscopy was performed before start of the study, at day 366 and at day 660 before termination. Blood and urine samples for haematology, clinical chemistry and urine analysis were collected from 10 males and 10 females per group pre-test, and after about 3, 6 and 12 months. For these same 10 males and 10 females per group, coagulation measurements in blood and corticosterone measurements in urine were performed at 1 year and they were killed for interim pathological examinations. From all other surviving rats in the control group and at the highest dose, blood smears collected at 23 months were evaluated for leukocyte differential count.

All interim-kill rats and rats surviving until 23 months were subjected to gross pathology and selected organs were weighed. A wide range of tissues collected from rats in the control group and at the highest dose were evaluated microscopically. In groups at the intermediate and lowest dose, microscopic examinations were also conducted on the adrenal glands of males from both scheduled sacrifices and on thyroid glands of females at the terminal sacrifice. Gross lesions from all rats were examined microscopically. All rats sacrificed in extremis, found dead, or accidentally killed were examined grossly and all collected tissues were evaluated microscopically. A portion of adrenal gland was evaluated by electron microscopy in four male rats in the control group and four male rats in the group at 20 000 ppm sacrificed after 1 year. Statements of adherence to QA and GLP were included.

Rats were sacrificed before 2 years due to declining survival in most groups in both sexes, including the controls. The deaths were not due to exposure to chlorantraniliprole as there was no statistically significant test substance-related effect on mortality. Survival on test day 693 (before scheduled sacrifice) in males at 0, 200, 1000, 4000, and 20 000 ppm was 37%, 42%, 45%, 37% and 47%, respectively. Survival on test day 686 (before scheduled sacrifice) in females at 0, 200, 1000, 4000, and 20 000 ppm was 34%, 45%, 33%, 40%, and 35%, respectively. No treatment-related effects on clinical signs, body weight, body-weight gain, food consumption, food efficiency, ophthalmology, haematology, coagulation, clinical chemistry, urine analysis, and urine corticosterone evaluations or on leukocyte differential counts were observed.

An increase in relative liver weights was observed in female rats at 4000 ppm (14%) and 20 000 ppm (24%) (only at interim sacrifice at 1 year), but was not associated with any findings indicative of liver toxicity. Therefore, these weight changes were considered non-adverse and consistent with a pharmacological response to metabolism. No gross pathology findings were attributed to exposure to chlorantraniliprole. Increased adrenal cortical microvesiculation due to lipid was present in the zona fasciculata region of the adrenal gland of some male rats at all doses in the 1-year study and in the main study. This finding was considered to be related to administration of chlorantraniliprole, but was not considered to be adverse since microscopic and electron microscopic examination showed that the adrenal morphology was generally in the range of what was observed in rats in the control group, and the finding was not associated with any indication of cytotoxicity or other evidence of structural or functional impairment (corticosterone concentrations in urine were normal)

of the adrenal gland. No other treatment-related microscopic changes were observed in males or females. At the doses tested, chlorantraniliprole was not carcinogenic in male or female rats.

The NOAEL was 20 000 ppm, equal to 805 mg/kg bw per day, the highest dietary concentration tested (MacKenzie, 2006).

2.4 Reproductive toxicity

(a) Multigeneration study

Rats

In a 2-generation study of reproductive toxicity, performed in accordance with OECD guideline 416, groups of 30 male and 30 female Crl:CD®(SD)IGS BR rats were given diets containing chlorantraniliprole (purity, 96.45%) at a concentration of 0, 200, 1000, 4000, or 20 000 ppm, equal to 0, 12, 60, 238 and 1199 mg/kg bw per day in males and 0, 16, 78, 318, 1594 mg/kg bw per day in females of the P generation and equal to 0, 18, 89, 370, 1926 mg/kg bw per day in males and 0, 20, 104, 406 and 2178 mg/kg bw per day in females of the F_1 generation (corrected for purity). The rats were observed daily for clinical signs and detailed clinical observations were performed at least once per week. Body weights and food consumption were recorded weekly. Body weights and food consumption were also recorded on days 0, 7, 14 and 21 of gestation, and days 0, 7, 14, and 21 of lactation for the P and F_1 females. In P and F_1 rats, estrus cycle parameters (percentage of days in diestrus, proestrus, and estrus) and estrus cycle length were evaluated for 3 weeks before cohabitation. The age at either vaginal opening or preputial separation was recorded for the F_1 generation. Sperm motility, morphology and concentration in the cauda epididymis, and spermatid concentration in the testis were determined for P and F_1 rats. On postnatal day 4, litters were culled to eight pups. Until weaning at postnatal day 21, litters were examined for number of live and dead pups, pup weight and sex, clinical signs and external alterations on postnatal days 0, 4, 7, 14 and 21. After litter production, all P and F_1 parents were subjected to gross pathology and reproductive organs and brain, liver, spleen, adrenals, pituitary and kidneys were weighed. The reproductive organs, adrenal glands and gross lesions of all P and F_1 parents and F_2 weanlings and gross lesions of all weanlings were histologically examined. As adrenals were identified as potential target organs, adrenal glands from two males in the control group and in the group at the highest dose were examined by electron microscopy. Statements of adherence to QA and GLP were included.

There were no adverse, test substance-related effects on body weight, body-weight gain, food consumption, or food efficiency, clinical signs of toxicity, or mortality in P and F_1 males during pre-mating or in P and F_1 females during pre-mating, gestation, or lactation.

There were no test substance-related effects on sperm motility, morphology, epididymal sperm or testicular spermatid numbers in the P and F_1 males, nor on the mean percentage days in estrus, diestrus or proestrus, mean cycle length, or mean precoital interval in the P or F_1 females. Mating, fertility, duration of gestation, number of implantation sites, and implantation efficiency in the P and F_1 generations were not affected by chlorantraniliprole at any dietary concentration.

An increase in absolute and relative liver weights (up to 19%) was observed in P and F_1 females at 4000 ppm and above and was attributed to a pharmacological increase in metabolism. Livers were not examined microscopically in this study. In addition, an increase in mean absolute and relative adrenal weight (4–22%) was observed at 4000 and 20 000 ppm P and F_1 adults. A test substance-related increase in the number of rats displaying a minimal to mild increase in the degree of adrenal cortical microvesiculation was observed in P adult males at doses of 1000 ppm and above, in F_1 adult males at 200 ppm and higher, and in F_1 females at 20 000 ppm. Electron microscopy of the adrenal gland of two P males in the group at 20 000 ppm did not reveal any adverse, test substance-related effect.

Since there was no evidence of toxicologically adverse histological changes, no impact on adrenal function (assessed in a separate study), and adrenal weights were unaffected in other dietary studies in which rats were exposed for between 90 days and up to 2 years at similarly high concentrations, the effects on the adrenals were considered to be not adverse.

A transient small reduction in body weight (up to 9%) of the F_1 pups at 20 000 ppm on days 7, 14, and 21 of lactation had recovered by day 35 after weaning and was considered to be not adverse.

The NOAEL for parental toxicity, offspring toxicity and reproductive toxicity was 20 000 ppm, equal to 1199 mg/kg bw per day, the highest dose tested (Malley, 2006a).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, performed in accordance with OECD guideline 414, groups of 22 time-mated female Crl:CD®(SD)IGS BR rats were given chlorantraniliprole technical (purity, 96.45%) at a dose of 0, 20, 100, 300, or 1000 mg/kg bw per day by oral gavage in 0.5% aqueous methylcellulose on days 6–20 of gestation.

The rats were examined twice per day for clinical signs of toxicity. Body weight was recorded daily. Food consumption was recorded every other day. At termination on day 21 of gestation, the number of live and dead fetuses and fetal resorptions were recorded, live fetuses were weighed, sexed, and external alterations, intrauterine location and identification number were recorded. Approximately one half of the fetuses from each litter were examined for visceral abnormalities. During the external examination, all live fetuses with malformations were also examined for soft tissue alterations. All remaining live fetuses were examined for skeletal alterations. Dams were necropsied. Statements of adherence to QA and GLP were included.

No test substance-related effects on maternal clinical observations, body weight, body-weight gain, food consumption, or gross post-mortem observations were detected at any dose.

The mean number of corpora lutea, implantation sites, resorptions, live fetuses, fetal weight, and sex ratio were comparable in all groups. There were no abortions, premature deliveries, or complete litter resorptions and no effects of treatment on the numbers of litters, postimplantation loss, or on gravid uterine weights.

There were no test substance-related fetal external, visceral, or skeletal malformations or variations or adverse effects on fetal skeletal ossification observed at any dose.

The NOAEL for maternal and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Malley, 2004a).

Rabbits

In a study of developmental toxicity, performed in accordance with OECD guideline 414, groups of 22 time-mated Hra:(NZW)SPF female rabbits were given chlorantraniliprole (purity, 96.45%) at a dose of 0, 20, 100, 300, or 1000 mg/kg bw per day (corrected for purity) by gavage in 0.5% aqueous methylcellulose on days 7–28 of gestation. The rats were examined twice per day for clinical signs of toxicity. Body weight was recorded daily. Food consumption was recorded at 2–3 day intervals. At termination on day 29 of gestation, the number of live and dead fetuses and fetal resorptions were recorded, live fetuses were weighed, sexed, and external/visceral alterations and intrauterine location were recorded. All live fetuses were subsequently examined for skeletal alterations. Dams were necropsied. Statements of adherence to QA and GLP were included.

No test substance-related effects on maternal clinical observations, body weight, body-weight gain, food consumption, or gross post-mortem observations were detected in the does at any dose. The mean number of corpora lutea, implantation sites, resorptions, live fetuses, fetal weight, and sex

ratio were comparable in all groups. There were no abortions, premature deliveries, or complete litter resorptions and no effects of treatment on the numbers of litters, postimplantation loss, or on gravid uterine weights.

There were no test substance-related fetal external, visceral, or skeletal malformations or variations or adverse effects on fetal skeletal ossification observed at any dose.

The NOAEL for maternal and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Mylchreest, 2005).

2.5 Genotoxicity

Chlorantraniliprole was tested for genotoxicity in a range of guideline-compliant assays, both in vitro and in vivo. No evidence for genotoxicity was observed in any test. In addition to this core battery of studies of genetic toxicology, there was also a 2-week study in male and female rats dosed orally with chlorantraniliprole at 0, 25, 100, or 1000 mg/kg bw per day (see above; Munley, 2006a). Bone-marrow smears were prepared from rats in the main study and examined for the presence of micronuclei to assess potential genetic toxicity, and an additional group of rats was given cyclophosphamide as a positive control for genetic toxicity. Reportedly, no increases in the micronucleated PCEs in the ratio of PCEs/NCEs were observed in any evaluated test substance-treated group of male or female rats (data not presented).

The results of the tests for genotoxicity are summarized in Table 3. The Meeting concluded that chlorantraniliprole is unlikely to be genotoxic.

2.6 Special studies

(a) Neurotoxicity

In a study of acute neurotoxicity, performed in accordance with OPPTS guideline 870.6200, groups of 12 male and 12 female Crl:CD®(SD)IGS BR rats were given chlorantraniliprole (purity, 95.9%) as a single dose at 0, 200, 700, or 2000 mg/kg bw (corrected for purity) by gavage in 0.5%

Table 3. Results of studies of genotoxicity with chlorantraniliprole

End-point	Test object	Concentration/dose	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537, *E. coli* WP2 *uvr*A	2.5–5000 µg/plate ± S9[a]	96.45	Negative	Wagner & Atta-Safoh (2004)
Gene mutation	CHO-K_1 cells, HGPRT test	15.6–250 µg/ml[b] ± S9[a]	96.45	Negative	San & Clarke (2004)
Chromosomal aberration	Human lymphocytes	125–500 µg/ml[c] ± S9	96.45	Negative	Gudi & Rao (2004)
In vivo					
Micronucleus formation	Mouse bone marrow	500–2000 mg/kg bw (gavage)	96.45	Negative	Donner (2006b)

Positive and negative (solvent) controls were included in all studies. In all studies, statements of adherence to GLP and QA were included.

[a] Precipitation at ≥ 1800 µg/plate

[b] Precipitation at 250 µg/ml

[c] Precipitation at 500 µg/ml

methylcellulose. The rats were checked daily for clinical signs of toxicity. Body weight and food consumption were recorded on days 1 (before treatment), 2, 8 and 15. A neurobehavioral test battery, consisting of motor activity and FOB assessments, was conducted on all rats before dosing, approximately 2 h after dosing on day 1, and on days 8 and 15. At termination on days 16 and 17, six male and six female rats were examined grossly. The central and peripheral nervous system and selected muscle tissues of the six males and six females from all groups were collected, and microscopic neuropathological evaluations were conducted on rats in the control group and at the highest dose. Statements of adherence to QA and GLP were included.

No adverse compound-related effects on mortality, clinical signs of toxicity, body weight, body-weight gain, food consumption, food efficiency, FOB parameters, motor activity, gross pathology, or neuropathology were observed at any dose in males or females.

The NOAEL was 2000 mg/kg bw, the highest dose tested (Malley, 2004b).

In a 90-day study of neurotoxicity, performed in accordance with OPPTS guideline 870.6200 (resembles OECD guideline 424), groups of 12 male and 12 female Crl:CD®(SD)IGS BR rats were given diets containing chlorantraniliprole (purity, 96.45%) at a concentration of 0, 200, 1000, 4000, or 20 000 ppm, equal to 0, 13, 64 and 255 mg/kg bw per day in males and 0, 15, 77 and 255 mg/kg bw per day in females (corrected for purity). The rats were checked twice per day for clinical signs of toxicity. A detailed physical examination was performed weekly. Body weight and food consumption were recorded weekly and on the days of FOB and motor activity testing. Assessments of FOB and motor activity were conducted on all rats before exposure (baseline) and during weeks 4, 8, and 13. At termination, six males and six females per group were examined grossly. The central and peripheral nervous system and selected muscle tissues of the six males and six females rats from all groups were collected, and microscopic neuropathological evaluations were conducted on rats in the control group and in the group at the highest dose. Statements of adherence to QA and GLP were included.

There were no test substance-related effects on mortality, clinical observations, body weight, body-weight gain, food consumption, food efficiency, FOB parameters, motor activity, or on gross or microscopic pathology in males or females.

The NOAEL was 20 000 ppm, equal to 1313 mg/kg bw per day, the highest dose tested (Malley, 2006b).

(b) Immunotoxicity

In a 28-day study of immunotoxicity, performed in accordance with OPPTS guideline 870.7800, groups of 10 male and 10 female Crl:CD-1®(ICR)BR mice were given diets containing chlorantraniliprole technical (purity, 96.45%) at a concentration of 0, 300, 1700, or 7000 ppm, equal to 0, 48, 264, or 1144 mg/kg bw per day for males and 0, 64, 362, or 1566 mg/kg bw per day for females (corrected for purity). The mice were checked daily for mortality and morbidity and weekly for clinical signs. Body weight and food consumption were recorded weekly. On day 23, the mice were injected intravenously with 0.2 ml of 1×10^9 sheep erythrocytes/ml. On day 28, sheep erythrocyte-specific IgM concentrations in blood were measured. Each mouse was examined grossly and the thymus, spleen, and brain were weighed. Statements of adherence to QA and GLP were included.

No treatment-related effects on body weight, food consumption, gross pathology, organ weight, or sheep erythrocyte-specific antibody (IgM) response were observed.

The NOAEL was 7000 ppm, equal to 1144 mg/kg bw per day, the highest dose tested (Munley, 2007).

In a 28-day study of immunotoxicity, performed in accordance with OPPTS guideline 870.7800, groups of 10 male and 10 female Crl:CD®(SD)IGS BR rats were fed diets containing chlorantra-

niliprole technical (purity, 96.45%) at a concentration of 0, 1000, 5000, or 20 000 ppm, equal to 0, 74, 363, or 1494 mg/kg bw per day for males and 0, 82, 397, or 1601 mg/kg bw per day for females (corrected for purity). The rats were checked daily for mortality and morbidity and weekly for clinical signs of toxicity. Body weight and food consumption were recorded weekly. On day 22, the rats were injected intravenously with 0.5 ml of 4×10^8 sheep erythrocytes/ml. On day 28, sheep erythrocyte-specific IgM concentrations in blood were measured. Each rat was examined grossly and the thymus, spleen, and brain were weighed. Statements of adherence to QA and GLP were included.

No treatment-related effects on body weight, food consumption, gross pathology, organ weight or sheep erythrocyte-specific antibody (IgM) response were observed.

The NOAEL was 20 000 ppm, equal to 1494 mg/kg bw per day, the highest dose tested (Munley, 2006b).

(c) Studies with metabolites

The rat metabolite IN-EQW78 is also a significant metabolite in soil, water, and sediment. The substances IN-ECD73 and IN-F6L99 are metabolites that are only observed at low concentrations in soil and as degradates in studies of high-temperature food processing.

(i) Acute toxicity

The results of studies of acute toxicity with metabolites of chlorantraniliprole are summarized in Table 4. No substance-related clinical signs of toxicity were observed in the studies of acute toxicity.

Table 4. Acute toxicity with metabolites of chlorantraniliprole

Species	Strain	Sex	Route	Metabolite	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	Crl:CD-1®(ICR) BR	Female	Oral	IN-ECD73[a]	99.8	> 2000	Finlay (2006e)[b,c]
Rat	Crl:CD®(SD)IGS BR	Female	Oral	IN-EQW78[d]	99.8	> 2000	Finlay (2006f)[b,c]
Mouse	Crl:CD-1®(ICR) BR	Female	Oral	IN-F6L99[e]	99.8	> 2000	Finlay (2006g)[b,c]

[a] IN-ECD73: 2,6-Dichloro-4-methyl-11*H*-pyrido[2,1-b]quinazolin-11-one.
[b] Performed according to the up-and-down procedure, OECD guideline 425.
[c] Statements of adherence to good laboratory practice and quality assurance were included.
[d] IN-EQW78: 2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3,8-dimethyl-4(3*H*)-quinazolinone.
[e] IN-F6L99: 3-Bromo-*N*-methyl-1*H*-pyrazole-5-carboxamide

Table 5. Results of studies of genotoxicity with metabolites of chlorantraniliprole

Metabolite	End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro						
IN-EQW78[a]	Reverse mutation	*S. typhimurium.* strains TA98, TA100, TA1535 and TA1537, and *E. coli* WP2 *uvr*A	0–3333 µg/plate ± S9	99.8	Negative	Ford (2006)
IN-ECD73[b]	Reverse mutation	*S. typhimurium.* strains TA98, TA100, TA1535 and TA1537, and *E. coli* WP2 *uvr*A	0–5000 µg/plate ± S9	99.8	Negative	Myhre (2006a)
IN-F6L99[c]	Reverse mutation	*S. typhimurium.* strains TA98, TA100, TA1535 and TA1537, and *E. coli* WP2 *uvr*A	0–5000 µg/plate ± S9	98.6	Negative	Myhre (2006b)

Positive and negative (solvent) controls were included in all studies. In all studies, statements of adherence to GLP and QA were included.
[a] IN-EQW78: 2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3,8-dimethyl-4(3*H*)-quinazolinone.
[b] IN-ECD73: 2,6-Dichloro-4-methyl-11*H*-pyrido[2,1-b]quinazolin-11-one.
[c] IN-F6L99: 3-Bromo-*N*-methyl-1*H*-pyrazole-5-carboxamide

(ii) Genotoxicity

The results of studies of genotoxicity with metabolites of chlorantraniliprole are summarized in Table 5. The Meeting concluded that these metabolites of chlorantraniliprole are unlikely to be genotoxic.

3. Observations in humans

Chlorantraniliprole has at present only been produced on a pilot scale. In the limited number of workers involved with the synthesis of this compound to date, no illnesses have been attributed to exposure associated with the handling, testing, or manufacturing of chlorantraniliprole.

Comments

Biochemical aspects

After oral administration, the extent of absorption of chlorantraniliprole is dependent on the dose administered. At a single dose of 10 mg/kg bw, absorption was about 73–85%, with 18–30% being excreted in the urine and 49–53% being excreted in the bile within 48 h. At a single dose of 200 mg/kg bw, absorption was about 14%, with 4% and 5–7% of the dose excreted in the urine and bile, respectively, within 48 h. Excretion in expired air was insignificant. Plasma half-lives were 38–43 h in males and 78–82 h in females. After multiple doses (10 mg/kg bw per day for 14 days) with chlorantraniliprole, peak plasma concentrations in males and females were about two and seven times higher than after a single dose at 10 mg/kg bw, respectively. Distribution in tissues was extensive, with 0.8% and 3% remaining in the tissues of males and females, respectively, 168 h after a single dose at 10 mg/kg bw.

Chlorantraniliprole is extensively metabolized through tolyl methyl and *N*-methyl carbon hydroxylation, followed by *N*-demethylation, nitrogen-to-carbon cyclization with loss of a water molecule resulting in the formation of the pyrimidone ring, oxidation of alcohols to carboxylic acids, amide-bridge cleavage, amine hydrolysis, and *O*-glucuronidation. The potential for hydroxylation of the tolyl methyl and *N*-methyl carbon groups was greater in males than in females. After a single dose at 200 mg/kg bw, excretion of the parent compound in the urine and faeces (78.9–85.5%) was 12 to16-fold that at 10 mg/kg bw (4.9–7.3%). The profile of metabolites after a single dose at 200 mg/kg bw or after repeated doses at 10 mg/kg bw per day was similar to the profile after a single dose at 10 mg/kg bw.

Toxicological data

The acute toxicity of chlorantraniliprole is low (oral and dermal LD_{50}, > 5000 mg/kg bw; inhalation LC_{50}, > 5.1 mg/l). Apart from ocular and nasal discharge observed in a study in which chlorantraniliprole was administered by inhalation, no clinical signs of toxicity were observed in studies of acute toxicity. Chlorantraniliprole is not irritating to the skin and eyes, and is not a skin sensitizer (Magnussen & Kligman test in guinea-pigs; local lymph node assay in mice).

Chlorantraniliprole shows low toxicity after repeated doses. Occasionally, reductions in body-weight gain were observed in studies with repeated doses. However, these reductions often did not occur on consecutive weeks but were seen sporadically, were not dose-related and were not consistently found in different studies at similar or higher doses. Therefore, the incidental changes in body-weight gain were not considered to be a compound-related effect.

In short-term studies with chlorantraniliprole administered orally (gavage or diet), no adverse effects were observed at any dose tested, i.e., up to 7000 ppm, equal to 1443 mg/kg bw per day, in feeding studies in mice, up to 20 000 ppm, equal to 1188 mg/kg bw per day, in a feeding study in rats, and up to 40 000 ppm, equal to 1164 mg/kg bw per day, in a 1-year feeding study in dogs.

In an 18-month feeding study in mice, the NOAEL was 1200 ppm, equal to 158 mg/kg bw per day, on the basis of presence of eosinophilic foci in the liver, accompanied by hepatocellular hypertrophy and increased liver weight at 7000 ppm, equal to 935 mg/kg bw per day, in males only. No information on the chemical-specific mechanism of action was available to evaluate the relevance of liver foci to exposure of humans. However, the Meeting noted that this is a possible species- and sex-specific response that is of questionable toxicological significance and relevance, and thus the NOAEL of 158 mg/kg bw per day on the basis of these end-points is likely to be conservative.

In a 2-year feeding study in rats, the NOAEL was 20 000 ppm, equal to 805 mg/kg bw per day, the highest dose tested.

No treatment-related changes in the incidence of tumours were observed.

The Meeting concluded that chlorantraniliprole is not carcinogenic in rodents.

Chlorantraniliprole was tested for genotoxicity in adequate range of studies of genotoxicity in vitro and in vivo. No evidence for genotoxicity was observed in any test. The Meeting concluded that chlorantraniliprole is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that chlorantraniliprole is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity with chlorantraniliprole in rats, the NOAEL for parental, offspring and reproductive toxicity was 20 000 ppm, equal to 1199 mg/kg bw per day, the highest dose tested.

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

In a study of acute neurotoxicity in rats given chlorantraniliprole orally by gavage, the NOAEL was 2000 mg/kg bw per day, the highest dose tested. In a 90-day dietary study of neurotoxicity in rats, the NOAEL was 20 000 ppm, equal to 1313 mg/kg bw per day, the highest dose tested.

In a dietary study of immunotoxicity in mice, the NOAEL was 7000 ppm, equal to 1144 mg/kg bw per day, the highest dose tested. In a dietary study of immunotoxicity in rats, the NOAEL was 20 000 ppm, equal to 1494 mg/kg bw per day, the highest dose tested.

To date, chlorantraniliprole has only been produced on a pilot scale. In the limited number of workers involved with the synthesis of this compound to date, no illnesses have been attributed to exposure associated with the handling, testing, or manufacturing of chlorantraniliprole.

The rat metabolite 2-[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3,8-dimethyl-4(3*H*)-quinazolinone (IN-EQW78) was also a significant metabolite in soil, water, and sediment. The substances 2,6-dichloro-4-methyl-11*H*-pyrido[2,1-*b*]quinazolin-11-one (IN-ECD73) and 3-bromo-*N*-methyl-1*H*-pyrazole-5-carboxamide (IN-F6L99) were metabolites only observed at low concentrations in soil and as degradates in studies of high-temperature food processing. In studies of acute toxicity, these three chlorantraniliprole metabolites had LD_{50}s of > 2000 mg/kg bw. These metabolites gave negative results in a test for reverse mutation.

The Meeting concluded that the existing database on chlorantraniliprole is sufficient to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for chlorantraniliprole of 0–2 mg/kg bw on the basis of eosinophilic foci accompanied by hepatocellular hypertrophy and increased liver weight in mice in an 18-month feeding study for which the NOAEL was 158 mg/kg bw per day, and using a safety factor of 100. There was no available information on the chemical-specific mechanism

of action with which to evaluate the relevance of the liver foci to exposure of humans. The Meeting noted, however, that this is a possible species- and sex-specific response that is of questionable toxicological significance and relevance, and thus the NOAEL of 158 mg/kg bw per day (and consequently the ADI) identified on the basis of these end-points is likely to be conservative.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for chlorantraniliprole in view of its low acute toxicity, the absence of developmental toxicity, and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

Levels relevant for risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month study of toxicity and carcinogenicity[a]	Toxicity	1200 ppm, equal to 158 mg/kg bw per day	7000 ppm, equal to 935 mg/kg bw per day
		Carcinogenicity	7000 ppm, equal to 935 mg/kg bw per day[c]	—[c]
Rat	Two-year study of toxicity and carcinogenicity [a]	Toxicity	20 000 ppm, equal to 805 mg/kg bw per day	—[c]
		Carcinogenicity	20 000 ppm, equal to 805 mg/kg bw per day[c]	—[c]
	Two-generation study of reproductive toxicity[a]	Parental	20 000 ppm, equal to 1199 mg/kg bw per day	—[c]
		Offspring toxicity	20 000 ppm, equal to 1199 mg/kg bw per day	—[c]
		Reproductive toxicity	20 000 ppm, equal to 1199 mg/kg bw per day	—[c]
	Developmental toxicity[b]	Maternal toxicity	1000 mg/kg bw per day	—[c]
		Foetotoxicity	1000 mg/kg bw per day	—[c]
	Acute neurotoxicity[b]	Neurotoxicity	2000 mg/kg bw per day	—[c]
	90-day neurotoxicity[a]	Neurotoxicity	20 000 ppm, equal to 1313 mg/kg bw per day	—[c]
Rabbit	Developmental toxicity[b]	Maternal toxicity	1000 mg/kg bw per day	—[c]
		Foetotoxicity	1000 mg/kg bw per day	—[c]
Dog	One-year study[a]	Toxicity	40 000 ppm, equal to 1164 mg/kg bw per day	—[c]

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.

Estimate of acceptable daily intake for humans

0–2 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 chlorantraniliprole

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of absorption	Rapid, incomplete and dose-dependent oral absorption (73–85% at 10 mg/kg bw; 14% at 200 mg/kg bw).
Distribution	Extensive (rats)
Potential for accumulation	Low in males, moderate in females (rats)
Rate and extent of excretion	Plasma half-lives: males, 38–43 h; females, 78–82 h
	At 10 mg/kg bw: 18–30% in urine, 49–53% in bile, within 48 h.
	At 200 mg/kg bw: 4% in the urine, 5–7% in bile, within 48 h.
Metabolism in animals	Extensive, through tolyl methyl and *N*-methyl carbon hydroxylation, followed by *N*-demethylation, nitrogen-to-carbon cyclization, formation of a pyrimidone ring, oxidation of alcohols to carboxylic acids, amide-bridge cleavage, amine hydrolysis, and *O*-glucuronidation.
Toxicologically significant compounds (animals, plants and environment)	Chlorantraniliprole
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.1 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Dermal sensitization	Not sensitizing (Magnussen & Kligman test in guinea-pigs; local lymph node assay in mice)
Short-term studies of toxicity	
Target/critical effect	None
Lowest relevant oral NOAEL	1443 mg/kg bw per day (mice), 1188 mg/kg bw per day (rats), 1164 mg/kg bw per day (dogs); highest doses tested
Lowest relevant dermal NOAEL	1000 mg/kg bw per day, the highest dose tested (rat)
Lowest relevant inhalatory NOAEC	No data available
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver: eosinophilic foci, hepatocellular hypertrophy, increased liver weight (mice)
Lowest relevant NOAEL	1200 ppm, equal to 158 mg/kg bw per day (mice)
Carcinogenicity	Not carcinogenic (mice, rats)
Genotoxicity	
	Not genotoxic in vitro or in vivo
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects (rats)
Lowest relevant reproductive NOAEL	20 000 ppm, equal to 1199 mg/kg bw per day, the highest dose tested (rats)
Developmental target	No developmental effects (rats, rabbits)
Lowest relevant developmental NOAEL	1000 mg/kg bw per day, the highest dose tested (rats, rabbits)
Neurotoxicity/delayed neurotoxicity	
Neurotoxicity	No neurotoxic effects
Lowest relevant oral NOAEL	2000 mg/kg bw, the highest dose tested (acute toxicity in rats treated by gavage)
	1313 mg/kg bw per day, the highest dose tested (90-day dietary study in rats)

Other toxicological studies	
Immunotoxicity	Not immunotoxic
Lowest relevant oral NOAEL	7000 ppm, equal to 1144 mg/kg bw per day, the highest dose tested (28-day study in mice)
	20 000 ppm, equal to 1494 mg/kg bw per day, the highest dose tested (28-day study in rats)
Medical data	
	No adverse effects observed in workers involved with the synthesis of this compound

Summary

	Value	*Study*	*Safety factor*
ADI	0–2 mg/kg bw	Mouse, 18-month study	100
ARfD	Unnecessary	—	—

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