AZOXYSTROBIN

First draft prepared by
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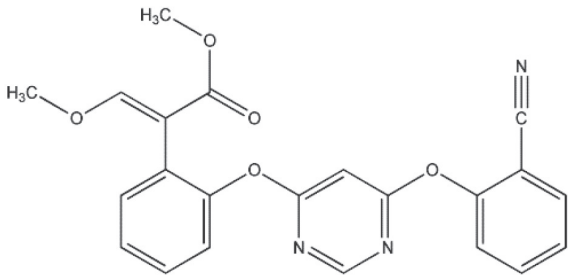
Explanation

Azoxystrobin is the ISO approved name for methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate, (IUPAC), for which the CAS No. is 131860-33-8. Azoxystrobin is a β-methacrylate compound that is structurally related to the naturally occurring strobilurins, which are compounds derived from some fungal species. Azoxystrobin is a broad-spectrum, systemic fungicide that acts by inhibiting electron transport in pathogenic fungi. It has the ability to provide

protection against the fungal diseases caused by *Ascomycota*, *Deuteromycota*, *Basidiomycota* and *Oomycota* groups.

Azoxystrobin has not been evaluated previously by JMPR and was evaluated by the present Meeting at the request of the Fortieth Session of the Codex Committee on Pesticide Residues (CCPR). All pivotal studies with azoxystrobin were certified as complying with good laboratory practice (GLP).

Figure 1. Chemical structure of azoxystrobin



Evaluation for acceptable daily intake

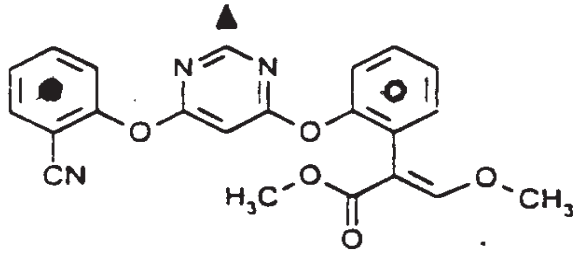
Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant Organization for Economic Co-operation and Development (OECD) and/or United States Environmental Protection Agency (EPA) test guideline(s).

1.1 Biochemical aspects: absorption, distribution and excretion

Rats

The absorption, distribution, and elimination of azoxystrobin was studied after oral dosing of azoxystrobin radiolabelled with ^{14}C as shown in Figure 2.

Figure 2. Position of the radiolabel on azoxystrobin used in pharmacokinetic studies in rats



▲ Denotes position of pyrimidinyl ring label

❍ Denotes position of phenylacrylate ring label

● Denotes position of cyanophenyl ring label

Studies using whole-body autoradiography were conducted using azoxystrobin labelled with ^{14}C in either the cyanophenyl, pyrimidinyl or phenylacrylate ring (see Figure 2). The excretion and

tissue distribution of radioactivity was investigated for 48 h in male and female rats given a single dose of azoxystrobin at 1 mg/kg bw by gavage. Treated rats were housed in metabolism cages to facilitate the collection of urine, faeces, exhaled air and volatiles. One male and one female rat receiving azoxystrobin radiolabelled in each position were killed at 24 h and 48 h after dosing. Each carcass was frozen and sectioned in preparation for whole-body radiography.

About 89% and 86% of the administered dose of [^{14}C]pyrimidinyl-labelled azoxystrobin was excreted within 48 h in the urine and faeces of male and female rats, respectively. Most of the radioactivity was excreted in the faeces, with < 17% in the urine. The male and female rats treated with [^{14}C] phenylacrylate-labelled azoxystrobin excreted about 80% and 97% of the administered dose within 48 h, respectively. Most of the radioactivity was excreted via the faeces with < 21% in the urine. At 48 h, males and females, excreted approximately 0.01% of the administered dose as carbon dioxide trap and approximately 0.01% as volatile metabolites. The male and female rats treated with]^{14}C]cyanophenyl-labelled azoxystrobin excreted about 95% and 98% of the administered dose within 48 h, respectively. Most of the radioactivity was excreted via the faeces, with < 16% in the urine. At 48 h, males and females excreted small amounts of radioactivity as carbon dioxide (< 0.3%) and as volatile metabolites (0.01%). For all radiolabels, the distribution of radioactivity was similar in males and females, as shown by whole-body autoradiography. At 24 h, most of the radiolabel was present in the alimentary canal, moderate amounts in the kidneys and small amounts in the liver. Forty-eight hours after dosing, the whole-body autoradiography results showed a marked reduction in radioactivity.

The results of these studies indicated that there were no significant differences between the rates and routes of excretion or tissue distribution of azoxystrobin labelled in one of three positions. No sex-related difference in excretion profile was evident. Minor differences in excretion were primarily due to the small numbers of rats used in the study. No significant differences in the amount of radioactivity recovered in the exhaled air and as volatiles were observed between the three radiolabels or between sexes. On the basis of the results of this study, other studies of excretion and tissue retention were conducted using only pyrimidinyl-labelled azoxystrobin (Lythgoe & Howard, 1993a).

In toxicokinetic studies, groups of male and female Alpk:APfSD rats (five to eight per group, depending on experiment) were given azoxystrobin (purity, 99%) with or without pyrimidinyl label as a single dose at 1 or 100 mg/kg bw by gavage or as 14 repeated doses of 1 mg/kg bw per day. Biliary metabolites were assessed using rats with cannulated bile ducts given a single dose at 100 mg/kg bw by gavage. The vehicle was polyethylene glycol (PEG 600) at 4 ml/kg bw. Treated rats were housed in stainless steel metabolism cages for 7 days. Urine was collected at 6 h, and urine and faeces were collected separately at 12, 24, 36, 48 h and at 24 h intervals until 7 days after dosing. At each collection, cages were rinsed with water and cage-washing collected together with the urine. At the end of the study, cages were thoroughly rinsed with ethanol/water (1 : 1 v/v) and retained for radiochemical analysis. Carbon dioxide and volatiles were trapped. After 7 days, various organs and tissues were removed and analysed for radioactivity. Recovery of radioactivity in selected tissues and excreta of rats treated with azoxystrobin at a single lower or higher dose and after repeated doses of azoxystrobin for 7 days is shown in Table 1.

For rats receiving a single lower dose (1 mg/kg bw), total excretion of radioactivity (urine, faeces, and cage wash) was 93.75% and 91.44% for males and females, respectively over the 7 days. Most (> 85%) of the urinary and faecal excretion took place during the first 36 h after dosing. In these rats, about 83.2% and 72.6% of the administered dose was excreted in the faeces of males and females within 7 days, respectively, and about 10.2% and 17.9% of the administered dose was excreted in the urine of the males and females within 7 days, respectively. Approximately 0.34% and 0.31% of the administered dose was found in the carcass and tissues within 7 days after dosing in males and females, respectively. For rats at this dose (1 mg/kg bw), the highest concentrations of radiolabel were found in the liver (mean for males and females, 0.009 µg equivalents/g) and in

the kidneys (males, 0.027 µg equivalents/g; and females, 0.023 µg equivalents/g). At termination, the total concentration of radioactivity in blood was 0.004 µg equivalents/g for males and females. Less than 0.6% of the administered dose was recovered in the expired air (Jones, 2004; Lythgoe & McAsey, 1995, 1993).

For rats receiving the single higher dose (100 mg/kg bw), total excretion of radioactivity (urine, faeces, and cage wash) was 98.29% and 97.22% for males and females, respectively, over the 7 days. Most (> 82%) of the urinary and faecal excretion took place during the first 48 h after dosing. At this dose, about 89.37% and 84.53% of the administered dose was excreted in the faeces of the males and females within 7 days, respectively, and about 8.54% and 11.54% of the administered dose was excreted in the urine of the males and females within 7 days, respectively. Approximately 0.33% and 0.33% of the administered dose was found in the carcass and tissues within 7 days after dosing in males and females rats, respectively. At this higher dose, the highest concentrations of radiolabel were found in the kidneys (males, 1.373 µg equivalents/g; and females, 1.118 µg equivalents/g) and in the liver (males, 0.812 µg equivalents/g; and females, 0.714 µg equivalents/g). At termination, the total concentration of radioactivity in blood was 0.389 µg equivalents/g for males and 0.379 µg equivalents/g for females (Lythgoe & Howard, 1995, 1993b).

Eight male and female rats were given 14 consecutive daily oral doses of unlabelled azoxystrobin at 1 mg/kg bw followed by a single oral dose of [^{14}C]pyrimidinyl-labelled azoxystrobin at 1 mg/kg bw. For the repeated doses, about 89.1% and 86.5% of the administered dose was excreted in the faeces of the males and females rats within 7 days, respectively, and about 12.5% and 17.0% of the administered dose was excreted in the urine of the males and females rats within 7 days, respectively. In males and females, excretion of radioactivity was rapid, with > 96% being excreted during the first 48 h. Approximately 0.62% and 0.39% of the administered dose was found in the carcass and tissues within 7 days after dosing in male and female rats, respectively. For the repeated dose, the highest concentrations of azoxystrobin-derived radioactivity were found in the kidneys (males and females, < 0.04 µg equivalents/g). The concentrations found in the liver were 0.02 and 0.01 µg equivalents/g for males and females, respectively. At termination, the total concentration of radioactivity in blood was 0.01 µg equivalents/g for males and females (Lythgoe & Howard, 1993c).

Bile-duct cannulated rats were given azoxystrobin radiolabelled in either the pyrimidinyl, cyanophenyl or phenylacrylate rings at 100 mg/kg bw by gavage. Comparison of the rates and routes

Table 1. Recovery of radioactivity in tissues and excreta of rats given ^{14}C-labelled azoxystrobin orally

Sample analysed	Recovery (% of administered dose)					
	Single lower dose		Repeated lower dose		Single higher dose	
	Male	Female	Male	Female	Male	Female
Expired air	NP	NP	NP	NP	NP	NP
Tissues	0.11 ± < 0.01	0.08 ± < 0.01	Approx. 0.12	Approx. 0.08	Approx. 0.07	Approx. 0.05
Carcass	0.23 ± 0.02	0.23 ± 0.06	0.50 ± 0.08	0.31 ± 0.05	< 0.26	< 0.27
Body, total	0.34	0.31	0.62	0.39	0.33	0.33
Cage wash	0.33 ± 0.13	0.93 ± 0.58	0.50 ± 0.5	0.10 ± 0.1	0.38 ± 0.13	1.15 ± 0.78
Urine	10.19 ± 1.53	17.89 ± 3.50	12.50 ± 3.4	17.00 ± 2.7	8.54 ± 1.03	11.54 ± 1.42
Faeces	83.24 ± 1.52	72.62 ± 5.40	89.10 ± 5.9	86.5 ± 1.7	89.37 ± 3.99	84.53 ± 1.98
Excreta; total	93.75	91.44	102.1	103.6	98.29	97.22
Total recovery	94.1	91.75	102.72	103.99	98.61	97.54

From Lythgoe & Howard (1993a, 1993b) and Lythgoe & McAsey (1993)
NP, not performed in the studies of excretion/distribution.

of excretion and the profile of the metabolites showed (as previously) that there were no significant differences in the metabolism of the three differently labelled forms, thus indicating that there was minimal cleavage of the ether linkages between the aromatic rings. Experiments designed to identify metabolites were therefore conducted in bile-duct cannulated rats given [^{14}C]pyrimidinyl labelled azoxystrobin by gavage. In the bile-duct cannulated rats, excreta, bile, and cage wash were collected at 6, 12, 24, 36, and 48 h and stored at –20 °C. Samples of bile, faeces and urine were collected between 0 h and 48 h and pooled. Samples for males and females were separated. Urine and faeces were collected at up to 168 h after dosing from rats given the single dose (higher or lower) and from rats receiving repeated doses for 14 days, and were used for quantification of metabolites. Some bile samples were enzymatically digested using cholylglycine hydrolase at 30 units/ml, pH 5.6 at 37 °C overnight. Metabolites were identified using various analytical techniques, such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), proton nuclear magnetic resonance spectroscopy (NMR) and mass spectrophotometry (MS).

On the basis of biliary excretion data for rats given a single dose of either [^{14}C]pyrimidinyl-, [^{14}C]phenylacrylate-, or [^{14}C]cyanophenyl-labelled azoxystrobin at 100 mg/kg bw, 74.4% (males) and 80.7% (females) of the pyrimidinyl-derived radioactivity was excreted in the bile after 48 h. For the cyanophenyl-derived radioactivity, 56.6% and 62.5% was excreted in the bile of males and females, respectively. For the phenylacrylate-derived radioactivity, 64.4% (males) and 63.6% (females) was excreted in the bile. Quantitatively, there were no significant differences in biliary excretion between males and females.

Azoxystrobin was found to undergo extensive metabolism in rats. A total of 15 metabolites were detected in the excreta and subsequently identified. Seven additional metabolites were detected but not identified. None of the unidentified metabolites represented more than 4.9% of the administered dose. The quantitative data for the various metabolites in the faeces, urine and bile of rats receiving a single dose of azoxystrobin at 100 mg/kg bw are shown in Table 2. The mass balance for the study of metabolite identification indicated that a substantial percentage of the administered radiolabel (45.6–73.6%) was unaccounted for, although the studies of excretion showed total recovery of 91.75–103.99%, with 72.6–89.3% being in the faeces. The percentage of unaccounted-for radiolabel was especially notable in the groups receiving a single lower dose and a repeated lower dose. The study authors indicated that the variable efficiency in recovery could be explained by the fact that, for metabolite identification, faeces were extracted with acetonitrile which allowed partitioning of the parent compound when it was present in the faeces (i.e. rats receiving the higher dose). For the groups receiving a single lower dose or repeated lower dose (where quantities of the parent compound were minimal), most of the faecal radiolabel was associated with polar metabolites that would not be present in the acetonitrile extract. The resulting concentration of radiolabel in the extract would, therefore, be very low. For the group receiving the higher dose, greater amounts of parent compound were left unabsorbed, thereby resulting in greater amounts of parent compound available for partitioning into the acetonitrile extract.

The glucuronide conjugate (metabolite V) was the most prevalent biliary metabolite in both males (29.3%) and females (27.4%). Metabolite I (parent compound) was not detected in the bile. Each of the other biliary metabolites accounted for between 0.9% and 9.0% of the administered dose. In the bile-duct cannulated rats, about 15.1% and 13.6% of the faecal radioactivity was metabolite I (parent compound) in male and female rats, respectively. No parent compound was detected in the urine of bile-duct cannulated male and female rats. The predominant metabolite in the urine of the bile-duct cannulated rats was unidentified metabolite 2, which accounted for about 1.8% and 2.0% of the administered dose in male and female rats, respectively.

There was no evidence for a dose-influencing metabolism, but a sex-specific difference in biotransformation was observed, with females producing more metabolites than did males. Biotransformation was unaffected by dose. The study authors suggested that absorption was dose-dependent.

The oral absorption at 1 mg/kg bw was nearly complete (100%) since no parent compound was detected. The oral absorption at the higher dose (100 mg/kg bw) was estimated to be approximately 74–81% since about 19–26% of the parent compound was detected. However, it is difficult to estimate the true oral absorption value owing to poor recoveries after extraction, especially at the lower dose.

The proposed metabolic pathway for azoxystrobin in rats is shown in Figure1. There were two principal metabolic pathway: hydrolysis to the methoxyacid, followed by glucuronide conjugation to give metabolite V; and glutathione conjugation of the cyanophenyl ring followed by further metabolism via a number of intermediates (VI, VII, and VIII) to the mercapturic acid metabolite IX. Azoxystrobin was also hydroxylated at the 8 and 10 positions on the cyanophenyl ring followed by glucuronide conjugation (metabolites II, III, IVa and IVb). There were several minor pathways involving the acrylate moiety, resulting in formation of the metabolite XIII and XIV. Three metabolites (X, XII, and XV) arising via the cleavage of the ether linkages were identified (Lappin & Gledhill, 1994).

In an additional metabolism study, [^{14}C]cyanophenyl-labelled azoxystrobin was given to bile-duct cannulated and non-cannulated rats at a dose of 100 mg/kg bw. Samples of urine, faeces and bile were collected for up to 72 h. The purpose of this study was to reevaluate certain plant and goat metabolites that were previously not identified in rats and further elucidate the metabolic pathway of azoxystrobin in rats.

Table 2. Identification and distribution of metabolites in bile-duct cannulated rats given azoxystrobin as a single dose at 100 mg/kg bw by gavage

Metabolite	Recovery (% of administered dose)							
	Males				Females			
	Faeces	Bile	Urine	Total	Faeces	Bile	Urine	Total
I (azoxystrobin)	15.1	—	—	15.1	13.6	—	—	13.6
II	—	6.5	—	6.5	0.1	6.8	0.3	7.2
III	—	—	0.1	0.1	—	1.7	—	1.7
IVA+IVA [a]	—	6.8	—	6.8	—	9.0	0.3	9.3
IVb + IVb [a]	—	—	—	—	0.1	1.4	0.2	1.7
V	—	29.3	0.1	29.4	—	27.4	1.7	29.1
VII + XI [a]	—	7.0	—	7.0	—	1.6	0.3	1.9
VIII = XIV [a]	—	3.2	0.1	3.3	—	6.1	0.3	6.4
IX	—	4.5	—	4.5	0.1	2.4	0.4	2.9
X	—	—	—	—	—	4.8	0.4	5.2
XIII	—	2.8	Trace	2.8	—	0.9	Trace	0.9
XV	0.2	4.1	0.3	4.6	0.2	1.5	0.4	2.1
Unidentified No. 1	—	4.4	—	4.4	—	2.1	—	2.1
Unidentified No. 2	—	2.5	1.0	3.5	—	1.3	1.8	3.1
Unidentified No. 3	—	1.1	0.2	1.3	—	1.1	0.4	1.5
Unidentified No. 4	—	—	—	—	—	—	0.1	0.1
Unidentified No. 5	—	—	0.1	0.1	—	1.3	0.3	1.6
Unidentified No. 6	0.1	—	0.1	0.2	0.1	4.4	—	4.5
Total identified	15.4	72.2	2.0	89.6	14.2	73.8	6.9	95.0

From Lappin & Gledhill (1994)

[a] Metabolites could not be fully resolved by high-performance liquid chromatography (HPLC) and individual quantitation was not possible.

Three further metabolites, previously detected in either plants or goats, were identified.

The IUPAC names of the selected metabolites are given in Table 3. Compound 13, resulting from cleavage of the diphenyl ether link, was detected in the bile and urine as the glucoronide conjugate at a concentration of up to 1.8% of the administered dose. Compound 20 was also detected in the bile and urine at a concentration of up to 1.3%. Compound 35 was detected in the urine, faeces and bile at a concentration of up to 0.6%. Compounds 24 and 30 were not detected (Joseph et al., 1995).

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of azoxystrobin is summarized in Table 4.

Table 3. IUPAC names of the selected metabolites of azoxystrobin reevaluated in a study in rats

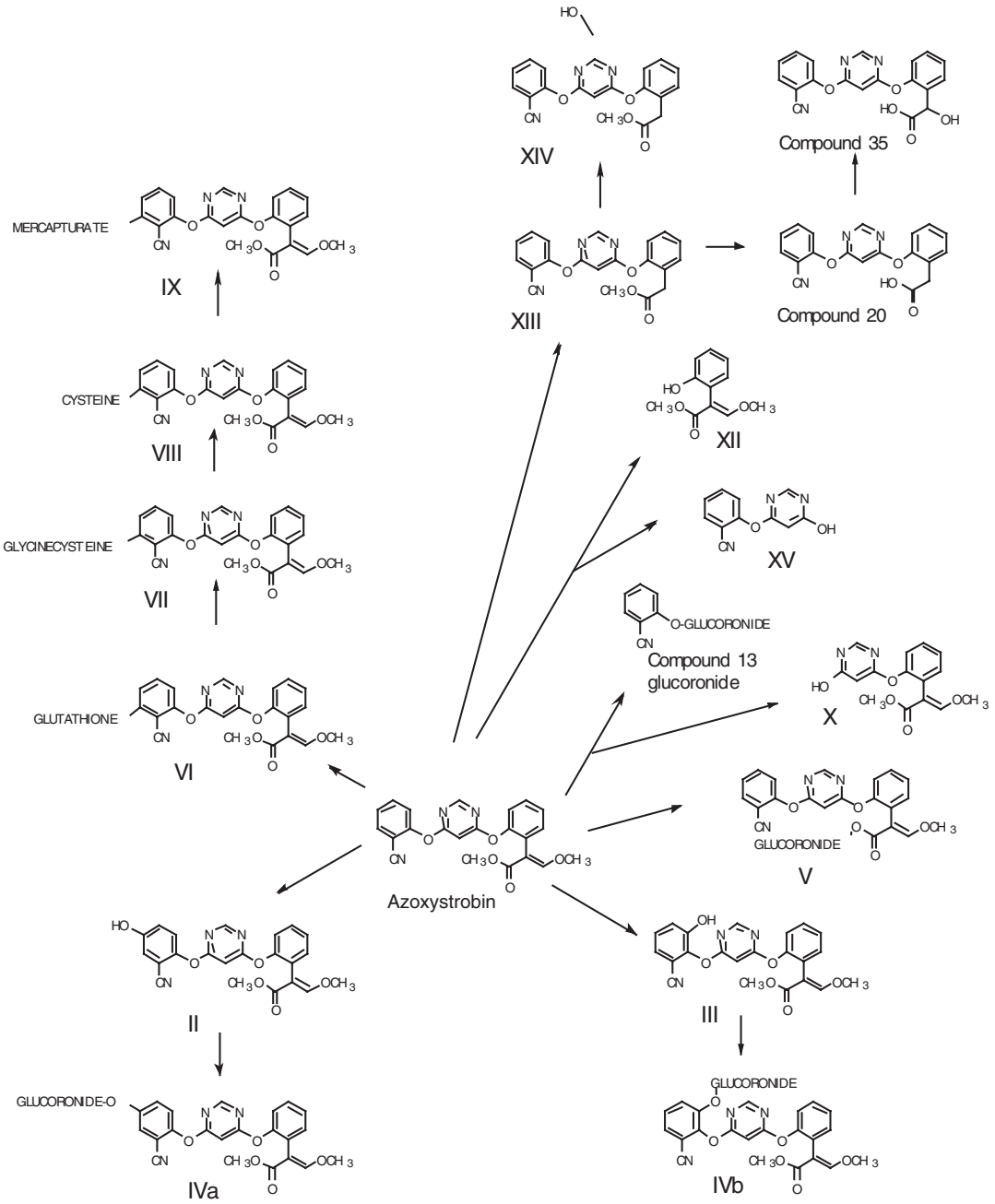
Compound No.	Code No.	IUPAC name
13	R71395	2-hydroxybenzonitrile
20	R400050	{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}acetic acid
24	R400753	Methyl 2-{2[6-(2-cyanophenoxy)pyrimidin-4-yloxy] phenyl}-glycolate
30	R402173	2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] benzoic acid
35	R402987	2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}glycolic acid

From Joseph et al. (1995)
IUPAC, International Union of Pure and Applied Chemistry

Table 4. Acute toxicity of azoxystrobin

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Reference
Mice	CD-1	Males and females	Oral	> 5000	—	Robinson (1991b, 1995b)
Rats	Wistar (Crl: (WI) BR)	Males and females	Oral	> 5000	—	Robinson, (1991a, 1995a)
Rats	Wistar (Crl: (WI) BR)	Males and females	Dermal	> 2000	—	Robinson (1991c, 1995c)
Rat	Wistar Alpk: APfSD	Males and females	Inhalation (4 h, nose-only)	—	Females: 0.698 Males: 0.962	Parr-Dobrzanski (1992, 1995)
Rat	Wistar Alpk:APfSD		Inhalation	—	≥ 4.7	Pinto (1997)
Rabbits	New Zealand White	Females	Dermal irritation	Slight irritation	—	Robinson (1991d, 1995d)
Rabbit	New Zealand White		Ocular irritation	Slight irritation		Robinson (1991f, 1995e)
Guinea-pig	Dunkin-Hartley		Dermal sensitization	Not sensitizing		Robinson (1991g, 1995f)

Figure 3. Proposed metabolic pathway of azoxystrobin in rats



Note: Metabolite numbers are shown as roman numerals.

(a) Oral toxicity

Mice

Groups of five male and five female young adult CD-1 mice were given azoxystrobin (purity, 95.2%) as a single dose at 0, or 5000 mg/kg bw by gavage in corn oil. Treated mice were subjected to gross necropsy after 14 days. Two male mice died owing to dosing accidents and were replaced. There were no treatment-related mortalities. Clinical observations were confined to slight piloerection and slight urinary incontinence in some mice. All clinical signs had regressed by day 6. There were no significant treatment-related clinical signs, necropsy findings or changes in body weight. The oral median lethal dose (LD_{50}) for azoxystrobin in mice was > 5000 mg/kg bw for males and females (Robinson, 1991b, 1995b).

Rats

Groups of five male and five female young adult Wistar rats (Crl:(WI)BR) rats were given azoxystrobin (purity, 95.2%) as a single dose at 0 and 5000 mg/kg bw by gavage in corn oil. Treated rats were subjected to gross necropsy after 14 days. One female rat died on day 2 from a dosing accident and was replaced. The female that died on study day 2 had excess watery fluid in the thoracic cavity consistent with inappropriate administration of the test material. All rats lost weight initially, due to pre-dose fasting, but most had exceeded their initial weight by day 8, and continued to gain weight until the end of the study. There were no significant treatment-related clinical signs, necropsy findings or changes in body weight. There were no treatment-related deaths. The oral LD_{50} of azoxystrobin in rats was > 5000 mg/kg bw for males and females (Robinson, 1991a, 1995a).

(b) Dermal toxicity

Rats

Five male and five female young adult Wistar (Crl:(WI)BR) rats were exposed dermally to azoxystrobin (purity, 95.2%) at 2000 mg/kg bw as a paste in corn oil applied to approximately 10% of the (shaved) body surface area. The test substance was maintained in contact with the skin for 24 h using an occlusive dressing. The rats were observed for 14 days. Body weights were recorded at intervals throughout the study. All rats were subjected to a post-mortem examination at termination. Slight skin irritation (slight erythema) was observed during the study, but there were no significant signs of systemic toxicity and none of the rats died. All rats lost weight initially, but all had exceeded their initial weights by day 6. Post-mortem examination did not reveal any treatment-related pathological effects. The dermal LD_{50} of azoxystrobin in rats was > 2000 mg/kg bw for males and females (Robinson, 1991c, 1995c).

(c) Exposure by inhalation

Rats

In a study of acute toxicity after inhalation, groups of five male and five female young adult Wistar rats (Alpk:APfSD) were exposed nose-only to azoxystrobin (purity, 96.2%) for 4 h at a concentration of 0.2, 0.5, or 0.8 mg/l. One additional group of five male rats was exposed to azoxystrobin at 1.0 mg/l. the rats were then observed for 14 days. The mean measured particulate concentrations were 0, 0.257, 0.511, 0.767 or 1.010 mg/l, which were chemically analysed as 0, 0.242, 0.481, 0.717 or 0.968 mg/l. Atmospheres generated had mean aerodynamic particle sizes of 1.13, 1.17, 1.35 and 1.17 µm. No mortality was observed at 0.2 mg/l. Mortality occurred at 0.5 mg/l (one male and one female), 0.8 mg/l (one male and three females) and 1.0 mg/l (three males). Most rats exposed to azoxystrobin at 0.5, 0.8, or 1.0 mg/l developed slow deep breathing, auditory hypoesthaesia, and breathing irregularities during and up to 4 days after exposure. In addition, many rats had a splayed

gait and reduced splay reflex immediately after exposure. Surviving rats showed rapid recovery, and all treatment-related clinical signs had disappeared by day 7. Body weight was reduced in surviving rats in all groups after exposure, but by day 8 most rats were gaining weight and had exceeded their initial weights. All rats that died during exposure had dark red or mottled lungs. No other treatment-related effects were observed.

The median lethal concentration (LC_{50}) of azoxystrobin at 4 h in rats was calculated to be 0.698 mg/l for females and 962 mg/l for males (Parr-Dobrzanski, 1992, 1995).

In a second study of acute toxicity after inhalation, groups of five male and five female young adult Wistar rats (Alpk: APfSD) were exposed nose-only to azoxystrobin technical grade active ingredient (purity, 96.2%) for 4 h at a target concentration of > 3.7 mg/l. The rats were then observed for 14 days. The mean measured particulate concentration was 4.7 mg/l. Atmospheres generated had mean aerodynamic particle size of 14.7 µm. The range of particles size was too large for a study of toxicity after inhalation. No mortality was observed during the study. After exposure, some rats had wet fur, piloerection and hunched posture, which subsided by day 4. Body weights of four male and four female rats were increased by day 8 and these rats continued to gain weight until the end of the study. No other treatment-related effects were observed at gross necropsy.

The LC_{50} of azoxystrobin technical grade active ingredient at 4 h in rats was > 4.7 mg/l for males and females (Pinto, 1997).

(d) Dermal irritation

In a study of primary dermal irritation, six young adult female New Zealand White rabbits were dermally exposed to 0.5 g of azoxystrobin (purity, 95.2%), moistened with distilled water, for 4 h. The treated area was covered by an occlusive dressing. The application site was washed after removal of the dressing and dermal irritation was assessed after 30–60 min and then daily for up to seven days. Very slight erythema and oedema were present for three days after dosing in one rabbit, and for 1 h in another. No other signs of irritation were observed. The Meeting concluded that azoxystrobin was a slight dermal irritant in rabbits given a single application for 4 h. The mean erythema and mean oedema scores over the first 3 days were calculated to be 0.2 and 0.2, respectively (Robinson, 1991d, 1995d).

(e) Ocular irritation

In a study of primary eye irritation, 0.1 ml of azoxystrobin (purity, 95.2%), was instilled into the conjunctival sac of one eye of each of six young adult female New Zealand White rabbits. The initial pain reaction was assessed immediately after treatment. Irritation was scored by the method of Draize at 1–2 h, and 1, 2, and 3 days after exposure. The test material induced slight to moderate erythema and slight chemosis in all rabbits within 1 h, but the effects resolved within 48 h after treatment. Additional signs of irritation included slight mucoid and Harderian discharge and partial haemorrhaging of the nictitating membrane. These effects had completely regressed 2 days after dosing. The Meeting considered that azoxystrobin was slightly irritating (class 3 on a 1–8 scale) to the eyes of rabbits (Robinson, 1991e, 1995e).

(f) Sensitization

In a study of dermal sensitization with azoxystrobin (purity, 95.2%) mixed with corn oil, young male and female Dunkin-Hartley guinea pigs were tested using the maximization method of Magnusson & Kligman. For the main study, 10 female guinea-pigs were assigned to a control group, and 10 female guinea-pigs to the treatment group. In this study, the test concentrations chosen were 10% for intradermal induction, 64% for topical induction, and 37% or 67% for the challenge. Skin reactions at the challenge sites were observed at 24 h and 48 h after removal of the patch. No mortalities or

clinical signs of toxicity were observed in the study. Challenge of previously induced guinea-pigs with a 67% or a 30% w/v preparation of azoxystrobin in corn oil caused light brown staining at some challenge sites, but this did not obscure the assessment of any erythematous response that may have been present. There were no signs of dermal reactions in any guinea-pigs in the induction or challenge phase. In a study designed to provide a positive control, challenge of previously induced guinea-pigs with a 10% w/v dilution of a 40% w/v aqueous formaldehyde solution elicited an extreme skin sensitization response. The Meeting concluded that azoxystrobin was not a dermal sensitizer in guinea-pigs, as determined by the maximization method (Robinson, 1991g, 1995f).

2.2 Short-term studies of toxicity

Rats

In a 21-day study of dermal toxicity after repeated doses, groups of five male and five female Wistar (Alpk:APfSD) rats received dermal applications of azoxystrobin (purity, 96.2%) at a dose of 0, 200, 500 or 1000 mg/kg bw per day formulated in deionized water, for 6 h/day, for a total of 21 days over a 30-day period. The hair was clipped from the back of each rat before the first application, then periodically as required. The application site was then wrapped in occlusive gauze bandage covered by a patch of plastic film and secured with polyvinylchloride (PVC) tape for 6 h. After the exposure, the gauze and tape were removed and the application site was cleansed free of any residual test material, using a clean swab of cotton wool soaked in warm water, and dried. The control group received distilled water applied with the same method. The rats were observed twice per day for signs of mortality, morbidity, toxicity, and the presence of dermal irritation. Dermal reactions at the application site were scored daily (before dosing) using the Draize score. Body weight was measured before dosing then daily thereafter. Food consumption was calculated on a daily basis. Blood was taken at the end of the study, and the standard test parameters were examined. At the end of the study, all rats were examined grossly post mortem. Testes, kidneys and liver were weighed. The adrenals, brain, kidneys, liver, testes, epididymides, treated skin, and untreated skin were removed and examined microscopically. This study was conducted in accordance with GLP regulations.

No mortality was observed and there were no significant treatment-related clinical abnormalities. There were no treatment-related effects on body weight, food consumption, organ weights, clinical biochemistry, or haematology. There were no treatment-related pathological abnormalities. Abdominal scabs and scabs at the edge of the application area were observed in all groups of females and were attributed to the bandaging method and were not of toxicological significance.

The no-observed-adverse-effect level (NOAEL) for systemic toxicity and dermal irritation with azoxystrobin was 1000 mg/kg bw per day (the highest dose tested). A lowest-observed-adverse-effect level (LOAEL) was not identified (Robinson, 1994).

In a 90-day study of toxicity, groups of 12 male and 12 female Wistar-derived (Alpk:APfSD) rats were given diets containing azoxystrobin (purity, 95.2%) diet at a concentration of 0, 200, 2000 or 4000 ppm (equal to 0, 20.4, 211.0 or 443.8 mg/kg bw per day for males and 0, 22.4, 223.0 or 448.6 mg/kg bw per day for females) for 13 weeks. The groups given diets at 4000 ppm were initially given diets at 6000 ppm, but this concentration was reduced after 15 days owing to reduced food consumption and a marked reduction in growth. After 5 days on control diet, this group was subsequently fed diets containing azoxystrobin at 4000 ppm for the rest of the study. Diets were prepared at the initiation of the study and stored frozen. Stability, homogeneity and dietary concentrations were confirmed analytically. Rats were inspected daily for signs of toxicity and mortality, with detailed cage-side observations done weekly. Body weight and food consumption were measured weekly. At termination, blood was taken for haematological and clinical chemistry analysis. Urine analysis and

ophthalmoscopic examinations were performed during the week of termination. All rats that died and those that were sacrificed on schedule were given gross pathological examinations and selected organs were weighed. Selected tissues were collected for histological examination.

Diets were stable for 64 days at room temperature. The test article homogeneity results were within the acceptable range (–0.8 to +1.4% deviation from the mean). The analysis of test substance concentration indicated that the measured concentrations of azoxystrobin ranged from 92% to 111% of the target concentrations.

No mortality occurred during the study. Distended abdomen was seen in males and females at 2000 and 4000 ppm, consistent with local gastrointestinal disturbances and reduced nutritional status. At termination, males (11 out of 12) and females (10 out of 12) in the group at 4000 ppm appeared to be of small size compared with rats in the control group or at 200 ppm. No other treatment-related clinical signs of toxicity were observed. Final body weights of males and females at 4000 ppm were reduced by 32% and 18%, respectively, and final body weights of males and females at 2000 ppm were reduced by 18% and 11%, respectively. Food consumption and food efficiency were reduced in males and females at 4000 ppm, particularly during weeks 1–2 or weeks 1–4. However, by the end of the study, food efficiency of females at 4000 ppm was not significantly reduced compared with that of controls.

The results of ophthalmological examination of rats at 4000 ppm were comparable to those for rats in the control group. Minimal reductions in haemoglobin, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) were observed only in females at 4000 ppm. Minor but statistically significant reductions in MCV at 200 and 2000 ppm and MCH at 2000 ppm were observed in females. Leukocyte count was statistically significantly increased in females at 4000 ppm. Platelet counts were slightly decreased in males and females at 4000 ppm. Clotting parameters were not affected. The changes in haematological parameters were small; these changes were therefore not considered to be toxicologically significant. Changes in clinical chemistry parameters such as reduced cholesterol (males), glucose (females), decreased triglycerides (males and females), and decreases in some plasma enzyme activities (males and females) were observed at 4000 ppm. All these findings were less marked in the groups at 2000 ppm and were absent in the groups at 200 ppm. The total urinary protein of males at 4000 ppm was reduced. Blood was present in the urine of males in the control group and in a number of male and female rats at 2000 and 4000 ppm. Increases in liver and kidney weights adjusted for body weight in rats at 2000 and 4000 ppm were attributable to treatment with azoxystrobin. Changes in organ weights were accompanied by histopathological findings in two males at 4000 ppm. Treatment-related effects in these males included marked elevations in total bilirubin, cholesterol, triglycerides, and plasma enzyme activities. The effect on the liver of these two rats was observed microscopically as proliferation of the intrahepatic bile duct/ductiles and oval cells. Hepatocellular hyperplasia and an enlarged hepatic lymph node were observed in one of the two males. There was a reduction in renal tubular basophilia in males at 4000 ppm.

The LOAEL was 2000 ppm, equal to 211.0 mg/kg bw per day, on the basis of decreased body weights and body-weight gains in males and females. The NOAEL was 200 ppm, equal to 20.mg/kg bw per day (Milburn, 1992, 1997).

Dogs

In a 90-day study of toxicity, groups of four male and four female beagle dogs were given capsules containing azoxystrobin (purity, 96.2%) at a dose of 0, 10, 50, or 250 mg/kg bw per day for 92 or 93 days. Equal numbers of dogs in each group were treated for each number of days. The dogs were inspected twice per day for clinical or behavioural abnormalities. A detailed physical examination was performed before the start of treatment and at termination. Eyes were examined by indirect ophthalmoscopy at week 1 and before termination. Body weight and food consumption were measured weekly. Blood for measurement of haematological and clinical chemistry parameters

was collected from all dogs before the test, and after 4, 8 and 13 weeks of treatment. Urine analysis was performed on all dogs at termination. At the end of the study, a complete gross post mortem was done. The adrenals, brain, kidneys, liver, epididymides, testes and thyroid glands were weighed. The organs specified were examined microscopically.

No dogs died during the study. Treatment-related clinical observations in males and females included increases in salivation at dosing and increased incidence of salivation, fluid faeces, vomiting, and regurgitation primarily in dogs at 250 mg/kg bw per day (statistical analysis was not performed). All males and three of the females at 250 mg/kg bw per day exhibited salivation and/or salivation at dosing. Isolated occurrences of salivation/salivation at dosing were seen in one female at 50 mg/kg bw per day and one male in each group at 50 and 10 mg/kg bw per day. The incidence of salivation and gastrointestinal findings at 10 mg/kg bw per day was minimal. There was a dose-related increase in the incidence of fluid faeces, which was prominent in dogs at 250 mg/kg bw per day. Minor increases were seen in the incidence of regurgitation and vomiting in males and females at 250 mg/kg bw per day. The Meeting considered that these clinical signs were treatment-related but not relevant for the identification of a NOAEL, being judged to be secondary to local gastrointestinal irritation/disturbances and bolus dosing (capsule).

The weekly body weights of males and females differed statistically significantly from those of dogs in the control group for most weeks at 250 mg/kg bw per day and in females at 50 mg/kg bw per day, although values were within 9% of controls ($p \leq 0.05$ or 0.01). Total body-weight gains were 34% and 38% lower than those of dogs in the control groups for males and females, respectively, at the highest dose. Haematological alterations in one or both sexes at 250 mg/kg bw per day were small, sporadic compared with values for concurrent controls and/or pre-treatment values and not toxicologically relevant. Clinical chemistry parameters that were altered statistically significantly for one or more weeks in males and females at the highest dose compared with those in dogs in the control group included plasma cholesterol (13–26% increase), triglycerides (42–89% increase), alkaline phosphatase activity (24–87% increase), and plasma albumin (7.9–11.6% decrease). Cholesterol was increased in males at the intermediate and lowest dose (17–25%). These results were accompanied by increased absolute liver weight in females at the intermediate and lowest dose (6.3% and 9.3%, respectively), and are consistent with an adverse effect on liver and possibly biliary function. The lack of histopathological correlates and of a clear dose- and time-related response in some cases indicated that the clinical and liver-weight changes were an adaptive response in the liver of dogs at the lowest and intermediate doses. Other clinical chemistry changes did not appear to be treatment-related (plasma sodium, creatinine, and total protein). There were no treatment-related effects on gross or microscopic pathology, food consumption, ophthalmology, or urine analysis. In the absence of histological changes, the increased thyroid weight found in females at the highest dose (37%) was of uncertain toxicological significance.

The LOAEL was 250 mg/kg bw per day on the basis of treatment-related changes in clinical chemistry parameter (cholesterol, triglycerides and alkaline phosphatase) associated with increases in absolute liver weights and decreases in body weights and body-weight gains in males and females. The NOAEL was 50 mg/kg bw per day. The study author identified a NOAEL of 10 mg/kg bw per day, probably on the basis of gastrointestinal findings seen at the next higher dose and above (Allen, 1993; 1995a, 1995b).

In a 1-year study of oral toxicity, groups of four male and four female beagle dogs were given capsules containing azoxystrobin (purity, 96.2%) at a dose of 0, 3, 25, or 200 mg/kg bw per day for 52 weeks. The dogs were inspected twice per day for morbidity or mortality, with clinical signs being checked daily. Thorough examinations were given weekly. Body weights were recorded weekly and food consumption was measured daily. Eyes were examined by indirect ophthalmoscopy at weeks

13, 26, 39 and before termination. Clinical examinations, including cardiac and pulmonary auscultation, were conducted at weeks 13, 26, 39, and before termination. Blood was collected from all dogs before the test, and during weeks 4, 13, 26 and 52 for measurement of haematological and clinical parameters. Urine analysis was performed on all dogs before the test, at week 26 and at termination. At the end of the study, a complete gross post mortem was done. The adrenals, brain, epididymides, kidneys, liver, thyroid and parathyroid, and testes/ovaries were weighed. The organs specified were examined microscopically.

No dogs died before the scheduled termination date. There were no effects on body weight or food consumption related to the administration of azoxystrobin. There were no treatment-related findings noted at the veterinary or ophthalmic examinations. The most notable treatment-related clinical observation was an increase in the incidence of fluid faeces in males and females at 200 mg/kg bw per day: there were 414 occurrences in 4 out of 4 males and 115 occurrences in 4 out of 4 females compared with 3 occurrences in 2 out of 4 males and 6 occurrences in 2 out of 4 females in the control group (statistical analysis was not performed). Females at the highest dose had minor increases in salivation (21 occurrences in 3 out of 4 females at the highest dose compared with 0 out of 4 in the control group) and salivation at dosing (80 occurrences in 3 out of 4 females at the highest dose, compared with 0 out of 4in the control group), although the combined frequency was similar to that of males in the concurrent control group. The Meeting considered that these clinical signs were treatment-related, but not relevant for the identification of a NOAEL, being judged to be secondary effects attributable to local gastrointestinal irritation/disturbances and bolus dosing (capsule).

Minor changes in MCV, MCH, prothrombin time, leukocyte count, neutrophils, kaolin-cephalin time, platelets, lymphocytes, and monocytes were observed; however, these changes in haematological parameters were not considered to be toxicologically significant, being small in magnitude, without a dose–response relationship and transient in nature. Treatment-related clinical chemistry changes at 200 mg/kg bw per day ($p \leq 0.05$ or 0.01) for one or more weeks included increased concentrations of plasma cholesterol (males and females, 14–48%), triglycerides (males and females, 65–124%,), alkaline phosphatase activity (males and females, 17–156%), gamma-glutamyl transferase activity (females, 74–112%) and lowered plasma albumin (males, 9.4–13%). Males at the intermediate dose had increased concentrations of cholesterol (23–27%) and triglycerides (65%). These results suggest that azoxystrobin has an effect on liver and possibly biliary function. Minor and/or transient alterations ($p \leq 0.05$ or 0.01) in total plasma protein, bilirubin, calcium, phosphorus, urea, potassium, and sodium concentrations were observed in one or both sexes. Clinical chemistry changes observed in males and females lacked histopathological correlates There was a small decrease in absolute brain weight in males at the highest dose (6.5%, $p \leq 0.05$) that is of uncertain biological significance. This small decrease in absolute brain weight was of unknown etiology and uncertain biological significance: it was not correlated with any histopathological findings, and the relative-to-body brain weight was not clearly affected. There was a dose-related increase in liver weight in males at the highest dose (15%, $p \leq 0.01$) and in females at the intermediate (12%, $p \leq 0.05$) and highest dose (19%, $p \leq 0.01$). Increases in liver weights seen in females at the intermediate dose were considered to be an adaptive response because liver-enzyme parameters were not affected and no liver pathology was seen. There were no treatment-related effects on urine analysis and gross or microscopic pathology in male or female dogs.

The NOAEL was 25 mg/kg bw per day on the basis of alterations in clinical chemistry parameters and increases in liver weights seen at the LOAEL of 200 mg/kg bw per day, the highest dose tested. The study author identified the no-observed-effect level (NOEL) as 3 mg/kg bw per day (Allen, 1994), although the sponsor contended that there were no adverse effects were identified in the study (Syngenta, 2007).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity, groups of 55 male and 55 female C57BL/10JfAP/Alpk mice were given diets containing azoxystrobin (purity, 96.2%) at a concentration of 0, 50, 300, or 2000 ppm (equal to 0, 6.2, 37.5, or 272.4 and 0, 8.5, 51.3, or 363.3 mg/kg bw per day for males and females, respectively) for 104 weeks. Additional groups of five males and five females were used as microbiological sentinels and fed either control diet or diet containing azoxystrobin at 2000 ppm. Prepared diets were stored at room temperature. Stability, homogeneity and dietary concentrations were confirmed analytically. The mice were inspected daily for mortality and morbidity. Changes in clinical condition or behaviour were recorded daily. Body weights were measured weekly for the first 12 weeks, then every 2 weeks thereafter and at termination. Food consumption was measured weekly for the first 12 weeks, then every 4 weeks thereafter until termination. Water consumption was not measured. An ophthalmoscopic examination was not done. Blood was collected from 11 males and 11 females per group at weeks 53, 79 and at termination. Differential leukocte counts and erythrocyte morphology were performed on mice in the control group and mice at 2000 ppm. Clinical chemistry and urine analysis were not performed. All mice that died and those that were sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed (adrenals, brain, kidneys, liver and testes/ovaries). Tissues were collected for histological examination.

Azoxystrobin was homogenously distributed in the diet and was stable in the diet for 56 days storage at room temperature. The measured test concentrations were within the range of 10% of the target concentrations except for one diet containing azoxystrobin at 2000 ppm that was 117% of the target concentration.

At the end of the study, the survival of males was 58%, 61%, 60% and 63% (control, lowest, intermediate and highest dose, respectively). The survival of was similar at 47%, 38%, 45% and 56% (control, lowest, intermediate and highest dose, respectively). No effects were observed on mortality, clinical signs, haematology, or gross or microscopic pathology. Mean body weights of males at 2000 ppm were significantly ($p \leq 0.01$) lower (5–12%) than those of mice in the control group from week 2 and continuing until the end of the study. Final body weights of males at the highest dose were 94% those of the controls. Body weights of males at 300 ppm were also statistically significantly lower compared with those of the controls at weeks 2, 3, 35, and 61–83; however, final body weights gave evidence for recovery (101% of mice in the control group). No differences in body weights were observed for males at 50 ppm when compared with mice in the control group. Females at 2000 ppm had significantly ($p \leq 0.01$ at week 8 only, $p \leq 0.05$) lower mean body weights (2–7%) compared with those of the controls from study week 3 and continuing until the end of the study. Final body weights of females at the highest dose were 93% those of females in the control group. Although food consumption was similar in treated and control groups, overall food utilization was significantly ($p \leq 0.01$) lower in males and females at the highest dose during weeks 1–12 (the only interval for which food utilization values were calculated). Absolute kidney weights of males at 2000 ppm were significantly ($p \leq 0.05$) less than those of the controls. Absolute kidney weights of females at 2000 ppm were slightly lower than those of the controls (not significant). No significant differences in adjusted kidney weights (organ weight adjusted for body weight) were observed in males and females at 2000 ppm. Absolute liver weights were not affected at any dose tested. However, adjusted liver weights were increased compared with those of mice in the control group for males (14%) and females (18%) at the highest dose. In the absence of any histopathological findings, the Meeting considered that these changes in liver weights were adaptive. The changes in kidney weights were not considered to be adverse because there were no histological findings in the kidney. No evidence of carcinogenicity was observed at the doses tested.

The LOAEL for systemic toxicity was 2000 ppm, equal to 272.4 mg/kg bw per day, on the basis of reduced body weights in males and females. The NOAEL for systemic toxicity was 300 ppm, equal to 37.5 mg/kg bw per day (Moxon, 1995a).

Rats

In a combined long-term study of toxicity and carcinogenicity, groups of 52 male and 52 female Alpk:APfSD rats were given diets containing azoxystrobin, (purity, 96.2%) at a concentration of 0, 60, 300 or 750 ppm/1500 ppm (males/females), equal to 0, 3.6, 18.2, and 34.0 mg/kg bw per day for males and 0, 4.5, 22.3, and 117.1 mg/kg bw per day for females, for 104 weeks. An additional 12 males and 12 females per group were designated for interim sacrifice at week 52. Owing to excessive mortality, the highest dose was reduced to 750 ppm, equal to 34 mg/kg bw per day, in males from week 52 and the rats in this group designated for interim sacrifice were retained with the main study. Additional groups of seven males and seven females were used as microbiological sentinels and fed either control diet or diet containing azoxystrobin at 1500/750 ppm. Diets were prepared in batches of 30 or 60 kg throughout the study. Stability, homogeneity and dietary concentrations were confirmed analytically. the rats were inspected twice per day for mortality and morbidity. Changes in clinical condition and behaviour were recorded daily. Detailed clinical observations were recorded weekly. Body weights were measured weekly for the first 14 weeks, then every 2 weeks for the rest of the study. Food consumption was measured for the first 14 weeks, at week 16, and every fourth week thereafter. Water consumption was not measured. An ophthalmoscopic examination was performed before treatment and at week 54. The eyes of rats in the control group and the group at the highest dose were examined before termination. Blood was collected at weeks 14, 27, 53, 78 and week 105 of treatment. Urine was collected at weeks 13, 26, 52, 78 and 104. Haematological parameters examined were erythrocyte count, leukocyte count, haematocrit (erythrocyte volume fraction), haemoglobin concentration, platelet count, differential leukocyte count and cell morphology. Standard clinical chemistry and urine analysis parameters were examined. At weeks 53 or 105, the designated rats were necropsied and examined histopathologically. Liver, kidney, brain, testes, ovaries, and adrenals were removed and weighed. All tissues were examined histologically, except oral and nasal cavities, which were stored. The common bile duct and intraduodenal bile duct were taken from all rats with bile-duct distension from approximately week 39 and from all rats killed or found dead from week 53.

Azoxystrobin was uniformly distributed in the diet and was stable at room temperature for 66 days. The measured test concentrations ranged from 91.2% to 110.7% of the nominal values. Overall mean achieved dietary concentrations were within ± 2.0% of the nominal concentrations.

Distended abdomens were observed in males starting from week 17, with 5, 0, 5, and 15 rats affected in the control group, and at 60, 300, and 1500/750 ppm, respectively. Hunched posture was observed in males in a dose-related manner, with 3, 11, 12, and 17 rats affected, respectively. There was an apparent increased incidence of opaque eyes in males (0, 4, 2, and 5 rats in the control group, and at 60, 300 and 1500/750 ppm, respectively). No treatment-related clinical signs were observed in females at any dose.

By week 52, survival rates of the males receiving the diets containing azoxystrobin at 0, 60, 300, or 1500 ppm were 97%, 100%, 98%, and 86%, respectively, prompting the dose reduction for the group receiving the highest dietary concentration. Survival rates at week 104 for the control group, and at the lowest, intermediate and highest dose were 37%, 38%, 29%, and 30%, respectively, for males and 45%, 62%, 62%, and 68%, respectively, for females. The lower survival rate for females in the control group did not develop until after week 100.

Males at the highest dose had statistically significantly lower body weights (92–95%) compared with those of males in the control group beginning at week 2 and continuing until week 101 (except for week 87, when no difference occurred). Females at the highest dose had statistically significantly lower body weights (87–94%) than the controls beginning at week 2 and continuing until

study termination. Food consumption was significantly lower (95%) in males at the highest dose at weeks 1–20, 48, and 96 when compared that for controls. Food consumption for females at the highest dose was significantly less (91–96%) than that of females in the control group at weeks 1, 3–11, 13–36, 44, 56, and 68. Food utilization was significantly ($p \leq 0.01$) reduced in males at the highest dose for each of the intervals calculated: weeks 1–4, 5–8, 9–12, and 1–12. Females at the highest dose had significantly ($p \leq 0.01$) reduced food utilization compared with that of controls for weeks 1–4 and 1–12.

Several haematological parameters for rats at the intermediate and highest dose were occasionally statistically significantly different than the values for rats in the control group, but no dose- or treatment-related pattern was observed. Reduction in the activity of alkaline phosphatase, plasma alanine aminotransferase and aspartate aminotransferase was observed at various time-points and doses. These changes were considered not to be toxicologically relevant since they were small in magnitude and lacked any clear dose–response relationship.

Several urinary parameters for the treated groups were occasionally significantly different from the values for controls, but there were no dose- or treatment-related trends apparent for males or females. At weeks 52–54 there was a dose-related increase in the number of males with minute lens opacity, with 1, 2, 5, and 7 rats affected in the groups at 0, 60, 300, and 1500 ppm, respectively. No treatment-related ophthalmoscopic findings were observed in females at weeks 52–54 or in males or females at weeks 103–104. Adrenal weights were statistically significantly lower than those of controls in females at 1500 ppm at week 53. At terminal sacrifice, males and females at the highest dose had significantly lower adrenal gland weights (84% of values for controls) and kidney weights (83% and 89% of values for controls, respectively) compared with controls. Absolute liver weights were increased in females at the highest dose at week 53, but not at terminal sacrifice. In the common bile duct of males at the highest dose, there were significant increases ($p \leq 0.01$) in the rates of distension (13 out of 47), cholangitis (13 out of 47), thickening of the wall (11 out of 47), and epithelial hyperplasia (9 out of 47); these lesions were not observed in rats in the control group (0 out of 34) or males and females in any other group receiving azoxystrobin.

There was no evidence of carcinogenic activity in this study. Among female rats, there was a significant dose-related decrease in the incidence of benign fibroadenomas of the mammary gland with 10 out of 52, 3 out of 52, 2 out of 52 ($p \leq 0.05$), and 1 out of 52 ($p \leq 0.01$) affected in the control group, and at 60, 300, and 1500 ppm, respectively.

The NOAEL was 300 ppm, equal to 18.2 mg/kg bw per day, on the basis of reduced body weights, food consumption and food efficiency, and bile-duct lesions (males only) seen at the LOAEL of 750 ppm, the highest dose tested (equal to 34.0 mg/kg bw per day). The study author identified a NOEL of 300 ppm, equal to 18.2 mg/kg bw per day (Milburn, 1995).

2.4 Genotoxicity

Azoxystrobin gave mixed responses in a battery of assays for genotoxicity. Negative results were obtained in the Ames test, and tests for unscheduled DNA synthesis (UDS) and for micronucleus formation in vivo. Azoxystrobin gave a weak positive response in two studies in mammalian cells (mouse lymphoma cells and human lymphocytes). The latter findings suggest that azoxystrobin has a clastogenic potential in vitro since the increased occurrence of small colonies in the mouse lymphoma cell assay is considered to be indicative of chromosome aberrations rather than of point mutations. However, azoxystrobin has shown to give negative results in assays for chromosomal damage (i.e. clastogenicity) in vivo and for general DNA damage at high doses of 2000 mg/kg bw or above. The Meeting concluded, therefore, that the clastogenic effects seen in vitro are not expressed in the whole animal. Furthermore, long-term studies have not shown any evidence of carcinogenicity

in mice or rats. Based on the overall weight of evidence, the Meeting concluded that azoxystrobin is unlikely to be genotoxic.

2.5 *Reproductive toxicity*

(a) Multigeneration studies

Rats

In a two-generation study of reproductive toxicity, groups of 26 male and 26 female Alpk:APfSD (Wistar-derived) rats were given diets containing azoxystrobin (purity, 96.2%) at a concentration of 0, 60, 300, or 1500 ppm. The average achieved intake of azoxystrobin during the premating interval for the F_0 and F_1 generations was as follows: 0 6.4, 32.3, or 165.4 mg/kg bw for males and 0, 6.8, 33.8, or 175.0 mg/kg bw per day for females. All rats were mated on a 1 : 1 ratio. All rats were exposed continuously to diets containing the test material throughout the study. Diets were prepared in batches of 60 kg and stored at room temperature. Stability, homogeneity and dietary concentrations were confirmed analytically. The rats were inspected daily for clinical observations, mortality, and morbidity. Physical examinations were performed weekly. Body weights were recorded weekly during the mating period. Females were weighed on days 1, 8, 15, and 22 of gestation, and days 1, 5, 11, 16, 22 and 29 of lactation. Food consumption was measured weekly throughout the mating

Table 5. Results of studies of genotoxicity with azoxystrobin

End-point	Test system	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a] (Ames test)	*S. typhimurium* strains TA98, TA100, TA1535 and TA1537 *E. coli* WP2P, WP2P *uvrA*	100–5000 µg/plate ±S9, in DMSO	97.2	Negative	Callander (1992)
Forward mutation[b]	Mouse lymphoma L5178Y cells	8–60 µg/ml (test 1) 34–80 µg/ml (test 2) 26–80 µg/ml (test 3) ±S9, in DMSO	96.2	Weakly positive	Callander & Clay (1993); Fox & Callander (1995)
Chromosomal aberration[b]	Human lymphocytes	1–20 µg/ml (–S9) 25–200 µg/ml (+S9)	95.2	Weakly positive	Fox & Mackay (1992, 1995a)
In vivo					
Micronucleus formation[c]	C57 BL/6JfBL10/AlpK mice (male and female)	5000 mg/kg bw (single oral dose)	97.2	Negative	Jones & Mckay (1992); Fox & Mackay (1995b)
Unscheduled DNA synthesis	Hepatocytes from male Aldereley Park (Alpk:APfSD) rats	1250 and 2000 mg/kg bw (single oral dose)	97.2	Negative	Lane & Kennelly (1992); Fox & Mackay (1995c)

S9, 9000 × *g* supernatant from livers of male rats.

[a] Precipitation at concentrations of 2500 and 5000 µg/plate.

[b] Higher concentrations were limited by cytotoxicity.

[c] Groups of five males and five females per dose. Azoxystrobin administered via gavage in corn oil. Bone marrow collected at 24 h and 48 h. Clinical signs after dosing included tiptoe gait, piloerection, diarrhoea and urinary incontinence on day of dosing.

period and for females during gestation and lactation. Estrous cycles were monitored with vaginal smears taken during the mating period and until mating was confirmed. The duration of gestation was calculated. Females were allowed to deliver normally and rear young to weaning on day 29. Litters were examined after delivery and pups were sexed, examined for gross abnormalities and the number of stillborn and live pups recorded. Litters were then examined daily for survival. The number, sex and weight of pups were recorded on postnatal days 1, 5, 11, 16, 22, and 29. All parental (F_0) and F_1 rats and those found dead and killed in extremis were necropsied and examined macroscopically. All pups that were not selected for the next generation were killed on postnatal day 29. Selected male and female pups received a full examination post mortem. Selected tissues (liver, uterus, cervix, vagina, ovaries, mammary glands, testes, epididymides, prostate, seminal vesicle and pituitary gland) were examined histopathologically. Liver, epididymides, and testes/ovaries were weighed.

The analytical data indicated that the mixing procedure for the diets was adequate and that the variation between nominal and actual dietary concentrations received was within 10% of the nominal values.

There were no treatment-related clinical signs of toxicity or increases in mortality noted at any dose. However, one F_0 male and one F_1 male from the groups at 1500 ppm were sacrificed in a moribund condition and exhibited treatment-related distention of the common bile duct. At 1500 ppm, systemic toxicity in the F_0 and F_1 adults (males and females) was apparent as reduced adjusted body weights (3–12%, $p \leq 0.01$ or 0.05) and food consumption (5–14%, $p \leq 0.05$ or 0.01) during the pre-mating intervals. At 1500 ppm, gestational body weights were reduced (within 5% of values for controls) for F_0 and F_1 females. In addition, treatment-related increases in liver weights adjusted for final body weights were noted in the F_0 and F_1 males and females (15–38%, $p \leq 0.01$ or 0.05) at 1500 ppm. Treatment-related distention of the common bile duct was also noted in 3 and 11 of the F_0 and F_1 males at 1500 ppm, respectively, on examining grossly. Treatment-related histopathological lesions of the common bile duct in the adult males at the highest dose were characterized as epithelial hyperplasia of the intraduodenal portion, cholangitis, ulceration of the dilated region, and small basophilic deposits in the lumen. Treatment-related increases in severity of proliferative cholangitis were also observed in the livers of the F_0 and F_1 males at 1500 ppm. Males and female in the F_1a and F_2a at 1500 ppm had treatment-related increases in the adjusted (for final body weight) liver weights (10–13%, $p \leq 0.01$).

No treatment-related clinical signs of toxicity were observed in pups of either generation. Treatment-related reductions in adjusted (for initial weight) pup body weights were observed in the F_0a and F_2a pups at 1500 ppm (8–21%, $p \leq 0.05$ or $p \leq 0.01$). Treatment-related increases in the adjusted mean liver weights were noted in the F_1a and F_2a males and females at 1500 ppm (10–13%, $p \leq 0.01$). There were no treatment-related macroscopic or microscopic findings in the F_1a or F_2a litters. None of the reproductive parameters were affected at any of the dietary concentrations tested.

The LOAEL for systemic toxicity was 1500 ppm, equal to165.4 mg/kg bw per day, on the basis of reduced adjusted body weight, feed consumption, feed utilization, an increased in adjusted liver weights and histopathology (males). The NOAEL for systemic toxicity was 300 ppm, equal to 32.3 mg/kg bw per day. The NOAEL for offspring toxicity was 300 ppm, equal to 32.3 mg/kg bw per day, on the basis of reduced pup body weight and increased liver weights seen at the LOAEL of 1500 ppm, equal to 165.4 mg/kg bw per day. The NOAEL for reproductive toxicity was ≥ 1500 ppm, equal to ≥ 165.4 mg/kg bw per day, the highest dose tested (Moxon, 1994b, 1995b, 1997).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 24 female Alpk:APfSD rats were given azoxystrobin (purity, 95.2%) at a dose of 0, 25, 100 or 300 mg/kg bw per day by gavage in corn oil (dosing volume, 1 ml/100 g bw) from day 7 to 16 of gestation, inclusive. Stability, homogeneity and dose

concentrations were confirmed analytically. All rats were observed daily for clinical signs of toxicity, mortality and moribundity. Maternal body weights were recorded on days 1, 4, 7–16, 19 and 22 of gestation. Food consumption was determined at 3-day intervals until day 22 of gestation. On day 22 of gestation, all surviving dams were sacrificed and subjected to gross necropsy. Examinations at sacrifice comprised uterine weight, number and positions of implantations; corpora lutea in each ovary, individual fetal weights, percentage preimplantation loss, percentage postimplantation loss, early and late intrauterine deaths. All fetuses were weighed, sexed, and examined for external malformations/variations. Each fetus was examined viscerally by fresh dissection and the sex was verified. The fetuses were then eviscerated and fixed in 70% industrial methylated spirits. After approximately 24 h, the brain was examined for macroscopic abnormalities and the carcasses were stained with Alizarin Red S for evaluation of the skeleton.

Analysis of the dosing solution indicated that the test material was homogenously distributed and was stable for 25 days. The achieved concentrations were within 4% of the nominal values.

Three rats at 300 mg/kg bw per day were found dead after receiving two daily doses and one rat was killed in extremis. Severe signs of toxicity were observed in another 12 rats. Dosing of the remaining rats in this group was suspended. These rats were able to recover and continue to scheduled termination. The remaining 12 rats at the highest dose did not start treatment and no assessment of developmental toxicity was made at this dose. Gross necropsy of rats at the highest dose that were found dead revealed red areas and thin walls in the stomach or jejunum. In the groups at the intermediate dose, two animals showed haemorrhagic areas in the stomach at terminal necropsy; these findings were considered to be likely to be related to local irritation caused by the administration of azoxystrobin by gavage.

At 100 mg/kg bw per day, minimally reduced body weights (< 2%) were observed ($p < 0.05$), although body-weight gain and food consumption were not affected. Clinical signs during dosing included diarrhoea (42%), urinary incontinence (17%) and salivation between days 9 and 16 (71%). At 25 mg/kg/bw per day, salivation was observed in 29% of rats between days 11 and 16. The Meeting considered that these clinical signs were treatment-related but not relevant for the identification of a NOAEL, being considered to be secondary effects caused by local gastrointestinal irritation/disturbances and bolus dosing by gavage in corn oil.

In the conceptus, no significant adverse developmental effects were observed. General reduced ossification was seen at all doses, the incidence of which was not statistically different between the controls group and groups receiving azoxystrobin. There was, however, a statistical increase (6.9% vs 2.6%, $p < 0.05$) in the rats with a PES score of 6 at 100 mg/kg bw per day vs controls. Since this possible minimal increase in reduced ossification was not supported by increases in other related end-points, and was not statistically different from controls, the Meeting considered that it was not of toxicological significance.

The NOAEL for maternal and developmental toxicity was 100 mg/kg bw per day, the highest dose tested. The study author concluded that the NOAEL for maternal and developmental toxicity was 25 mg/kg bw per day, considering that minimal reduction in ossification was a treatment-related effect (Moxon, 1994a).

Rabbits

In a study of developmental toxicity, groups of 20 pregnant New Zealand white rabbits were given azoxystrobin (purity, 96.2%) at a dose of 0, 7.5, 20, or 50 mg/kg bw per day by gavage in corn oil (dosing volume, 2 ml/kg bw) on days 8–20 of gestation, inclusive. Test substance formulations were prepared daily. Stability, homogeneity and dose concentrations were confirmed analytically. All rabbits were observed twice per day for mortality or clinical signs of toxicity. Maternal body weights were recorded on days 0 and 4 of gestation and daily on days 8–20, and on days 23, 26 and 30 of gestation. Food consumption was measured on days 8, 11, 14, 17, 20, 23 and 26 of gestation. On

day 30 of gestation, all surviving does were killed and subjected to gross necropsy. The uterus and ovaries were excised and the number of corpora lutea on each ovary was recorded. Gravid uteri were weighed, opened, and the location and number of viable and nonviable fetuses, early and late resorptions, and the total number of implantations were recorded. All fetuses were weighed and examined for external malformations/variations. Each fetus was examined viscerally by fresh dissection and the sex determined. The brain from each fetus was examined by mid-coronal slice. All carcasses were eviscerated and processed for skeletal examination.

The analytical data indicated that the mixing procedure for the dosing solution was adequate and that the variation between nominal and actual doses received by the rabbits was acceptable (within 3% of nominal values).

There were two, four, three and seven deaths in the control group and at 7.5, 20 and 50 mg/kg bw per day, respectively. One rabbit at 50 mg/kg bw per day was found dead; the other deaths resulted from premature termination after abortion or deterioration in clinical condition, usually between days 12 and 20 of gestation. The following clinical observations were reported: blood on tray, sporadic in two, one, two, six rabbits in the control group and at 7.5, 20 and 50 mg/kg bw per day, respectively; general coat staining, two, two, three, five rabbits in the control group and at 7.5, 20 and 50 mg/kg bw per day, respectively; diarrhoea, two, four, seven, seven rabbits in the control group and at 7.5, 20 and 50 mg/kg bw per day, respectively. While these occurrences were observed in a dose-related manner, their toxicological significance is uncertain. At the start of dosing, slight loss of body weight for all groups including the controls was observed. The loss in body weight was more severe at 20 and 50 mg/kg bw per day than in the control group. Five rabbits at 50 mg/kg bw per day showed progressive bosy-weight loss from which they did not recover and were therefore killed. However, two, three and two rabbits were killed for similar reasons in the control group and at 7.5 and 20 mg/kg bw per day, respectively. Food consumption data were inconclusive owing to food wastage and other factors. Some rabbits showed very little food consumption. The number of rabbits showing negligible food consumption during the dosing period was five, six, eight and nine in the control group and at the lowest, intermediate and highest dose, respectively. No dose-related adverse effects were noted during necropsy, either in rabbits that died during the study, or in rabbits sacrificed at the end of the study.

The incidence of fetuses with major defects was 8, 0, 2 and 13 in the control group and at 7.5, 20 and 50 mg/kg bw per day, respectively. At the highest dose, nine fetuses (8.6%) from two litters (16.7%) had open eye, the majority being bilateral. One fetus at the highest dose had cleft palate. Other effects were of low occurrence, were not dose-related, and were not associated with treatment. These effects included internal hydrocephaly, encephalocoele, fenestration in parietal, reduced pulmonary artery, enlarged aorta. Many of these effects occurred in the control group only. Fused sternebrae (third and fourth, or fourth and fifth) was noted in the group at the highest dose. The occurrence was statistically ($p < 0.01$) above control values and involved twelve fetuses from four litters. Most of the affected fetuses were from the litters with open eye. While these changes were considered to be compound-related, their biological significance was uncertain.

Owing to excessive food wastage, maternal death and other unidentified factors, the Meeting did not consider that the results of this study were appropriate for the identification of an acceptable daily intake (ADI) or an acute reference dose (ARfD).

The NOAEL for maternal toxicity was 7.5 mg/kg bw per day on the basis of decreased body weights, clinical signs of toxicity and marked reduction in food consumption seen at the LOAEL of 20 mg/kg bw per day and above. The NOAEL for developmental toxicity was 20 mg/kg bw per day on the basis of fused sternebrae, open eyes and cleft palates seen at 50 mg/kg bw per day (Moxon, 1994c).

In a second study of developmental toxicity, groups of 21 female New Zealand White rabbits were given azoxystrobin (purity, 96.2%) at a dose of 0, 50, 150 or 500 mg/kg bw per day by gavage in corn oil (dosing volume, 1 ml/kg bw) from days 8 to 20 of gestation, inclusive. Rabbits in the control

group received the appropriate volume of corn oil only. All other experimental details were similar to those for the study of developmental toxicity by Moxon (1994c, described above).

The analytical data indicated that the mixing procedure for was adequate and that the variation between nominal and actual doses given to the rabbits was acceptable (within 3% of nominal values). None of the intercurrent deaths in the study was considered to be associated with the administration of azoxystrobin. The occurrence of intercurrent deaths was one, two, one, and two in the control group and at 50, 150 and 500 mg/kg bw per day, respectively. One rabbit at 500 mg/kg bw per day was killed on day 11 and was found to have an intussusception of the colon and severe body-weight loss. This death was not considered to be treatment-related. Two rabbits in the group at 150 mg/kg bw per day were killed; one was killed on day 21 of gestation after abortion and other rabbit on day 17 of gestation because of excessive body-weight loss starting from day 8 of gestation.

Clinical signs included diarrhoea and/or staining in the genital area in 1, 7, 15, and 18 rabbits in the control group and at 50, 150 and 500 mg/kg bw per day, respectively, beginning generally around days 9 and 10 of gestation. No other treatment-related signs were observed. The Meeting considered that these clinical signs were treatment-related but not relevant for the identification of a NOAEL, being considered to be secondary effects caused by local gastrointestinal irritation/disturbances and a bolus dosing by gavage in corn oil.

At 150 mg/kg bw per day and 500 mg/kg bw per day, significant ($p < 0.01$) but transient reductions (–33%, –51%, respectively) in food consumption were observed during the first 3 days of dosing. At 500 mg/kg bw per day, decreased body-weight gain (–45%) was observed during the dosing period. Slight reductions in body weights were observed on days 9–12 at 50 and 150 mg/kg bw per day; however, the decrease in body weights did not occur in a dose-related manner. No treatment-related increases in gross lesions were observed.

No dose-related or statistically significant increases in external or visceral anomalies were observed. Although skeletal anomalies, mainly variations, were common in all groups (including concurrent controls), there were no statistically significant increases, nor were there any trends in dose–response observed. The LOAEL for developmental toxicity was > 500 mg/kg bw per day. The NOAEL for developmental toxicity was 500 mg/kg bw per day.

The NOAEL for maternal toxicity was 150 mg/kg bw per day on the basis of decreased body-weight gain seen at the LOAEL of 500 mg/kg bw per day. The NOAEL for developmental toxicity was 500 mg/kg bw per day, the highest dose tested. The study author identified the NOAEL for maternal toxicity as 50 mg/kg bw per day owing to marginal decreases in body weight, diarrhoea and food consumption at 150 mg/kg bw per day (Moxon, 1995c).

A technical review by Lewis (1995) evaluated the results of the two studies of developmental toxicity in rabbits, described above. Lewis (1995) cites several studies in pregnant and non-pregnant rabbits carried out to determine a suitable vehicle for the administration of azoxystrobin and also the appropriate volume of the vehicle, and concludes that the results of the first study of developmental toxicity (Mowon, 1994) in rabbits given azoxystrobin at a dose of 50 mg/kg bw per day were invalid owing to difficulty in selecting appropriate doses with respect to acceptable maternal toxicity, lack of any clear dose–response relationship, and variability in response within the experimental groups. This review also states that the evaluation of these data suggested that the dosing vehicle, corn oil, used at 2 mg/kg bw may have contributed to the pattern of observed effects.

2.6 Special studies

(a) Acute neurotoxicity:

In a study of acute neurotoxicity, three groups of 10 male and 10 female Alpk:ApfSD rats were given a single dose of azoxystrobin (purity, 96.2%) at 0, 200, 600 or 2000 mg/kg bw by gavage in corn

oil (dosing volume, 10 ml/kg bw) and observed for the following 14 days. All the rats were evaluated in functional observational battery (FOB) and motor activity tests on days –7, 1 (2 h after dosing; time of peak effects), 8 and 15. All rats were observed before the study start and daily throughout the study for any changes in clinical condition. Body weights and food consumption were measured weekly throughout the study. Five males and females in the control group and at the highest dose were perfused in situ and evaluated for microscopic neuropathology.

Measurements of dosing solutions indicated that dosing solutions were homogeneous, were stable for at least 8 days and that the rats received appropriate doses.

At doses of 200 mg/kg and higher, diarrhoea/signs of diarrhoea were observed at 2 h after dosing in males and females. Tip-toe gait and hunched posture at 2 h were also observed in rats receiving azoxystrobin but not in rats in the control group (no dose–response relationship observed). No apparent dose-related increase in incidence of clinical signs was evident. Recovery from all of these findings was usually apparent by day 2. The Meeting considered that the diarrhoea was treatment-related but not relevant for the identification of a NOAEL, this effect being considered as a secondary effect caused by local gastrointestinal irritation/disturbances. No treatment-related effects on survival, food consumption, motor activity, brain weight/dimensions, or gross/microscopic pathology were observed. Body weights of males at 2000 mg/kg bw were slightly decreased (2.9% and 2.6% at day 8 and 15). Statistically significantly decreased body weight in the males at 200 mg/kg bw was observed on day 15. However, a dose–response relationship was not evident and the decrease was considered to be incidental. There was no effect on the amount of food consumed in males or females at any dose.

Statistically significant increases in landing foot splay on day 8 in females at 600 and 2000 mg/kg bw were noted (23.7% and 20.5% higher than controls, respectively; on day 1, females at 600 and 2000 mg/kg bw had non-statistically significantly increased values of 11.8% and 12.5%, respectively). The findings of hunched posture, tip-toe gait and increased landing-foot splay were not considered indicative of neurotoxicity owing to the lack of any effect on the day of dosing (only marginal non-significant increase seen) and to lack of a clear dose–response relationship and the association with marked gastrointestinal disturbance. There were no effects on brain weight, length or width. There were no treatment-related macroscopic or histopathological changes, including in the nervous system.

The NOAEL for systemic toxicity was 2000 mg/kg bw. No LOAEL was identified. The NOAEL for neurotoxicity was ≥ 2000 mg/kg bw (Horner, 1994 and 1996).

(b) Short-term study of neurotoxicity:

In a short-term study of neurotoxicity, groups of 12 male and 12 femaleAlpk:APfSD rats were given diets containing azoxystrobin (purity, 96.2%) at a concentration of 0, 100, 500 or 2000 ppm (equal to 0, 8.0, 38.5 or 161 mg/kg bw per day in males and 0, 9.1, 47.9 or 201.5 mg/kg bw per day in females) for 13 weeks. All rats were evaluated in FOB and motor activity tests in weeks –1, 5, 9 and 14. All rats were observed before the study start and daily throughout the study for any changes in clinical condition. Body weights and food consumption were measured weekly throughout the study. Six male and six female rats from the control group and at the highest dose were perfused in situ and evaluated for microscopic neuropathology.

Analysis of the dietary preparations showed that diets were stable at room temperature for 56 days and the test article was homogeneously distributed in the diet. The overall mean dietary concentrations were within 8% of the nominal values.

There were no deaths or treatment-related changes in clinical condition observed during the study. At 2000 ppm, mean body weights of males were statistically significantly decreased throughout the study (at week 13, 12.6% less than controls). Mean body weights of females were slightly decreased (at week 13, 5.1% less than controls; significant only at week 2). Cumulative body-weight

gains were 18% lower (males) and 10% lower (females) than those of the controls. Food consumption was statistically significantly lower than those of the controls in males (5.4% to 15.4%) but not females. Food utilization in males at 2000 ppm was statistically significantly decreased during weeks 1–4 (9.7%) and 1–13 (11.7%) and was non-significantly less in females during the same periods (11.8% and 14.4%, respectively).

There were no consistent indications of treatment-related neurotoxicity (clinical signs, qualitative or quantitative neurobehavioral effects, brain weight/ dimensions, or gross/microscopic pathology). Statistically significant decreases in landing foot splay in males (week 5: 19%, 16.4% and 24.1%, for the lowest to the highest dose, respectively; week 9, 18% at the highest dose), forelimb grip strength (males: week 5, 14.3%, 14.3% and 19%, for the lowest to the highest dose, respectively; and females, week 14, 12.9%, highest dose), hind-limb grip strength in males (week 5, 13.3%, 15.3% and 12.9%, for the lowest to the highest dose, respectively) and motor activity in females (21%, week 9) weer noted but not considered to be treatment-related owing to lack of a dose–response relationship, inconsistency of observations at different time-points, variability of pre-treatment values and/or small magnitude of response. Brain weight and length were unaffected by treatment. There were no macroscopic or treatment-related microscopic findings at the end of the study.

The LOAEL for systemic toxicity was 2000 ppm, equal to 161 mg/kg bw per day, on the basis of decreased body weight/body-weight gain and food utilization in males and females (marginal in females). The NOAEL was 500 ppm, equal to 38.5 mg/kg bw per day. The NOAEL for neurotoxicity was ≥ 2000 ppm, equal to ≥ 161 mg/kg bw per day (Rattray, 1994, 1996).

(c) Studies on metabolites

No studies on metabolites were submitted by the sponsor.

3. Observations in humans

No observations in humans were provided by the sponsor.

Comments

Biochemical aspects

In an autoradiography study in rats, groups of one male and one female were given azoxystrobin labelled with ^{14}C in either the cyanophenyl, pyrimidinyl or phenylacrylate ring as a single dose at 1 mg/kg bw by gavage. The results of this study indicated that the position of the radiolabel had no significant effect on the rates and routes of excretion or tissue distribution of azoxystrobin, therefore, further metabolism studies were conducted using azoxystrobin labelled in the pyrimidinyl position. In studies in rats given a single oral dose of radiolabelled azoxystrobin, 73–89% of the administered dose was recovered in the faeces and 9–18% in the urine (1 and 100 mg/kg bw) after 7 days. The extent of oral absorption at 1 mg/kg bw was nearly complete since no parent compound was found in the excreta. At least 74–81% of the administered dose was absorbed at 100 mg/kg bw, based on recoveries of radioactivity in the bile and urine. Between 82% and 96% of the administered dose was excreted within the first 48 h. Regardless of the dose administered, residues remaining in the carcass (including organs and tissues) were between 0.31% and 0.62% of the administered dose after 7 days. The highest concentrations were found in the liver (0.009–0.72 µg equivalents/g) and in the kidneys (0.023–1.12 µg equivalents/g) at 7 days. No significant quantities of radiolabel were detected in exhaled air. In a study of biliary excretion, about 57–74% of the administered dose was recovered in the bile within 48 h after administration of a single dose at 100 mg/kg bw by gavage. No parent compound was detected in the bile.

Systemically absorbed azoxystrobin was extensively metabolized. The mass balance for the metabolism study indicated that a substantial percentage (45.6–73.6%) of the radiolabel was unextracted, although the excretion studies showed a total recovery of 91.8–104%, with 72.6–89.3% being in the faeces. Fifteen metabolites were identified in the excreta and seven additional metabolites were detected but not identified (< 4.9% of the administered dose). The major metabolites of azoxystrobin in the bile, urine and faeces resulted from hydrolysis followed by glucuronide conjugation. Azoxystrobin was also hydroxylated at the 8 and 10 positions on the cyanophenyl ring, followed by glucuronide conjugation. A minor pathway involving the cleavage of the ether linkage was identified. Approximately 15–32% of the unchanged azoxystrobin was detected in the faeces of bile-duct cannulated rats and rats at the highest dose. Absorption, distribution, excretion and metabolite profiles were essentially similar in males and females, but sex-specific differences in biotransformation were observed, with the number of metabolites produced being greater in females than in males.

Toxicological data

Azoxystrobin has low acute toxicity when administered by the oral, dermal or inhalation routes. The LD_{50} in rats treated orally was > 5000 mg/kg bw. The LD_{50} in rats treated dermally was > 2000 mg/kg bw. The LC_{50} in rats treated by inhalation (nose only) was 0.7 mg/l. Azoxystrobin was slightly irritating to the eyes and skin of rabbits. Azoxystrobin was not a skin sensitizer as determined by the Magnusson & Kligman (maximization) test in guinea-pigs.

In short-term studies in rats and dogs and long-term studies in mice and rats, the major toxicological findings included decreased body weight and body-weight gains, often accompanied by decreased food consumption and utilization. The major target organs in rats were the liver, kidney and bile duct as shown by changes in organ weights, histopathology, and clinical chemistry parameters. Changes in liver weights, often accompanied by changes in clinical chemistry, were also observed in dogs and mice. Kidney-weight changes in mice were not accompanied by any histopathological findings.

In a 90-day dietary study of toxicity in rats, decreased body weights and body-weight gains were seen at 2000 ppm (equal to 221.0 mg/kg bw per day) and 4000 ppm (equal to 443.8 mg/kg bw per day). At 4000 ppm, decreased food consumption, food utilization, changes in clinical chemistry parameters, increased liver and kidney weights, hepatocellular hyperplasia and enlarged lymph nodes, and reduction in total urinary protein were seen in males. The no-observed-adverse-effect level (NOAEL) was 200 ppm, equal to 20.4 mg/kg bw per day.

In a 90-day and a 1-year study in dogs, clinical observations included increased salivation at dosing, and increased incidences of salivation, vomiting, regurgitation and fluid faeces, beginning in week 1 and occurring throughout the study in some cases. These signs were considered to be treatment-related; however, they were not considered to be relevant for identification of a NOAEL for systemic toxicity because these effects were secondary to local gastrointestinal irritation/disturbances and bolus dosing (capsules). In a 90-day study of toxicity in dogs, decreases in body weights were observed in males and females at 250 mg/kg bw per day, the highest dose tested. Changes in liver weights and in clinical chemistry parameters were observed at the intermediate and the highest dose, indicating adverse effects on the liver and possibly on biliary function. The changes in the liver at 50 mg/kg bw per day were small and without histological correlates, therefore, the Meeting considered that they were not toxicologically relevant. In a 90-day study in dogs, the NOAEL was 50 mg/kg bw per day on the basis of alterations in clinical chemistry (cholesterol, triglycerides and alkaline phosphatase activity), and decreases in body weights seen at the LOAEL of 250 mg/kg bw per day, the highest dose tested. Similar findings were observed in a 1-year study of toxicity in dogs but were mainly confined to the highest dose of 200 mg/kg bw per day. In a 1-year study of toxicity in dogs, the NOAEL was 25 mg/kg bw per day on the basis of changes in clinical chemistry and increases in liver weights seen at 200 mg/kg bw per day. The overall NOAEL in dogs was 50 mg/kg bw per day on the basis of the similarity of effects in the two studies in dogs.

The carcinogenic potential of azoxystrobin was studied in mice and rats. In a study of carcinogenicity in mice, reduced body weights were observed at 2000 ppm, equal to 272.4 mg/kg bw per day. The NOAEL was 300 ppm, equal to 37.5 mg/kg bw per day. There were no treatment-related neoplastic findings in the bioassay in mice.

In a long-term combined study of toxicity and carcinogenicity in rats, the highest dose of 1500 ppm, equal to 108.6 mg/kg bw per day, was excessively toxic in males and was reduced to 750 ppm, equal to 34 mg/kg bw per day, after 1 year. Reduced body weights, food consumption, and food-conversion efficiency was observed in males and females at the highest dose tested. In the common bile duct of males at the highest dose only, there were significant increases in the incidences of distension, cholangitis, thickening of the wall, and epithelial hyperplasia. The NOAEL was 300 ppm, equal to 18.2 mg/kg bw per day. There were no treatment-related neoplastic findings in rats.

Azoxystrobin gave a mixed response in a battery of tests for genotoxicity. It gave a weak positive response in two studies in mammalian cells (mouse lymphoma cells and human lymphocytes). The latter findings suggest that azoxystrobin has a clastogenic potential in vitro since the increased occurrence of small colonies observed in the mouse lymphoma-cell assay is considered to be indicative of chromosome aberrations rather than of point mutations. However, azoxystrobin has been shown to give negative results in assays for chromosomal damage in vivo (i.e., clastogenicity) and for general DNA damage at high doses of 2000 mg/kg bw or above. Therefore, the Meeting concluded that the clastogenic effects seen in vitro are not expressed in the whole animal.

The Meeting concluded that azoxystrobin is unlikely to be genotoxic.

In view of the lack of evidence for a genotoxic potential in vivo and the absence of carcinogenicity in rats and mice, the Meeting concluded that azoxystrobin is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, reproductive parameters were not affected at the highest dose tested (1500 ppm, equal to 165.4 mg/kg bw per day). The NOAEL for parental systemic toxicity was 300 ppm, equal to 32.3 mg/kg bw per day, on the basis of reduced adjusted body weight, feed consumption, feed utilization, and an increase in liver weights and the frequency of histopathological findings in the liver (males only). Offspring toxicity was manifested as a decrease in pup body weights, and a decrease in adjusted mean liver weights was observed in pups of both generations at 1500 ppm, equal to 165.4 mg/kg bw per day. The NOAEL for offspring toxicity was 300 ppm, equal to 32.3 mg/kg bw per day.

In a study of developmental toxicity in rats, treatment at the highest dose (300 mg/kg bw per day) was terminated as this dose was toxic; at this dose, three rats died and one was killed in extremis after two doses. Clinical signs of diarrhoea, salivation and urinary incontinence were seen at 25 and/or at 100 mg/kg bw per day. The Meeting considered these effects to be treatment-related but not relevant for the identification of a NOAEL for systemic toxicity, being considered to be secondary to local gastrointestinal irritation/disturbances and dosing by gavage. There were no effects on fetuses at any doses tested. The NOAEL for maternal and developmental toxicity was 100 mg/kg bw per day, the highest dose tested.

Two studies of developmental toxicity in rabbits were available. The results of the first study were considered to be invalid because of the adverse effects of administration of high volumes of corn oil as a vehicle. Several special studies were conducted in pregnant and non-pregnant rabbits to evaluate the influence of the type and volume of vehicle used for administration by gavage. The results of these studies showed that corn oil at volumes greater than 2 ml/kg bw was harmful. The NOAEL for maternal toxicity in rabbits was 150 mg/kg bw per day (identified in the study using the lowest volume of corn oil for dosing) on the basis of decreased body-weight gain seen at the LOAEL of 500 mg/kg bw per day. There were no effects on fetuses. The NOAEL for developmental toxicity in rabbits was 500 mg/kg bw per day, the highest dose tested.

Azoxystrobin was not embryotoxic, fetotoxic or teratogenic at doses of up to 300 and 500 mg/kg bw per day in rats and rabbits, respectively.

The Meeting concluded that azoxystrobin is not teratogenic.

In a study of acute neurotoxicity in rats, no treatment-related effects on motor activity parameters, brain measurements (weight, length and width) or neurohistopathology were observed at doses of up to and including 2000 mg/kg bw. Increased incidences of transient diarrhoea, tip-toe gait, hunched posture and landing-foot splay were observed in all groups receiving azoxystrobin, although these effects were not dose-related. They were considered to be treatment-related but not relevant for identification of a NOAEL for systemic toxicity, being considered to be secondary to local gastrointestinal irritation/disturbances and bolus dosing by gavage. The NOAEL for systemic toxicity was 2000 mg/kg bw, the highest dose tested. In a short-term study of neurotoxicity in rats, no treatment-related changes in mortality, clinical signs, FOB, motor activity, brain measurements (weight, length, and width), gross necropsy, or neurohistopathology were observed at doses of up to 2000 ppm, equal to 161 mg/kg bw per day, the highest dose tested. The NOAEL for systemic toxicity was 500 ppm, equal to 38.5 mg/kg bw per day, on the basis of decreased body weight and body-weight gain and food utilization in males and females seen at the LOAEL of 2000 ppm, equal to 161 mg/kg bw per day.

Azoxystrobin was not considered to be neurotoxic on the basis of the available data.

No significant adverse effects were reported in personnel working in plants producing azoxystrobin.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.2 mg/kg bw based on a NOAEL of 300 ppm (equal to 18.2 mg/kg bw per day) in a 2-year study of carcinogenicity in rats, identified on the basis of reduced body weights, food consumption and food efficiency, and bile-duct lesions seen at 750 ppm (equal to 34 mg/kg bw per day) and above, and using a safety factor of 100.

The Meeting concluded that it was unnecessary to establish an ARfD for azoxystrobin because no toxicity could be attributable to a single exposure in the available database, including a study of developmental toxicity in rats and rabbits and a study of acute neurotoxicity in rats. The mortality seen in the study of developmental toxicity in pregnant rats at 300 mg/kg bw per day was associated with gross local gastrointestinal pathology and was not seen in pregnant rabbits. The Meeting considered that clinical signs observed in dogs and rats were related to local gastrointestinal effects seen after bolus dosing by gavage in rats or bolus dosing (capsules) in dogs, since these signs were not seen in the dietary studies. Therefore, the Meeting considered that these effects were not relevant for the establishment of an ARfD.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	104-week study of toxicity and carcinogenicity[a]	Toxicity	300 ppm, equal to 37.5 mg/kg bw per day	2000 ppm, equal to 272.4 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 272.4 mg/kg bw per day[c]	—

Species	Study	Effect	NOAEL	LOAEL
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	300 ppm, equal to 18.2 mg/kg bw per day	750 ppm, equal to 34 mg/kg bw per day[c]
		Carcinogenicity	750 ppm, equal to 34 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	300 ppm, equal to 32.3 mg/kg bw per day	1500 ppm, equal to 165.4 mg/kg bw per day[c]
		Offspring toxicity	300 ppm equal to 32.3 mg/kg bw per day	1500 ppm, equal to 165.4 mg/kg bw per day[c]
	Developmental toxicity[b]	Maternal toxicity	100 mg/kg bw per day[c]	—
		Embryo and fetal toxicity	100 mg/kg bw per day[c]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	150 mg/kg bw per day	500 mg/kg bw per day[c]
		Embryo and fetal toxicity	500 mg/kg bw per day[c]	—

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.2 mg/kg bw per day

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 exposure

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

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid and nearly complete absorption
Distribution	Widely distributed in tissues
Potential for accumulation	Low, no evidence of significant accumulation
Rate and extent of excretion	Approximately 82–90% (73–89% in faeces and 9–18% in urine) within 48 h
Metabolism in animals	Extensive; metabolic pathways include hydrolysis followed by glucuronide conjugation and minor pathway included cleavage of the ether
Toxicologically significant compounds (animals, plants and environment)	Azoxystrobin

Acute toxicity

Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	0.7 mg/l , dust (4 h exposure, nose only)
Rabbit, dermal irritation	Slight irritation
Rabbit, ocular irritation	Slight irritation
Guinea-pig, dermal sensitization	Not a sensitizer (Magnusson & Kligman test)

Short-term studies of toxicity

Target/critical effect	Body-weight effects
Lowest relevant oral NOAEL	20.4 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day; highest dose tested
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	Unlikely to be genotoxic

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Liver-weight increases and bile-duct lesions
Lowest relevant NOAEL	18.2 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic in mice and rats

Reproductive toxicity

Reproduction target/critical effect	No toxicologically relevant effects
Lowest relevant reproductive NOAEL	165.4 mg/kg bw per day (rats; highest dose tested)
Developmental target/critical effect	No developmental toxicity in rats and rabbits
Lowest relevant developmental NOAEL	100 mg/kg bw per day (rats; highest dose tested)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity	No sign of specific neurotoxicity

Mechanistic data

	No studies were submitted

Medical data

	No significant adverse health effects reported

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.2 mg/kg bw per day	Rat, 2-year study of toxicity	100
ARfD	Unnecessary	—	—

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