CHLOROTHALONIL METABOLITE R611965 (addendum)

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

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

Explanation 3
Evaluation for acceptable daily intake 4
1. Biochemical aspects 4
1.1 Absorption, distribution and excretion 4
2. Toxicological studies 4
2.1 Acute toxicity 4
2.2 Short-term studies of toxicity 4
2.3 Long-term studies of toxicity and carcinogenicity 8
2.4 Reproductive toxicity 9
(a) Multigeneration studies 9
(b) Developmental toxicity 10
2.5 Genotoxicity 12
Comments 12
Toxicological evaluation 14
References 16

Explanation

R611965 (3-carbamyl-2,4,5-trichlorobenzoic acid, formerly known as SDS-46851) is a chlorothalonil metabolite that is formed in the soil and taken up through the roots by crops. The present Meeting evaluated R611965 for the first time.

Chlorothalonil (Chemical Abstracts Service [CAS] No. 1897-45-6) and SDS-3701, a chlorothalonil metabolite that is found in plants, soil and ruminants, were last evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2009.

The present Meeting evaluated the newly submitted studies on R611965 at the request of the 2009 JMPR, to address the toxicological relevance of this soil degradation product.

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

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

A single dose of ^{14}C-ring-labelled R611965 (radiochemical purity 98.6%) and unlabelled R611965 (purity 99.3%; Lot No. 0207) was administered to Sprague-Dawley (Crl:CD Br VAF/Plus) rats (five of each sex per dose) at 10 or 1000 mg/kg body weight (bw). Two males and two females received vehicle only (0.75% methylcellulose). Expired air was collected at 6, 12 and 24 hours after dosing. Urine and faeces were collected at 6 (urine only), 12 and 24 hours and daily thereafter. After 7 days, the animals were killed, and levels of radioactivity were determined in a range of organs and tissues. Statements of adherence to quality assurance (QA) and GLP were included.

Average recovery ranged from 91% to 99%. Following administration, 90% of the administered dose was excreted within 48 hours (10 mg/kg bw) or 96 hours (1000 mg/kg bw). At both doses, the major route of excretion was faeces (68–77% of the dose), with 16–27% being excreted in urine. Less than 0.02% of the administered dose was expired, and after 7 days, less than 0.3% of the dose was recovered from tissues and carcass. At termination, highest concentrations were found in liver (0.02–0.05% of the dose). No obvious sex differences were observed (Ho, Marciniszyn & Killeen, 1990).

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with R611965 are summarized in Table 1.

2.2 Short-term studies of toxicity

Mice

In a range-finding study, Crl:CD-1(ICR) VAF/Plus mice (five of each sex per dose) were fed diets containing R611965 (purity 99.3%; Lot No. 0207) at 0, 250, 500, 1000, 5000 or 10 000 parts per million (ppm), equal to 0, 46, 96, 188, 963 and 2028 mg/kg bw per day in males and 0, 54, 108, 217, 1023 and 2112 mg/kg bw per day in females, for 28 days. Clinical observations were made daily, and a complete physical examination was performed weekly. Body weights and feed consumption were measured weekly. At termination after 28 days, haematology was performed, and the animals were necropsied. Selected organs were weighed, and lungs, liver, kidneys, testes and ovaries were examined histologically. Statements of adherence to QA and GLP were included.

The only finding in this study was slight to mild hyperplasia of the renal tubule epithelium in males at 250 (1/5), 1000 (1/5) and 10 000 ppm (2/5), but not at 500 or 5000 ppm. The severity of the lesion did not increase with dose, and there was no dose–response relationship. Moreover, in an 18-month dietary study in mice of the same strain, using 60 animals of each sex per dose, no treatment-related effects on kidneys were found at doses up to about 1000 mg/kg bw per day (Lucas & Laveglia, 1994). Therefore, it is concluded that the slight to mild hyperplasia observed in the present study is likely to be an incidental finding. Other parameters were also not affected by treatment (Mizens & Killeen, 1991).

Table 1. Results of studies of acute toxicity with R611965

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw)	Reference
Rat	Sprague-Dawley	Male/female	Oral	0.5% methylcellulose	~95	> 5000	Shults & Killeen (1985)[a,b]

LD_{50}, median lethal dose

[a] Statements of adherence to GLP and QA were included.

[b] Only two animals of each sex per dose were used. Soft stool and mucus in the stool were noted in two rats dosed at 1000 mg/kg bw. Soft stool, mucus and tan-coloured particles in the stool were noted in all rats dosed at 5000 mg/kg bw. One rat dosed at 5000 mg/kg bw also exhibited anogenital staining. Red nasal discharge and/or chromodacryorrhoea were observed in one male on day 7 and in one female on day 10. There was no effect on body weight, and no compound-related gross pathological changes were noted.

In a 90-day dietary study, Crl:CD-1 (ICR) VAF/Plus mice (10 of each sex per dose) received diets containing R611965 (purity 99.3%; Lot No. 0207) at 0, 250, 750, 2200 or 7500 ppm, equal to 0, 41, 122, 368 and 1270 mg/kg bw per day for males and 0, 47, 145, 456 and 1532 mg/kg bw per day for females. The animals were observed daily for mortality and clinical signs and were given a detailed physical examination weekly. Body weights and feed consumption were measured weekly. At the end of the treatment period, blood was collected for haematology. At the end of the scheduled period, the animals were killed and necropsied. Brain, liver, kidneys and testes were weighed. An extensive range of tissues was examined histologically. Statements of adherence to QA and GLP were included.

One low-dose moribund female was killed. The poor physical state of the animal was considered not related to treatment. There were no treatment-related effects on clinical signs, body weight, body weight gain, feed consumption, organ weights or macroscopic abnormalities. Isolated statistically significant differences in haematological parameters between control and treated groups were not dependent on dose and were considered not treatment related. Slight to moderate renal tubular epithelial hyperplasia was observed in some male mice in the 250 (1/10), 750 (2/10) and 7500 ppm (4/10) dose groups, but not in controls or at 2200 ppm. There was no evidence of a dose–response relationship for this effect or an increase in the severity of the lesion with increasing dose. Given that no treatment-related effects on kidneys were found at doses up to about 1000 mg/kg bw per day in an 18-month dietary study in mice of the same strain, using 60 animals of each sex per dose (Lucas & Laveglia, 1994), it is concluded that the renal hyperplasia observed in the present study is likely to be an incidental finding.

The no-observed-adverse-effect level (NOAEL) was 7500 ppm, equal to 1270 mg/kg bw per day, the highest dose tested (Fillmore, Mizens & Killeen, 1991).

Rats

In a range-finding study, CD Sprague-Dawley rats (five of each sex per dose) were fed diets containing R611965 (purity > 94%; Lot No. 0202) at concentrations of 0, 125, 250, 500, 1000 or 2000 mg/kg bw per day (the dietary concentrations were adjusted based on body weight and feed consumption to maintain the target doses) for at least 14 days. Clinical observations (daily), complete physical examinations, body weights and feed consumption (weekly), haematology, clinical chemistry and urinalysis (at termination) were assessed. At the end of the scheduled period, the animals were killed and subjected to a full postmortem examination. Selected organs were weighed, and a limited range of specified tissues was taken for subsequent histopathological examination. Statements of adherence to QA and GLP were included.

The animals consumed between 83% and 90% of the target doses of R611965. There were no treatment-related effects on mortality, clinical findings, body weight, body weight gain, feed consumption, haematology, urinalysis or organ weights, and there were no macroscopic or histopathological abnormalities. Isolated statistically significant differences, observed in some clinical chemistry

parameters at 125 and 250 mg/kg bw per day, were small and not observed at higher doses and were considered not to be treatment related (Wilson, Sadler & Killeen, 1985).

CD Sprague-Dawley rats (five of each sex per dose) received R611965 (purity > 94%; Lot No. 0202) in the diet at 0, 500 or 2000 mg/kg bw per day (the dietary concentrations were adjusted based on body weight and feed consumption to maintain the target doses) for at least 30 days. Clinical observations (daily), complete physical examinations, body weights and feed consumption (weekly), haematology, clinical chemistry and urinalysis (at termination) were assessed. At the end of the scheduled period, the animals were killed and subjected to a full postmortem examination. Selected organs were weighed, and an extensive range of tissues was examined histologically. Statements of adherence to QA and GLP were included.

The animals consumed between 92% and 105% of the target doses of R611965. There were no treatment-related effects on mortality, clinical findings, body weight, body weight gain, feed consumption, haematology, clinical chemistry, urinalysis or organ weights, and there were no macroscopic abnormalities. Relative liver weights were increased in both sexes at 500 mg/kg bw per day (10–13%, statistically significant for females) and 2000 mg/kg bw per day (17–18%, statistically significant in both sexes). It was noted, however, that the relative liver weights of controls were below the historical control range and that only the relative liver weights of males at 2000 mg/kg bw per day exceeded the historical control range. Very slight to slight centrilobular hypertrophy was seen in all high-dose animals and in one male at 500 mg/kg bw per day. In the absence of histopathological damage and relevant clinical chemistry changes, the increased relative liver weight and hepatocellular hypertrophy should not be considered adverse effects.[1]

The NOAEL was 2000 mg/kg bw per day, the highest dose tested (Wilson & Ignatoski, 1986).

A combined 90-day toxicity and single-generation reproductive toxicity study with R611965 was performed in which 35 rats of each sex per dose were given feed containing R611965. Twenty-five rats of each sex per dose were used to study the reproductive effects of R611965. The findings of the 90-day study are summarized here. Sprague-Dawley rats (10 of each sex per dose) received R611965 (purity > 99%; Lot No. 46851-0204) in the diet at 0, 250, 750 or 2000 mg/kg bw per day (the dietary concentrations were adjusted based on body weight and feed consumption to maintain the target doses) for a duration of 90 days. The animals were subjected to clinical observations (daily), physical examinations, body weight and feed consumption measurements (weekly), haematology and clinical chemistry (weeks 6 and 13), urinalysis (weeks 5 and 12) and ophthalmoscopy (day 75). At the end of the scheduled period, the animals were killed and necropsied. Selected organs were weighed, and an extensive range of tissues was examined histologically. Statements of adherence to QA and GLP were included.

From week 7 of treatment onward, occasional soft stools were observed in animals fed 2000 mg/kg bw per day, more commonly in males than in females. However, induction of soft stools by R611965 was not reported in a 2-year study in rats (Killeen, Laveglia & Serrone, 1993) and in studies of reproductive toxicity (Serrone & Killeen, 1988; Lucas & Killeen, 1993), and therefore these findings are considered not toxicologically significant. An increase (19%) in prothrombin levels was seen in high-dose males at week 6, but not at week 13. At the high dose, increases in blood glucose in males (29%) and potassium in females (9%) and a decrease in alanine aminotransferase (ALT) activity in females (21%) were significantly different from control at week 6, but not at week 13. These findings in blood are therefore considered fortuitous and not related to treatment. No toxicologically

[1] Guidance on the interpretation of hepatocellular hypertrophy, General consideration 2.7 in Annex 1, reference 107.

relevant macroscopic or microscopic findings were observed. Statistically significant increases in relative liver weights (9–10%) and relative kidney weights (10–12%) in mid- and high-dose males, in relative liver weights (10%) in high-dose females and in relative adrenal weights (20–27%) in females of all dose groups were observed. These weight changes were not accompanied by histological changes. In the absence of associated histopathological findings, these changes were considered not to be adverse.

The NOAEL was 2000 mg/kg bw per day, the highest dose tested (Serrone & Killeen, 1988).

Dogs

In a preliminary feeding study, R611965 (purity 98%; Lot No. 0202) was administered by capsule to Beagle dogs (two of each sex per dose) at 0, 100, 500 or 1000 mg/kg bw per day for up to 38 days. All animals were observed daily for mortality and clinical signs. Detailed physical examinations were conducted weekly. Body weight was measured weekly, and feed consumption was measured daily. Ophthalmoscopy was performed prior to the start of the study and prior to termination. Blood and urine samples were collected pretest and at the end of the treatment period for haematology, clinical chemistry and urinalysis. At termination, the dogs were killed and subjected to gross examinations. Selected organs were weighed. An extensive range of organs was examined histologically. Statements of adherence to QA and GLP were included.

There were no mortalities. At 1000 mg/kg bw per day, one male and one female were lethargic during week 5, with red faeces and red emesis seen in the female and tarry faeces seen in the male. Both of these animals, in particular the male, lost weight during the study. The male dog showed an increased haemoglobin, haematocrit and total erythrocyte count and low sodium, potassium and chloride levels, indicative of a dehydrated condition and a disturbed electrolyte balance. One mid-dose male showed body weight loss, and one mid-dose female had a low weight gain that was outside the normal limits of variability. Overall, the feed consumption coincided with the body weight pattern of the dogs. In high-dose dogs, urinary pH was decreased from around 8.0 in controls to 6.0. Reductions in absolute (60%) and relative (38%) testes weights in the two high-dose dogs were found. Relative liver weight was increased in all dogs at 1000 mg/kg bw per day (43–61%) and in one dog of each sex at 500 mg/kg bw per day (31% and 75%). Microscopic examination showed a dose-related centrilobular to diffuse hypertrophy in the livers of all treated dogs. The testes of both dogs at 1000 mg/kg bw per day showed diffuse hypoplasia of the seminiferous tubules with aspermatogenesis. Both dogs at 500 mg/kg bw per day had reduced spermatogenesis compared with controls. The prostates of both dogs at 1000 mg/kg bw per day had moderately severe diffuse hypoplasia. The prostates of both dogs at 500 mg/kg bw per day and of one dog at 100 mg/kg bw per day had minimal to slight diffuse hypoplasia. At 1000 mg/kg bw per day, the ovaries and uterus of the female dog that lost body weight during the study had moderately severe diffuse hypoplasia. It is unclear whether these effects were related to treatment or were a secondary consequence of body weight loss (Serrone & Killeen, 1989a).

In a 90-day study, performed in accordance with European Commission guideline 87/302/EEC B.27, which resembles Organisation for Economic Co-operation and Development (OECD) guideline 409, groups of four male and four female Beagle dogs were dosed daily via gelatine capsules with R611965 (purity > 99%; Lot No. 0207) at 0, 5, 15, 50 or 500 mg/kg bw per day. All animals were observed daily for mortality and clinical signs. Detailed physical examinations were conducted weekly. Body weight was measured weekly, and feed consumption was measured daily. Ophthalmoscopy was performed prior to the start of the study and prior to termination. Blood and urine samples were collected pretest, after about 45 days of treatment and at the end of the treatment period for haematology, clinical chemistry and urinalysis. At termination, the dogs were killed and subjected to gross examinations. Selected organs were weighed. An extensive range of organs was examined histologically. Statements of adherence to QA and GLP were included.

There were no mortalities. Increased incidences of watery stools were seen in both sexes at 500 mg/kg bw per day (observed in four of four males and females, 88 observations, compared with three of four males and females in the control group, 25 observations). At the high dose, watery stools were first observed at day 9 of treatment. Body weight gain was reduced in males at the top dose to 1.6 kg (0.5–3.2 kg) compared with 3.2 kg (2.2–3.9 kg) in controls (body weight gain in three high-dose males was outside the control range), but no such effect was observed in females. The reduction in body weight gain in the three high-dose males was most marked during the first 6 weeks of treatment. Feed consumption and haematological and urinalysis parameters were not affected by treatment. Relative liver weights were increased in males and females at the high dose by 28% and 23%, respectively, reaching statistical significance in males only. Absolute liver weights at this dose were increased by 10% and 23% (not statistically significant), respectively. In the absence of changes in clinical chemistry parameters and microscopic changes, the increased liver weights are considered an adaptive response.[1] Increases in glucose levels were observed at doses of 15 mg/kg bw per day and higher at week 8, but not at week 13. The differences were small, not always dose dependent and reported to be within the historical control range (data not shown), and they are not considered toxicologically relevant. No treatment-related findings on ophthalmoscopy or macroscopic and microscopic examination were reported. The effects on the testes reported in the preliminary dog study were not confirmed.

The NOAEL was 50 mg/kg bw per day, based on reduced body weight gain in males and watery stools in both sexes at 500 mg/kg bw per day (Serrone & Killeen, 1990).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In an 18-month carcinogenicity feeding study, performed in accordance with European Commission guideline 87/302/EEC, which resembles OECD guideline 451, groups of Crl:CD-1 (ICR) BR VAF/Plus mice (60 of each sex per dose) were fed diets containing R611965 (purity 99.3%; Lot No. 0207) at 0, 1000, 3500 or 7000 ppm. Based on the lowest measured weekly compound intake, this is equal to 0, 136, 488 and 1022 mg/kg bw per day in males and 0, 155, 566 and 1123 mg/kg bw per day in females. Animals were checked twice daily for clinical signs, and a detailed clinical examination was performed weekly. Body weights and feed consumption were recorded weekly for the first 13 weeks and every other week thereafter. Differential leukocyte counts were conducted in 10 mice of each sex of the control and high-dose groups at 12 months and at termination. At termination, gross examination was performed on all animals. Brain, liver, kidneys and testes of 10 animals of each sex per dose were weighed. An extensive number of tissues were examined microscopically. Statements of adherence to QA and GLP were included.

No treatment-related effects on mortality or body weight gain were observed. The observed slight increases in aggression, activity and anogenital staining in high-dose males might be treatment related. Feed consumption in the high-dose group tended to be higher than in controls, which may reflect the lower nutritional value due to the high concentration of R611965. No effects of treatment on differential leukocyte count, necropsy, organ weights or histology (including neoplastic changes) were observed.

The NOAEL was 1022 mg/kg bw per day, the highest dose tested (Lucas & Laveglia, 1994).

Rats

In a combined chronic toxicity and carcinogenicity feeding study, R611965 (purity 99.3%; Lot No. 0207) was administered in the diet to Sprague-Dawley (Crl CD VAF/Plus) rats (60 rats of each sex per dose) for 105 weeks. Concentrations of R611965 in the diet were adjusted based on body

[1] Guidance on the interpretation of hepatocellular hypertrophy, General consideration 2.7 in Annex 1, reference *107*.

weight and feed consumption data in order to achieve desired intakes of 0, 80, 200, 500 and 1000 mg/kg bw per day. The animals were checked daily for clinical signs. The physical condition of the animals was monitored weekly. Body weight and feed consumption were measured weekly for the first 13 weeks and once every month thereafter. Ophthalmoscopy was performed prior to treatment and at months 12, 18 and 24. For haematological and clinical chemistry examinations and urinalysis, blood and urine samples were collected from 10 animals of each sex per group after 3, 6, 12 and 18 months and at termination. All rats were subjected to gross pathology, and liver, kidneys, brain and testes were weighed. A wide range of tissues was evaluated microscopically. Statements of adherence to QA and GLP were included.

Overall mean compound intake was greater than 95% of target intake for all doses. No treatment-related effects on mortality, clinical signs, ophthalmoscopy, body weight gain or organ weights were observed. Slightly higher feed consumption in treated animals was considered to be compensating for the lower nutritional value of the feed. Incidental findings on haematological, clinical chemistry and urinalysis parameters were considered not treatment related. Histological examination revealed increased incidences of bilateral retinal atrophy. For the 0, 80, 200, 500 and 1000 mg/kg bw per day groups, the incidences out of 60 rats were 1, 1, 2, 3 and 6 for males and 1, 2, 2, 6 and 9 for females, respectively. The increased incidences in high-dose males and in females at 500 and 1000 mg/kg bw per day are considered related to treatment. The incidences at 80 and 200 mg/kg bw per day are considered not to be different from control. In males of all treatment groups, slight, not dose-dependent increases in the incidence of severe chronic progressive nephropathy were observed, which could be related to treatment. However, chronic progressive nephropathy is a common finding in ageing rats that is not considered toxicologically relevant for humans (Hard & Khan, 2004). No other treatment-related histological changes, including neoplastic changes, were seen.

The NOAEL was 200 mg/kg bw per day, based on bilateral retinal atrophy observed at 500 mg/kg bw per day (Killeen, Laveglia & Serrone, 1993).

2.4 Reproductive toxicity

(a) Multigeneration studies

Rats

In a one-generation study of reproductive toxicity, as part of a combined study with a 90-day toxicity study (see section 2.2), Sprague-Dawley rats (25 of each sex per dose) were fed diets containing R611965 (purity > 99%; Lot No. 0204) at 0, 250, 750 or 2000 mg/kg bw per day (the dietary concentrations were adjusted based on body weight and feed consumption to maintain the target doses) during the 14-week premating phase. From the start of the reproduction phase to the end of the lactation phase, males received diets with constant concentrations of 0, 5200, 15 500 or 40 000 ppm in males, and females received diets with concentrations of 0, 4000, 12 000 or 32 000 ppm (i.e. the dietary concentrations fed during week 13 of the premating phase). The parental clinical condition, body weights and feed consumption were monitored throughout the treatment period. Mating, fertility and gestation indices, length of gestation, number of implants, number of live and dead pups, sex of pups, pup weights and pup abnormalities were recorded. At postnatal day (PND) 4, litters were culled to 10 pups. At the end of the scheduled period, the animals were killed and necropsied. Statements of adherence to QA and GLP were included.

In the parental animals, no effects of treatment on clinical signs, body weights, feed consumption, fertility or reproductive performance, or necropsy were observed. At PND 21, high-dose pups showed a small (9%) reduction in body weight compared with controls. As pups will have started eating test diet, this may be the result of the decreased nutritional value of the food as a result of the high test compound concentration. Moreover, this finding was not observed in the two-generation study of

reproductive toxicity (see below). Therefore, the slight reduction in body weight in high-dose pups is not considered toxicologically relevant. No other effects on pups were observed.

The NOAEL for parental, reproductive and offspring toxicity was 2000 mg/kg bw per day, the highest dose tested (Serrone & Killeen, 1988).

In a dietary two-generation study of reproductive toxicity, performed in accordance with European Commission guideline 87/302/EEC B.35, which resembles OECD guideline 416, R611965 (purity 99.3%; Lot No. 0207) was administered to Sprague-Dawley (CD-VAF/Plus) rats (35 rats of each sex per dose) at dietary concentrations of 0, 2000, 6000 or 20 000 ppm. Based on the lowest weekly compound intake, this is equal to 0, 93, 276 and 943 mg/kg bw per day in males and 0, 121, 370 and 1246 mg/kg bw per day in females of the P generation and 0, 88, 269 and 911 mg/kg bw per day in males and 0, 131, 390 and 1323 mg/kg bw per day in females of the F_1 generation. The rats were observed daily for clinical signs. Detailed physical examinations were performed weekly for all males throughout the study and for all females prior to mating and during the resting week in between weaning of the F_{1a} generation and mating for the F_{1b} generation. Body weights and feed consumption were recorded weekly in males, except during the mating periods. In females, body weights and feed consumption were measured weekly prior to mating and during the resting periods. Body weights were also recorded on gestation days (GDs) 0, 7, 14 and 20 and PNDs 0, 7, 14 and 21 for the P and F_1 females. On PND 4, litters were culled to eight pups. Until weaning at PND 21, litters were examined for number of live and dead pups, litter weight, pup weight and sex, clinical signs and external alterations. After the F_{1b} or F_{2b} litter production, all P and F_1 parents were subjected to gross pathology. The reproductive organs, liver, kidneys, lungs and all gross lesions of all P parents of all dose groups and F_1 parents of the control and high-dose groups were examined histologically. In addition, kidneys, stomachs and gross lesions of parental animals of the low- and mid-dose groups, the epididymis, prostate and seminal vesicles of the P adults and the testes of the F_1 adults were examined. Statements of adherence to QA and GLP were included.

In parental animals (P and F_1), there were no treatment-related effects on mortality, clinical signs, body weight, feed consumption, reproductive parameters or macroscopic and microscopic parameters. In mid-dose and high-dose P females, an increase in incidence, but not severity, of regenerative tubular epithelium was observed, which was statistically significant for high-dose females only. This increase was not found in F_1 females or in P or F_1 males. It was noted that the incidence in females of the P control group may have been unusually low. It is concluded that the kidney findings in the P females were not treatment related. In the offspring, no treatment-related effects on any of the evaluated parameters were noted.

The NOAEL for parental, reproductive and offspring toxicity was 20 000 ppm, equal to 911 mg/kg bw per day, the highest dose tested (Lucas & Killeen, 1993).

(b) Developmental toxicity

Rats

In a developmental toxicity study performed in accordance with European Commission guideline 87/302/EEC B.31, which resembles OECD guideline 414, R611965 (purity 99%; Lot No. 0204) was administered in 1% aqueous methylcellulose by oral gavage to pregnant Sprague-Dawley rats (25 per dose group) on GDs 6–15 at doses of 0, 500, 1000 or 2000 mg/kg bw per day. Animals were examined daily for clinical signs. A detailed physical examination and body weight measurements were performed on GDs 0, 6, 9, 12, 16 and 20. Feed consumption was recorded over the intervals between body weight measurements. At termination on GD 20, numbers of live and dead fetuses, corpora lutea, implantation sites, and early and late resorptions were recorded, live fetuses were weighed and sexed, and external alterations and intrauterine locations were recorded. Approximately one half of the fetuses from each

litter were examined for skeletal alterations. All remaining live fetuses were examined for visceral abnormalities. Dams were necropsied. Statements of adherence to QA and GLP were included.

No effects of treatment on any of the examined maternal parameters were noted. No treatment-related effects on any of the fetal parameters tested were noted.

The NOAEL for maternal and developmental toxicity was 2000 mg/kg bw per day, the highest dose tested.

No teratogenic properties of R611965 were identified (Chun, Wilson & Killeen, 1989).

Rabbits

In a developmental toxicity study performed in accordance with European Commission guideline 87/302/EEC B.31, which resembles OECD guideline 414, R611965 (purity > 99%; Lot No. 0202) was administered in 0.5% aqueous methylcellulose by gavage to pregnant New Zealand White rabbits (20 per dose) on GDs 7–19 at doses of 0, 250, 500 or 1000 mg/kg bw per day. Animals were examined daily for clinical signs. On GDs 0, 7, 13, 16, 19, 24 and 29, a detailed physical examination was performed, and body weights were recorded. Feed consumption was recorded over the intervals between body weight measurements. At termination on GD 29, numbers of live and dead fetuses, fetal resorptions, implantation sites and corpora lutea were recorded. Live fetuses were weighed and sexed, and external/visceral alterations and intrauterine locations were recorded. All live fetuses were subsequently examined for skeletal alterations. Dams were necropsied. Statements of adherence to QA and GLP were included.

In the low-, mid- and high-dose groups, two, one and seven dams were killed, respectively, after showing signs of abortion, compared with none in the control group. Three deaths in the control group and one death in the low-dose group were attributed to gavage error. Two high-dose animals were found dead, and one moribund animal was killed. These deaths were not attributed to a specific pathological finding or dosing error.

Clinical observations are presented in Table 2. Increased incidences of mucus-like faeces, few or no faeces, soft faeces, anorexia and thinness (increases in both number of animals and total number of clinical observations) were found in all treated groups. No historical control data were provided.

Effects on body weight gain are presented in Table 3. During the treatment period (GDs 7–19), the high dose group animals showed a body weight loss (0.27 kg), whereas control animals gained weight (0.11 kg) over the same period. In the low-, mid- and high-dose groups, overall body weight gains (0.16, 0.18 and 0.13 kg, respectively) were lower compared with controls (0.28 kg), although the differences were not statistically significant. Over the entire treatment period, feed consumption of the high-dose group was decreased (1062 g) compared with control (1930 g), low-dose (1956 g) and mid-dose animals (1826 g). At 500 mg/kg bw per day, feed consumption was decreased during GDs 17–20. Necropsy showed gall bladder lesions, enlarged gall bladder, reddened and gaseous intestines, pale liver and foci and erosions of the gastric mucosa in the high-dose group. At 1000 mg/kg bw per day, the number of live fetuses per litter (n = 6.6, 82% of total number of fetuses) and fetal weight (34.7 g) were slightly decreased (not statistically significantly) compared with control (n = 8.5, 98% of total number of fetuses, and 39.2 g, respectively). No other effects were observed upon fetal examination.

A NOAEL for maternal toxicity could not be determined. The lowest-observed-adverse-effect level (LOAEL) is 250 mg/kg bw per day, based on abortions, clinical signs (increased incidences of mucus-like faeces, few or no faeces, soft faeces, anorexia and thinness) and slightly reduced body weight gain.

The NOAEL for fetal toxicity is 500 mg/kg bw per day, based on a decreased number of live fetuses and decreased fetal weight observed at 1000 mg/kg bw per day, in the presence of maternal toxicity.

No teratogenic properties of R611965 were identified (Serrone & Killeen, 1989b).

Table 2. Clinical observations in pregnant rabbits treated from GD 7 to GD 19 with R611965

	Dose (mg/kg bw per day)			
	0	250	500	1000
Found dead or moribund	3[a]	1[a]	0	3
Abortions	0	2	1	7
Mucus-like faeces	0 (0)[b]	0 (0)	3 (6)	13 (39)
Few or no faeces	10 (34)	17 (91)	19 (143)	20 (183)
Soft faeces	2 (6)	6 (20)	6 (17)	16 (65)
Anorexia	0 (0)	6 (35)	10 (56)	18 (153)
Thinness	0 (0)	1 (1)	1 (1)	6 (20)

From Serrone & Killeen (1989b)
[a] Deaths due to gavage error.
[b] Number of animals showing effect out of 20 (total number of days observed).

Table 3. Body weight gain in rabbits during pregnancy

Gestation days	Body weight gain (kg)			
	Dose (mg/kg bw per day)			
	0	250	500	1000
0–7	0.14	0.16	0.14	0.18
7–13	0.02	0.01	0.03	−0.11
13–16	0.05	0.01	0.02	−0.07
16–19	0.04	0.01	−0.03	−0.09
19–24	0.05	0.03	−0.02	−0.07
24–29	−0.03	−0.04	0.01	0.03

From Serrone & Killeen (1989b)

2.5 Genotoxicity

The results of genotoxicity tests with R611965 are summarized in Table 4.

Comments

Biochemical aspects

Following a single oral dose of R611965 at 10 or 1000 mg/kg bw in rats, at least 16–27% is absorbed (based on urinary excretion within 48–96 hours). Excretion is rapid, with 90% being excreted within 48–96 hours, predominantly in faeces (68–77% of the dose). Biliary excretion was not assessed. Seven days after administration, less than 0.3% of the administered dose was found in tissues and carcass; highest levels were observed in liver. No obvious sex differences in kinetics were observed.

Toxicological data

The acute oral toxicity of R611965 in rats is low (LD_{50} > 5000 mg/kg bw). No data on acute dermal or inhalation toxicity, eye or skin irritation or skin sensitization were available.

Repeated-dose toxicity studies showed that R611965 had low oral toxicity in mice, rats and dogs. The overall NOAEL in short-term (28 and 90 days) and chronic studies (18 months) in mice was 1022 mg/kg bw per day, the highest dose tested in an 18-month carcinogenicity study. In rats, the

Table 4. Results of studies on the genotoxicity of R611965[a]

End-point	Test object	Concentration	Purity (%)	Results	Reference[b]
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	39–3900 µg/plate (±S9)[c]	99.4	Negative	Jones & Killeen (1985a)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	100–10 000 µg/plate (±S9)[d]	99.4	Negative	Jones & Killeen (1985b)
Gene mutation	Mouse lymphoma L5178Y TK+/− cells	75–1000 µg/ml (±S9)[e]	≥ 98	Negative	Mizens & Killeen (1988)
Sister chromatid exchange	K1-BH4 cells (Chinese hamster ovary)	200–2000 µg/ml (±S9)[f]	99.4	Negative	Jones & Killeen (1985c)
Unscheduled DNA synthesis	Rat hepatocyte primary cultures	0.008–240 µg/well[f]	99.4	Negative	Jones & Killeen (1985d)
In vivo					
Micronucleus formation	Mouse bone marrow (male and female)	500, 2500 or 5000 mg/kg bw (by gavage)[g]	≥ 92.3	Negative	Mizens, Killeen & Siou (1985)

DNA, deoxyribonucleic acid; S9, 9000 × *g* rat liver supernatant

[a] Positive and negative (solvent) controls were included in all studies.

[b] Statements of adherence to QA and GLP were included for all studies.

[c] Lot No. 0202. The highest dose was selected on the basis of a preliminary assay using TA100 and TA1538, in which slight toxicity was observed at 5000 µg/plate.

[d] Lot No. 0202. S9 homogenate was prepared from kidneys from Aroclor 1254–treated rats.

[e] Lot No. 0202. In a preliminary assay, toxicity was observed at 10 000 µg/ml, but not at doses at or below 1000 µg/ml.

[f] Lot No. 0202.

[g] Lot No. 0202. In a pilot study at 10 000 mg/kg bw, no signs of toxicity were noted. At 20 000 mg/kg bw, diarrhoea was observed. In the main study, two males and one female at 2500 mg/kg bw and one female at 5000 mg/kg bw died. The treatment of mice with the test substance did result in slight, irregular increases in the number of micronuclei in the initial test. The increases were small, within the acceptable solvent control range and not considered indicative of a clastogenic effect. Polychromatic erythrocyte/normochromatic erythrocyte ratio was statistically significantly decreased at 5000 mg/kg bw 72 h after dosing. A repeat test, in which two females at 5000 mg/kg bw died, did not show the test substance–induced micronuclei.

overall NOAEL in short-term (14, 30 and 90 days) and chronic studies (2 years) was 200 mg/kg bw per day, based on bilateral retinal atrophy observed at 500 mg/kg bw per day in a 2-year combined chronic toxicity and carcinogenicity study.

In a 90-day study in dogs, the NOAEL was 50 mg/kg bw per day, based on reduced body weight gain in males and watery stools in both sexes at 500 mg/kg bw per day.

In an 18-month study in mice and a 2-year study in rats, no carcinogenic effects of R611965 were observed. The Meeting concluded that R611965 is not carcinogenic in rodents.

R611965 was tested in an adequate range of studies of genotoxicity in vitro and in vivo. There was no evidence for genotoxicity. The Meeting concluded that R611965 is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that R611965 is unlikely to pose a carcinogenic risk to humans.

In one-generation and two-generation studies of reproductive toxicity with R611965 in rats, the overall NOAEL for parental, reproductive and offspring toxicity was 20 000 ppm, equal to 911 mg/kg bw per day, the highest dose tested.

In a study of developmental toxicity in rats, the NOAEL for maternal and developmental toxicity was 2000 mg/kg bw per day, the highest dose tested. In a study of developmental toxicity in rabbits, a NOAEL for maternal toxicity could not be determined. The LOAEL for maternal toxicity was 250 mg/kg bw per day, the lowest dose tested, on the basis of abortions, clinical signs (increased incidences of few or no faeces, soft faeces, anorexia and thinness) and slightly reduced body weight

gain. The NOAEL for fetal toxicity was 500 mg/kg bw per day, based on a decreased number of live fetuses and decreased fetal weight observed at 1000 mg/kg bw per day.

No neurotoxicity studies with R611965 were available. In acute and repeated-dose oral studies in mice, rats, rabbits and dogs in which R611965 was administered in the diet, by gavage or by capsule, no neurotoxic signs were observed.

No data on R611965 in humans were provided.

The Meeting concluded that the existing database on R611965 was sufficient to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting noted that the soil metabolite R611965 is considerably less toxic than the parent compound chlorothalonil (e.g. NOAELs of 200 versus 1.8 mg/kg bw per day in 2-year rat studies, respectively). R611965 is not acutely toxic by the oral route. The metabolite induced adverse effects in only a few repeated-dose oral toxicity studies in rats and dogs, at levels of 250–500 mg/kg bw per day. In the majority of the repeated-dose studies in rodents, no effects were observed at doses of 911–2000 mg/kg bw per day.

In view of the lower toxicity of the metabolite R611965 in comparison with the parent compound chlorothalonil (acceptable daily intake [ADI] = 0–0.02 mg/kg bw; acute reference dose [ARfD] = 0.6 mg/kg bw), the Meeting considered it unnecessary to derive a separate ADI and ARfD for this metabolite for risk management purposes.

Levels relevant for risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of carcinogenicity	Carcinogenicity	1022 mg/kg bw per day[a]	—
Rat	Two-year study of toxicity and carcinogenicity[b]	Toxicity	200 mg/kg bw per day	500 mg/kg bw per day
		Carcinogenicity	1000 mg/kg bw per day[a]	—
	One- and two-generation studies of reproductive toxicity[b]	Parental toxicity	20 000 ppm, equal to 911 mg/kg bw per day[a]	—
		Offspring toxicity	20 000 ppm, equal to 911 mg/kg bw per day[a]	—
		Reproductive toxicity	20 000 ppm, equal to 911 mg/kg bw per day[a]	—
	Developmental toxicity[c]	Maternal toxicity	2000 mg/kg bw per day[a]	—
		Fetotoxicity	2000 mg/kg bw per day[a]	—
Rabbit	Developmental toxicity[c]	Maternal toxicity	—	250 mg/kg bw per day[d]
		Fetotoxicity	500 mg/kg bw per day	1000 mg/kg bw per day
Dog	Ninety-day study[e]	Toxicity	50 mg/kg bw per day	500 mg/kg bw per day

[a] Highest dose tested.
[b] Dietary administration.
[c] Gavage administration.
[d] Lowest dose tested.
[e] Capsule administration.

Estimate of acceptable daily intake for humans

Unnecessary

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 exposures in humans

Critical end-points for setting guidance values for exposure to chlorothalonil metabolite R611965

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of absorption	Rapid; at least 16–27% at 10 and 1000 mg/kg bw (rat)
Distribution	Highest concentration in liver (rat)
Potential for accumulation	Low (rat)
Rate and extent of excretion	90% excretion within 48–96 h (rat)
Metabolism in animals	No data
Toxicologically significant compounds (in animals, plants and the environment)	R611965
Acute toxicity	
LD_{50}, oral	> 5000 mg/kg bw (rat)
LD_{50}, dermal	No data
LC_{50}, inhalation	No data
Dermal irritation	No data
Ocular irritation	No data
Dermal sensitization	No data
Short-term studies of toxicity	
Target/critical effect	Body weight, clinical signs (dog)
Lowest relevant oral NOAEL	50 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Bilateral retinal atrophy (rat)
Lowest relevant NOAEL	200 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects (rat)
Lowest relevant reproductive NOAEL	20 000 ppm, equal to 911 mg/kg bw per day, highest dose tested (rat)

Developmental target	Decreased number of live fetuses, decreased fetal weight in the presence of maternal toxicity (rabbit); not teratogenic
Lowest relevant developmental NOAEL	500 mg/kg bw per day (rabbit)
Neurotoxicity/delayed neurotoxicity	
Neurotoxicity	No data; no indication of neurotoxic potential in acute and repeated-dose oral studies
Medical data	
	No data

References

Chun JS, Wilson NH, Killeen JC (1989) A teratology study in rats with 3-carbamyl-2,4,5-trichlorobenzoic acid (SDS-46851). Unpublished report No. CTL/C/3370 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Fillmore GE, Mizens M, Killeen JC (1991) A 90-day feeding study in mice with SDS-46851. Unpublished report No. CTL/C/3310 from Ricerca Inc., Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Hard GC, Khan KN (2004) Invited review: A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. *Toxicologic Pathology*, 32:171.

Ho DM, Marciniszyn JP, Killeen JC (1990) Study of the excretion and distribution of radiolabel following oral administration of ^{14}C-SDS-46851 to Sprague-Dawley rats. Unpublished report No. CTL/C/3375 from Ricerca Inc., Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Jones RE, Killeen JC (1985a) *Salmonella*/mammalian-microsome plate incorporation assay (Ames test) with and without renal activation with 2,4,5-trichloro-3-carboxy-benzamide. Unpublished report No. CTL/C/3307 from SDS Biotech Corporation, Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Jones RE, Killeen JC (1985b) *Salmonella*/mammalian-microsome plate incorporation assay (Ames test) with and without activation with 2,4,5-trichloro-3-carboxy-benzamide. Unpublished report No. CTL/C/3308 from SDS Biotech Corporation, Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Jones R, Killeen J (1985c) In vitro sister chromatid exchange assay in Chinese hamster ovary (CHO) cells with 3-carboxy-2,5,6-trichlorobenzamide. Unpublished report No. CTL/C/3306 from SDS Biotech Corporation, Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Jones R, Killeen J (1985d) DNA repair test in rat hepatocyte primary cultures with 3-carboxy-2,5,6-trichlorobenzamide. Unpublished report No. CTL/C/3699 from SDS Biotech Corporation, Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Killeen JC, Laveglia J, Serrone DM (1993) A combined chronic toxicity/oncogenicity study in rats with 3-carbamyl-2,4,5-trichlorobenzoic acid (SDS-46851). Unpublished report No. CTL/C/3312 from Ricerca Inc., Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Lucas F, Killeen JC (1993) A two generation reproduction study in rats with SDS-46851. Unpublished report No. CTL/C/3242 from Ricerca Inc., Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Lucas F, Laveglia J (1994) An oncogenicity study in mice with SDS-46851. Unpublished report No. CTL/C/3294 from Ricerca Inc., Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Mizens M, Killeen J (1988) L5178Y TK+/− mouse lymphoma forward mutation assay with 3-carboxy-2,5,6-trichlorobenzamide (SDS 46851). Unpublished report No. CTL/C/3241 from Ricerca Inc., Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Mizens M, Killeen JC (1991) A 28-day feeding study in mice with SDS-46851. Unpublished report No. CTL/C/3626 from Ricerca Inc., Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Mizens M, Killeen JC, Siou G (1985) The micronucleus test in mice with 2,5,6-trichloro-3-carboxy-benzamide (SDS-46851). Unpublished report No. CTL/C/3239 from SDS Biotech Corporation, Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Serrone DM, Killeen JC (1988) Combined 90-day feeding study and one-generation reproduction study in rats with 3-carboxy-2,5,6-trichlorobenzamide. Unpublished report No. CTL/C/3311 from Ricerca Inc., Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Serrone DM, Killeen JC (1989a) A 30-day oral toxicity study in dogs with 3-carbamyl-2,4,5-trichlorobenzamide (SDS-46851). Unpublished report No. CTL/C/3459 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Serrone DM, Killeen JC (1989b) A teratology study in rabbits with 3-carbamyl-2,4,5-trichlorobenzoic acid (SDS-46851). Unpublished report No. CTL/C/3371 from Ricerca Inc., Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Serrone DM, Killeen JC (1990) A 90-day oral toxicity study in dogs with 3-carbamyl-2,4,5-trichlorobenzamide (SDS-46851). Unpublished report No. CTL/C/3309 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Shults S, Killeen J (1985) Acute oral toxicity screening in rats with SDS-46851. Unpublished report No. CTL/C/3374 from SDS Biotech Corporation, Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Wilson NH, Ignatoski JA (1986) A 30-day feeding study in rats with 3-carboxy-2,5,6-trichlorobenzamide (SDS-46851). Unpublished report No. CTL/C/3704 from SDS Biotech Corporation, Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Wilson NH, Sadler EM, Killeen JC (1985) A 14-day feeding study in rats with 3-carboxy-2,5,6-trichlorobenzamide. Unpublished report No. CTL/C/3373 from SDS Biotech Corporation, Painesville, OH, USA. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.