CHLOROTHALONIL (addendum)

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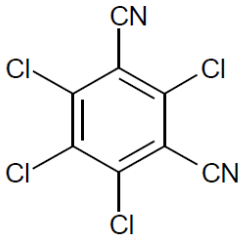
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Explanation

Chlorothalonil (tetrachloroisophthalonitrile) (Fig. 1) was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2009, when an acceptable daily intake (ADI) of 0–0.02 mg/kg body weight (bw) and an acute reference dose (ARfD) of 0.6 mg/kg bw were established. The 2009 Meeting also established an ADI of 0–0.008 mg/kg bw and an ARfD of 0.03 mg/kg bw for metabolite SDS-3701 (2,5,6-trichloro-4-hydroxyisophthalonitrile) (Annex 1, reference *118*). In 2010,

the Meeting evaluated metabolite R611965 (3-carbamyl-2,4,5-trichlorobenzoic acid; SDS-46851) and considered it unnecessary to establish a separate ADI and ARfD for R611965 because of its lower toxicity in comparison with the parent compound (Annex 1, reference *121*).

Fig. 1. Structure of chlorothalonil



Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, chlorothalonil was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review.

The new information on chlorothalonil included studies on absorption, distribution, metabolism and excretion, inhalation toxicity, acute and 90-day neurotoxicity, immunotoxicity, endocrine activity and phototoxicity. For six chlorothalonil metabolites and/or degradates, the new information included studies on acute toxicity, short-term toxicity and genotoxicity.

All critical studies contained statements of compliance with good laboratory practice (GLP) and were conducted in accordance with relevant national or international test guidelines, unless otherwise specified. No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Evaluation for acceptable intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

In a repeated-dose study, ^{14}C-labelled chlorothalonil (purity >98.0%) was administered to seven female Wistar rats by gavage in 14 consecutive daily doses of 1.5 mg/kg bw. One animal was euthanized at each of four different time points during the dosing period (days 2, 5, 8 and 11) and three different time points after the last dosing (days 15, 17 and 21). The [^{14}C]chlorothalonil-related residues in tissues and organs were determined by means of a quantitative whole-body autoradiography technique. The excreted radioactivity was determined in urine and faeces of three animals at daily intervals.

[^{14}C]Chlorothalonil was poorly absorbed from the gastrointestinal tract into the systemic circulation. A steady state in terms of excretion was reached within 2 days after the start of dosing. About 80% of the daily dose was excreted in faeces after 2 days and until the end of dosing, whereas only 7% of the daily dose was excreted in urine. After the dosing period, the daily excretion decreased rapidly.

The blood kinetics showed increasing concentrations of radioactivity with ongoing administration. A steady state was achieved 12 days after the start of dosing, at a plateau level of about 0.050–0.060 µg chlorothalonil equivalents (equiv) per gram. After the last of 14 consecutive daily doses, the concentration in whole blood decreased slowly. The terminal half-life in blood was calculated to be 89 hours.

The highest concentrations were found in organs involved in the elimination process (i.e. kidney and liver). The highest residue level was found in the kidney cortex 24 hours after the last dosing, accounting for 2.453 µg equiv/g. Besides these, quantifiable residues were also found in thyroid, lung, blood, adrenals and ovaries. In all tissues and organs in which radioactivity could be determined, the

steady state was achieved within 5 days. After the dosing period, the residues in tissues and organs, if quantifiable, depleted with half-lives of 2–10 days (Hassler, 2007).

In another study, the pharmacokinetics, absorption, metabolism and excretion of total radioactivity were investigated following a single oral gavage administration of [^{14}C]chlorothalonil (purity 99.2%) at 5 or 200 mg/kg bw in 0.5% aqueous carboxymethylcellulose to a group of six male and six female Han Wistar rats. Blood and plasma were collected at intervals up to 3 days post-dosing to characterize the pharmacokinetics of total radioactivity. To characterize the absorption and excretion of total radioactivity, urine and faeces were collected at intervals up to 7 days post-dosing. In a separate study, bile duct–cannulated rats (three of each sex) were administered a single gavage dose of chlorothalonil at 5 or 200 mg/kg bw, and bile, urine and faeces were collected for up to 72 hours.

Following a single 5 mg/kg bw dose of [^{14}C]chlorothalonil to male and female rats, radioactivity was detectable in blood from the first time point (0.5 hour). The maximum concentration (C_{max}) of radioactivity in blood was 0.21 µg equiv/g in males and 0.31 µg equiv/g in females, with a time to reach C_{max} (T_{max}) of 8 hours post-dosing in males and 4 hours post-dosing in females. Concentrations in males declined rapidly between 12 and 24 hours, then gradually to 54 hours post-dosing. Concentrations in females maintained a plateau between 8 and 12 hours post-dosing, before declining rapidly at 24 hours post-dosing. Concentrations then decreased steadily to 54 hours post-dosing. Radioactivity was still detected in blood in both male and female rats at 54 hours post-dosing. Concentrations of radioactivity were detectable in plasma from the first time point (0.5 hour). The C_{max} values in plasma were higher than those in blood, at 0.52 and 0.58 µg equiv/g in males and females, respectively. The T_{max} in plasma occurred at 8 hours post-dosing in both males and females, after which concentrations decreased steadily up to 72 hours post-dosing. Radioactivity was still detected in plasma in both male and female rats at 72 hours post-dosing.

Following a single 200 mg/kg bw dose of [^{14}C]chlorothalonil to male and female rats, radioactivity was detectable in blood from the first time point (0.5 hour). The C_{max} values in blood were 3.2 and 6.0 µg equiv/g in males and females, respectively, with a T_{max} of 12 hours post-dosing in both males and females. Concentrations in males declined steadily until 54 hours post-dosing and fell below the limit of detection by 72 hours post-dosing. Concentrations in females also declined steadily, but fell below the limit of detection by 48 hours post-dosing. Radioactivity was detectable in plasma from the first time point (0.5 hour). The C_{max} values in plasma were higher than those in blood, at 6.8 and 11.6 µg equiv/g in males and females, respectively. The T_{max} occurred at 12 hours post-dosing in males and 8 hours post-dosing in females, after which concentrations in both males and females decreased steadily up to 96 hours post-dosing.

Following a single oral administration of [^{14}C]chlorothalonil at 5 mg/kg bw, the major route of elimination was via the faeces, with 85–91% of the administered radioactivity recovered by 168 hours post-dosing. Urinary excretion accounted for 5.5–7.0% of the administered dose. Following a single oral administration of [^{14}C]chlorothalonil at 200 mg/kg bw, the major route of elimination was via the faeces, with 99–115% of the administered radioactivity recovered by 168 hours post-dosing. Urinary excretion accounted for less than 3.0% of the administered dose.

Following a single oral administration of [^{14}C]chlorothalonil at 5 mg/kg bw to bile duct–cannulated rats, the major route of elimination was via the faeces, with 75–80% of the administered radioactivity recovered by 72 hours post-dosing. Biliary elimination accounted for 12% of the administered dose, and urinary excretion accounted for 5.8–10% of the administered dose. Following a single oral administration of [^{14}C]chlorothalonil at 200 mg/kg bw to bile duct–cannulated rats, the major route of elimination was via the faeces, with 81–95% of the administered radioactivity recovered by 72 hours post-dosing. Biliary elimination accounted for 4.9–7.5% of the administered dose, and urinary excretion accounted for 2.9–4.3% of the administered dose (Punler, 2013).

In another study, [^{14}C]chlorothalonil (purity 98.6%) in 0.5% weight per volume (w/v) aqueous carboxymethylcellulose was dosed orally via gavage at 1.5 mg/kg bw or intravenously at 0.5 mg/kg bw

to groups of four male and four female Wistar rats per dose route. Excretion samples were obtained over a 7-day period. Blood samples were taken over a 3-day period to determine the pharmacokinetics of total radioactivity in blood.

Following a single oral administration of [^{14}C]chlorothalonil at 1.5 mg/kg bw, the majority of administered radioactivity (82–91%) was excreted in the first 24 hours, indicating that elimination was rapid. The [^{14}C]chlorothalonil that was systemically available, based on the urinary excretion ratio (oral:intravenous), accounted for 18–19% of the administered dose. Absorption was rapid, with peak mean measured blood concentrations observed at 2–4 hours. The oral bioavailability was approximately 2% for both sexes, suggesting that systemic availability was underestimated, as at least 5% of the dose was excreted in urine. No sex-related differences were observed in the pharmacokinetics or excretion of total radioactivity (Hutton, 2017).

1.2 Biotransformation

(a) In vivo

The nature and identity of the metabolites present in samples of plasma, urine, bile and faeces obtained from the study by Pulner (2013), described in section 1.1 above, were investigated.

Chlorothalonil was metabolized in the rat, forming up to seven metabolites through oxidation, hydroxylation and conjugation. For each matrix, metabolite profiles were qualitatively and quantitatively similar, regardless of sex and dose. The majority of the dose was excreted as unabsorbed parent in faeces. The major metabolites were identified as R611965 (3-carbamyl-2,4,5-trichlorobenzoic acid) and R613823 (2-acetamido-3-[3-(2-acetamido-3-hydroxy-3-oxo-propyl)sulfanyl-2,5-dichloro-4,6-dicyano-phenyl]sulfanyl-propanoic acid). Other identified components included SDS-3701 (R182281; 2,5,6-trichloro-4-hydroxyisophthalonitrile), R613825 (2-acetamido-3-[3,5-bis[(2-acetamido-3-hydroxy-3-oxo-propyl) sulfanyl]-4-chloro-2,6-dicyano-phenyl] sulfanyl-propanoic acid), R611966 (2,4,5-trichloro-3-cyanobenzamide), R417888 (2-carbamyl-3,5,6-trichloro-4-cyanobenzenesulfonic acid) and R419492 (4-carbamyl-2,5-dichloro-6-cyanobenzene-1,3-disulfonic acid). R613825 and R419492 had similar retention factors and could not be identified or quantified as individual metabolites.

The most abundant component excreted in urine was R613823 (≤3.3% of the dose). R611965 and the region comprising R613825 and/or R419492 accounted for 1.5% of the dose or less. A single component remained unidentified (≤1.0% of the dose), and origin-bound material accounted for 1.2% of the dose or less.

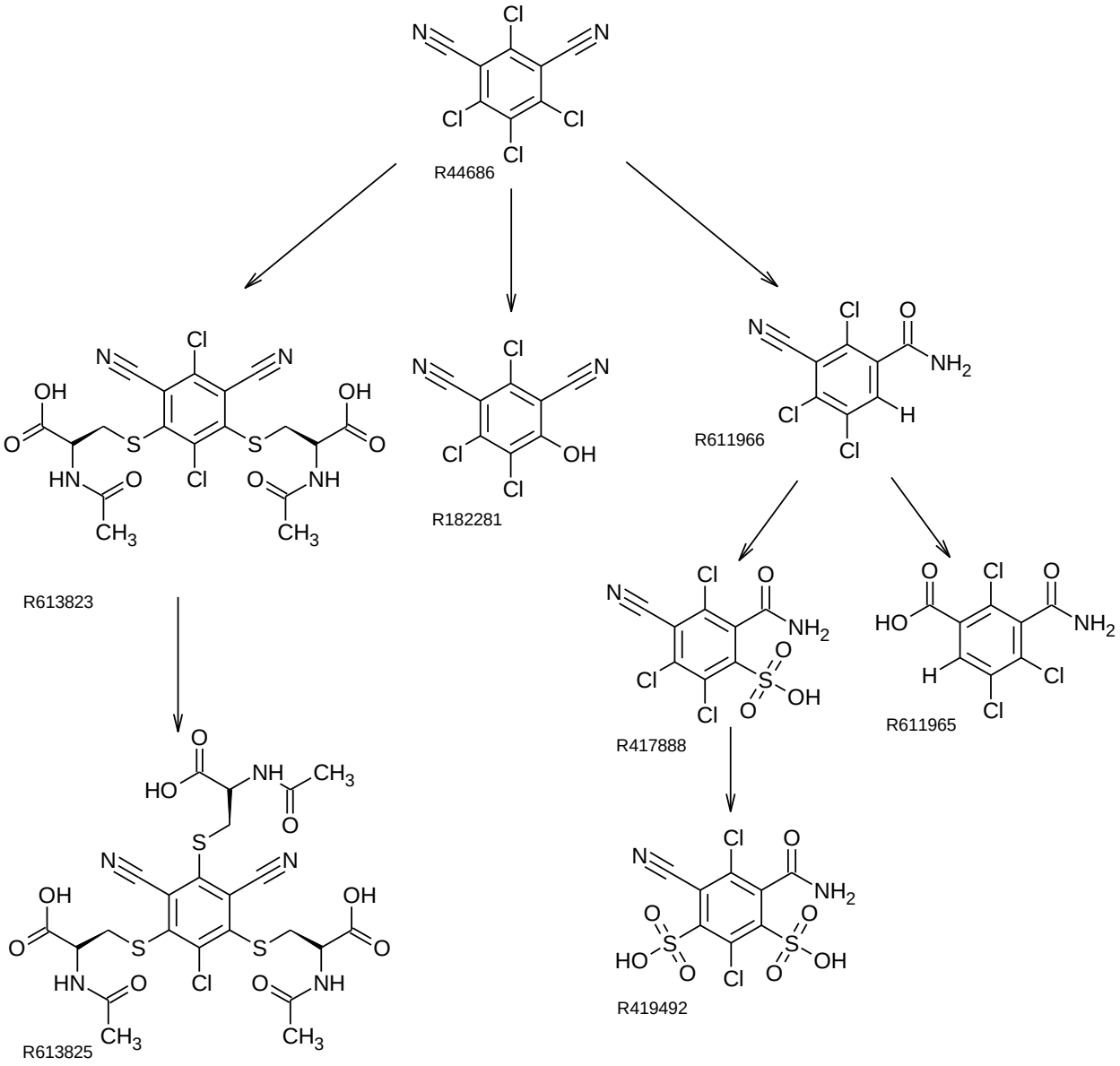
In bile, R613823 accounted for 1.7% of the dose or less, and R611965 and R613825/R419492 accounted for 1.4% of the dose or less. Three components in bile remained unidentified (≤2.0% of the dose), and origin-bound material accounted for 2.6% of the dose or less.

The most abundant component excreted in faeces was unabsorbed chlorothalonil (approximately 5.2–64.3% of the dose). R611966, SDS-3701, R611965, R613823 and R613825/R419492 were minor metabolites (≤5.4% of the dose). Two components remained unidentified (≤1.6% of the dose), and origin-bound material accounted for 4.4% of the dose or less.

The most abundant component circulating in plasma was SDS-3701, which accounted for 29–38% of the area under the concentration–time curve (AUC) for total radioactivity. The other circulating components observed were chlorothalonil, at 5.5–11% of the total radioactivity AUC, R611966, at 11–17% of the total radioactivity AUC, and R417888, at 4.7–12% of the total radioactivity AUC. Two components remained unidentified (4.1–13% of the total radioactivity AUC, ≤0.082 µg/g), and origin-bound material accounted for 7.8–16% of the total radioactivity AUC (≤0.181 µg/g) (Punler, 2013).

The proposed metabolic pathway for chlorothalonil in rats is shown in Fig. 2.

Fig. 2. Proposed metabolic pathway for chlorothalonil (R44686) in rats



Source: Punler (2013)

(b) In vitro

[^{14}C]Chlorothalonil (10 µmol/L) was incubated with rat and human liver microsomes at approximately 37 °C, and reactions were terminated following incubation for 0 and 60 minutes by the addition of acetonitrile acidified with formic acid (0.1% volume per volume). All samples were analysed by radio–high-performance liquid chromatography (HPLC), and fractions were labelled according to their approximate retention times. Each batch of microsomes was also incubated with [^{14}C]testosterone to assess the metabolic capacity of the test system used in the study.

The parent molecule accounted for more than 88% of the total chromatographic radioactivity after 60 minutes. The rank order for the disappearance of the [^{14}C]chlorothalonil parent molecule was female rat > male rat > human. Disappearance of the parent molecule was concomitant with the formation of up to six components across both species. All regions of radioactivity, except M3, M4 and M5, were considered to be of metabolic origin. M2 was the most notable component formed, accounting for 38.2% and 60.8% of the total chromatographic radioactivity following incubation for 60 minutes in

male and female rat liver microsomes, respectively. M2 was observed only in the 0-minute samples following incubation with human liver microsomes, and this component represented 3.93% of the total chromatographic radioactivity.

No unique or disproportionate human in vitro metabolites were considered to be formed under the experimental conditions employed (Sayer, 2017).

2. Toxicological studies

2.1 Short-term studies of toxicity

(a) Exposure by inhalation

In a 2-week inhalation toxicity study, a chlorothalonil formulation (Bravo WeatherStick 720 SC) containing 53.7% weight per weight (w/w) chlorothalonil was administered via nose-only inhalation (5 days per week, 6 hours per day) to six groups of 25 male Sprague Dawley rats at 0 mg/L (air control, group 1), 0 mg/L (blank formulation control, group 2), 0.001 mg/L (group 3), 0.003 mg/L (group 4), 0.009 mg/L (group 5) or 0.015 mg/L (group 6). Overall, the achieved chlorothalonil concentrations were 0.0011, 0.0029, 0.0096 and 0.0143 mg/L, respectively. The analytically measured mass median aerodynamic diameter was less than 4 μm.

Ten animals from each group were terminated after completion of 2 weeks of treatment, and five animals from each group were terminated at each of the designated recovery time points (48 hours, 7 days and 14 days after the last day of exposure). An additional group of five males (probe group 7) was exposed prior to all the other groups for a single 6-hour period to compare clinical signs with those observed in previous studies.

Animals were observed for clinical signs and mortality at selected time intervals. Body weights and feed consumption were measured at selected intervals. Ophthalmoscopy examinations were performed on all animals (except for probe group 7) pre-trial and on all control and high-dose animals during week 2 of treatment. Following termination after 2 weeks, all animals underwent a detailed necropsy examination, and the lungs were weighed. Respiratory tract tissues from all animals were retained and subjected to a comprehensive histopathological evaluation.

There were no premature decedents over the course of the study. There were some notable treatment-related clinical observations, including salivation (groups 2–6), staining of the muzzle (groups 5 and 6) and some respiratory signs (wheezing, gasping, irregular respiration; one or two animals in group 6 only). Concentration-dependent reduced body weight gains (Table 1) were found across all chlorothalonil treatment groups after the first and second weeks of exposure, with a net weight loss after the first week of exposure for group 6. During the recovery periods, the weight gain for the higher-concentration groups (groups 4–6) was slightly higher than for group 2.

Feed consumption followed a concentration-dependent trend, with statistically significant reductions for groups 4–6 during each of the two 5-day exposure periods. There were no ophthalmic findings considered to be related to treatment. No test item–related lung weight changes were noted. No treatment-related gross findings were noted. Microscopic findings in the larynx, lung, nasal cavity and trachea observed at all doses were indicative of irritation in the respiratory tract. At the end of the recovery period, the microscopic findings observed at the terminal euthanasia were not observed, were reduced in incidence and/or severity or showed partial recovery.

The no-observed-adverse-effect concentration (NOAEC) in the 2-week inhalation toxicity study was 0.001 mg/L, based on reduced body weight gain seen at 0.003 mg/L (Bain, 2013).

Table 1. Intergroup comparison of mean body weight change

Concentration (mg/L)	Mean body weight change (g)					
	Days 0–5	**Days 0–7**	**Days 0–12**	**Days 12–13**	**Days 12–19**	**Days 12–26**
0 (air control; group 1)	22	38	57	4	48	83
0 (blank formulation control; group 2)	18	35	54	5	45	79
0.001 (group 3)	17	34	50	5	47	84
0.003 (group 4)	10**	29*	43**	7	49	89
0.009 (group 5)	3**	25**	34**	8	56*	94
0.015 (group 6)	−3**	22**	25**	12**	60**	110

*: $P < 0.05$; **: $P < 0.01$
Source: Bain (2013)

2.2 Special studies

(a) Acute neurotoxicity

In a range-finding study of acute neurotoxicity, chlorothalonil (purity 99.3%) was administered orally by gavage as a single dose of 2000 mg/kg bw in 1.0% carboxymethylcellulose in water to male and female Crl:CD(SD) rats (four of each sex). A concurrent control group received the vehicle on a comparable dosing regimen. The dosing volume was 10 mL/kg bw for each group. All animals were observed twice daily for mortality and moribundity. Individual body weights and feed consumption were recorded daily beginning on the day of dose administration (study day 0). Detailed clinical observations were recorded for all animals at approximately 1, 2, 3, 4, 5, 6, 7 and 8 hours following dose administration on study day 0 and once daily thereafter until study day 6 (the day prior to scheduled euthanasia). All animals were euthanized and discarded without macroscopic examination on study day 7.

There were no treatment-related effects on clinical signs in rats of either sex. There was a statistically significant reduction in mean body weight in males on the first day of dosing compared with the control group. However, mean body weight gains for these animals were similar to those of the control group throughout the remainder of the study. Statistically significantly lower mean feed consumption was noted for 2000 mg/kg bw males and females following the first day of test substance administration, compared with the control group.

Based on the results of this study, the recommended high dose of chlorothalonil for evaluation in a subsequent acute neurotoxicity study was 2000 mg/kg bw, the limit dose for acute neurotoxicity testing (Herberth, 2014a).

In a study of acute neurotoxicity, chlorothalonil (purity 99.3%) was administered orally by gavage to groups of Crl:CD(SD) rats (10 of each sex per dose) at a dose of 100, 500 or 2000 mg/kg bw. A concurrent control group received the vehicle (1.0% carboxymethylcellulose in deionized water) on a comparable dosing regimen. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, except on days of functional observational battery (FOB) assessments. Individual body weights and feed consumption were recorded weekly beginning 1 week prior to dosing. FOB and locomotor activity data were recorded for all animals prior to dosing, at the time of peak effect (approximately 4–6 hours following dose administration) on study day 0, and on study days 7 and 14. On study day 15, five rats of each sex per group were deeply anaesthetized and perfused in situ; select tissues from the central and peripheral nervous systems were collected, and brain

weights and dimensions (excluding olfactory bulbs) were recorded. A neuropathological evaluation of nervous tissues collected from the five rats of each sex in the control and 2000 mg/kg bw groups was performed. The remaining (non-perfused) rats were euthanized and discarded without macroscopic examination.

There was no mortality. No test substance–related clinical findings were noted at any dose during the daily observations. Mean body weights, body weight gains and feed consumption were unaffected by test substance administration at all doses. No treatment-related effects on FOB or locomotor activity parameters were observed. No treatment-related effects on macroscopic or microscopic findings were observed.

The no-observed-adverse-effect level (NOAEL) for systemic toxicity and neurotoxicity was 2000 mg/kg bw, the highest dose tested (Herberth, 2014b).

(b) Short-term studies of neurotoxicity

In a 90-day neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing chlorothalonil (purity 99.52%) at a concentration of 0, 30, 300 or 3000 parts per million (ppm) (equal to 0, 2.1, 22.0 and 232.1 mg/kg bw per day for males and 0, 2.4, 24.2 and 243.2 mg/kg bw per day for females, respectively). All animals were observed prior to the study start and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, were performed in weeks −1, 2, 5, 9 and 14. Locomotor activity was also monitored in weeks −1, 2, 5, 9 and 14. Body weights and feed consumption were recorded weekly throughout the study, and feed utilization was calculated over selected periods. At the end of the scheduled period, five rats of each sex per group were killed by in situ perfusion fixation, and selected nervous system tissues were removed, processed and examined microscopically.

No treatment-related mortality was observed. At 3000 ppm, there was a generalized yellow staining of the coat, limbs and ears and staining of the tray papers under the cages of these animals. Clinical signs of toxicity were confined to staining around the mouth and nose, which was also noted at necropsy. At the high dose, male body weights (adjusted for initial weight) were statistically significantly lower than those of controls (by up to 17% in weeks 10–14). The body weights of females fed 3000 ppm were 12% lower than those of controls in week 2 and remained statistically significantly lower than those of controls from week 5 onwards. There were no treatment-related effects on body weight in rats fed 30 or 300 ppm. At 3000 ppm, feed consumption was lower than that of the controls throughout the study in males and in week 1 and then from week 5 onwards in females. There was no effect of treatment on ophthalmoscopic examinations, FOB or motor activity parameters, brain weights, macroscopic findings or microscopic findings.

The NOAEL for systemic toxicity was 300 ppm (equal to 22.0 mg/kg bw per day), based on decreased body weights and reduced feed consumption seen at 3000 ppm (equal to 232.1 mg/kg bw per day). The NOAEL for neurotoxicity was 3000 ppm (equal to 232.1 mg/kg bw per day), the highest dose tested (Brammer, 2004, 2005).

(c) Immunotoxicity

In an immunotoxicity study, groups of 10 female CD-1 mice were fed diets containing chlorothalonil (purity 99%) at a concentration of 0, 50, 750 or 2000 ppm (equal to 0, 8.2, 120.9 and 345.1 mg/kg bw per day, respectively) for 28 days. An additional group of 10 females (positive controls) received cyclophosphamide, a known immunosuppressant, at 10 mg/kg bw per day by oral gavage for the same period. All animals received a single intravenous injection of the antigen, sheep red blood cells, with cell numbers of 2×10^8, on day 25. Animals were observed for adverse clinical signs throughout the study. Body weight and feed and water consumption were measured at several intervals. Blood was taken on day 29 for analysis of specific immunoglobulin M (IgM) to sheep red blood cells.

At terminal sacrifice, selected organs (liver, gall bladder, spleen, thymus and kidneys) were removed, weighed and preserved, but were not examined histopathologically.

There were no unscheduled deaths or clinical signs of toxicity. No treatment-related effects on body weight, feed consumption or water intake were observed. After administration of sheep red blood cells, there was no evidence that chlorothalonil had an immunosuppressant effect on the humoral system. The absolute kidney weights were statistically significantly increased at 750 and 2000 ppm. The kidney weights adjusted for body weight (covariate analysis) were 22% and 29% higher than the control values at 750 and 2000 ppm, respectively. There were no differences noted in liver, spleen or thymus weights. There were no abnormal findings noted at necropsy. Animals that received cyclophosphamide had a statistically significantly lower serum antibody-specific IgM, when compared with controls that received untreated diet alone.

The NOAEL for immunotoxicity was 2000 ppm (equal to 345.1 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity was 50 ppm (equal to 8.2 mg/kg bw per day), based on increased kidney weights seen at 750 ppm (equal to 120.9 mg/kg bw per day) (Donald, 2012).

(d) Endocrine disruption

In an estrogen receptor binding assay, uterine cytosol from Sprague Dawley rats was used as the source of estrogen receptor to conduct binding experiments. A competitive binding experiment was conducted to measure the binding of a single concentration of [^{3}H]17β-estradiol (1 nmol/L) in the presence of increasing concentrations of chlorothalonil (purity 99.0%; logarithmic increase from 10^{-10} to 10^{-3} mol/L or 10^{-11} to 10^{-4} mol/L). Dimethyl sulfoxide (DMSO) was used as the vehicle at a final concentration of 2%. The assay included 19-norethindrone as a weak positive control, octyltriethoxysilane as a negative control and 17β-estradiol as the natural ligand reference material.

The reference compounds performed as expected in the assay. In the competitive binding experiment, chlorothalonil had no effect on specific binding at concentrations at or below 10^{-4} mol/L in runs 1 and 2 and at or below 10^{-5} mol/L in run 3. Precipitation occurred at 10^{-3} mol/L in runs 1 and 2 and at 10^{-4} mol/L in run 3.

Based on the results from the three runs, it was concluded that chlorothalonil is not active in the estrogen receptor binding assay (Willoughby, 2011a).

In an estrogen receptor transcriptional activation assay, hERa-HeLa-9903 cells cultured in vitro were exposed to chlorothalonil (purity 99.0%) at logarithmically increasing concentrations from 10^{-11} to 10^{-4} mol/L in cell culture medium containing 0.1% DMSO for 24 hours in two independent tests. The experiments were performed using 96-well plates, and each chlorothalonil concentration was tested in six replicates per plate. Cells were exposed to the test agent for 24 ± 2 hours to induce reporter (luciferase) gene products. Luciferase expression in response to activation of the estrogen receptor by chlorothalonil was measured using a proprietary luciferase assay.

The highest soluble concentration of chlorothalonil for use in the transcriptional activation assays was 10^{-4} mol/L, whereas the range of cytotoxicity was between 10^{-4} and 10^{-6} mol/L, as identified in the range-finding test and both independent runs. Because the maximum response relative to the positive control was less than 10% in at least two assay runs, chlorothalonil was considered to be negative for estrogen receptor transcriptional activation in this test system. The data indicate that chlorothalonil is not an estrogen receptor alpha agonist under the conditions of this study (Willoughby, 2011b).

In an androgen receptor binding assay, ventral prostate cytosol from Sprague Dawley rats was used as the source of androgen receptor to examine the ability of chlorothalonil to bind to the androgen receptor. The competitive binding assay was conducted to measure the binding of a single concentration of [^{3}H]R1881 (methyltrienolone; Chemical Abstracts Service No. 965-93-5; 10^{-9} mol/L; positive

control) in the presence of increasing concentrations (logarithmic increase from 10^{-11} to 10^{-3} mol/L) of chlorothalonil (purity 99.0%). DMSO was used as the solvent vehicle at a final assay concentration of approximately 3.2%. Three runs were performed in total, and each run included dexamethasone as a weak positive control and R1881 as the ligand reference standard.

The mean relative binding affinity (calculated by dividing the median inhibitory concentration [IC_{50}] of the positive control R1881 by the IC_{50} of the control/test material and multiplying by 100) was 0.0023% for the weak positive control (dexamethasone). As chlorothalonil was not classified as a "binder" (mean specific binding > 50%) for any of the runs, the relative binding affinity could not be calculated.

Based on the results from the three runs, it was concluded that chlorothalonil is a non-binder in the androgen receptor binding assay (Willoughby, 2012).

In a steroidogenesis assay, H295R cells cultured in vitro in 24-well plates were incubated with chlorothalonil (purity 99.0%) at logarithmic concentrations ranging from 10^{-10} to 10^{-4} mol/L for 48 ± 2 hours in triplicate for four independent experiments. DMSO was used as the solvent vehicle at a final concentration of 0.05%. A quality control plate containing two doses of the reference chemicals, forskolin (known inducer) and prochloraz (known inhibitor), was run each time the assay was performed. One independent experiment was not analysed because one of the forskolin concentrations on the quality control plate was different from the concentration specified in the test guideline (3.3 µmol/L was used instead of 1 µmol/L). Testosterone and estradiol levels were measured using HPLC with tandem mass spectrometry with internal standards.

Precipitation of the test compound was observed microscopically at 10^{-4} mol/L in runs 2 and 4. Cytotoxicity greater than 20% was observed at chlorothalonil concentrations of 10^{-5} and 10^{-4} mol/L in all three valid runs.

In three independent assays in H295R cells, chlorothalonil treatment did not result in statistically significant or reproducible alterations in testosterone or estradiol production (Wagner, 2012).

In an in vitro aromatase (cytochrome P450 19 [CYP19]) assay, chlorothalonil (purity 99%) in DMSO at logarithmic concentrations ranging from 10^{-10} to 10^{-3} mol/L was incubated with human recombinant aromatase and tritiated androstenedione ([1β-^{3}H]androst-4-ene-3,17-dione) for 15 minutes at 37 °C to assess the potential of chlorothalonil to inhibit aromatase activity. Aromatase activity was determined by measuring the amount of tritiated water produced at the end of a 15-minute incubation period for each concentration of chemical. Tritiated water was quantified using liquid scintillation counting.

Chlorothalonil was soluble in the assay buffer at concentrations of 10^{-4} mol/L and below. In three independent runs of the assay, increasing concentrations of chlorothalonil showed little decrease in aromatase activity. Chlorothalonil exhibited a mean aromatase activity of 89% (±1% standard deviation) at the highest soluble test concentration of 10^{-4} mol/L.

Under the study conditions utilized, chlorothalonil was not an inhibitor of aromatase activity (Wilga, 2011).

In a uterotrophic assay conducted to screen for potential estrogenic activity, chlorothalonil (purity 99.0%) in aqueous 1% carboxymethylcellulose was administered daily via oral gavage to groups of six ovariectomized Sprague Dawley rats at a dose of 0 (vehicle), 300 or 1000 mg/kg bw per day on postnatal days (PNDs) 65–67 or 66–68. A positive control group was treated with a daily dose of 17α-ethynyl estradiol in corn oil at 0.3 mg/kg bw per day by oral gavage. All animals were terminated and necropsied on PND 68 or 69 approximately 24 hours after administration of the last of the three doses to determine wet and blotted uterine weights.

Uterine weights in the chlorothalonil-treated groups at 300 and 1000 mg/kg bw per day were comparable to those of the vehicle controls. Absolute wet and blotted uterine weights for the positive control (17α-ethynyl estradiol) group were increased ($P \leq 0.01$) by 8.52- and 3.21-fold, respectively, as expected.

Based on the results of this study, it was concluded that chlorothalonil is negative in the uterotrophic assay (Sawhney Coder, 2012a).

In a Hershberger assay screening for androgenic activity, chlorothalonil (purity 99.0%) in aqueous 1% carboxymethylcellulose was administered daily via oral gavage to groups of six 54- to 56-day-old, castrated male Sprague Dawley rats at a dose of 300 or 1000 mg/kg bw per day (groups 4 and 5) for 10 days. Testosterone propionate (in corn oil), a known androgenic substance, was administered via subcutaneous injection at a dose of 0.2 mg/kg bw per day and a dosing volume of 0.5 mL/kg bw to group 2 males, which served as an androgenic comparator group for the test substance groups; the group 2 males also received a daily oral dose of the vehicle. Chlorothalonil and testosterone propionate were co-administered to groups 6, 7 and 8 at 100/0.2, 300/0.2 and 1000/0.2 mg/kg bw per day, respectively. The group 3 males received testosterone propionate comparably to the group 2 males, in conjunction with 3 mg/kg bw per day of flutamide (in corn oil), a known anti-androgenic substance, by daily oral gavage (at a dosing volume of 5 mL/kg bw), which served as an anti-androgenic comparator group for groups 6–8 to evaluate the potential of chlorothalonil to diminish the androgenic effects of testosterone propionate. A concurrent vehicle control group (group 1) received 0.5% carboxymethylcellulose orally by gavage.

All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and feed consumption were recorded daily. Blood was collected for possible future hormone analysis, and the bulbourethral glands, glans penis, liver, levator ani and bulbocavernosus muscle, seminal vesicles with coagulating glands and ventral prostate were examined, weighed and retained in 10% neutral-buffered formalin for possible future histopathological examination, with the following exception: the right portion of the medial lobe of the liver was flash-frozen and stored at approximately −70 °C for possible future biochemical analysis.

No test substance–related effects on mean body weight or body weight gain were noted in the 300 or 1000 mg/kg bw per day groups (groups 4 and 5). Co-administration of testosterone propionate at 0.2 mg/kg bw per day with chlorothalonil at 1000 mg/kg bw per day (group 8) resulted in slightly lower mean body weight gains sporadically throughout the treatment period (study days 0–10), compared with vehicle controls. As a result, the overall mean body weight gain in this group was lower (not statistically significantly) than in the testosterone propionate positive control group (group 2), and mean body weights were up to 6.22% lower than those in the testosterone propionate positive control group. Mean body weights and body weight gains were unaffected by co-administration of testosterone propionate at 0.2 mg/kg bw per day with chlorothalonil at 100 or 300 mg/kg bw per day (groups 6 and 7, respectively); differences from the testosterone propionate positive control group (group 2) were slight and not statistically significant. When the testosterone propionate positive control group (group 2) was compared with the vehicle control group (group 1), mean overall body weight gain in the testosterone propionate positive control group (78.0 g; study days 0–10) was statistically significantly higher than in the vehicle control group (65.5 g). No effects on mean body weight or body weight gain were noted when the testosterone propionate/flutamide positive control group (group 3) was compared with the testosterone propionate positive control group (group 2).

There was no effect of chlorothalonil treatment on the weight or histopathology of the bulbourethral glands, glans penis, liver, levator ani and bulbocavernosus muscle, seminal vesicles with coagulating glands or ventral prostate. No evidence of androgenic agonism or antagonism was noted following chlorothalonil administration at 300 or 1000 mg/kg bw per day alone (groups 4 and 5, respectively) or following co-administration of testosterone propionate at 0.2 mg/kg bw per day with chlorothalonil at 100, 300 or 1000 mg/kg bw per day (groups 6, 7 and 8, respectively).

Administration of the positive control substances, testosterone propionate and flutamide, gave the expected responses.

In conclusion, statistically significant changes were not seen in any of the five androgen-sensitive tissue weights (ventral prostate, seminal vesicle plus fluids and coagulating glands, levator ani and bulbocavernosus muscle, bulbourethral glands and glans penis). Chlorothalonil was negative for androgenicity and anti-androgenicity in the Hershberger assay (Sawhney Coder, 2012b).

(e) Phototoxicity

The phototoxic potential of chlorothalonil was determined in an in vitro cytotoxicity assay with the BALB/3T3 mouse fibroblast cell line. In this study, chlorothalonil (purity 98.6%) was dissolved in DMSO and diluted in a 1:100 ratio in Earle's balanced salt solution. The mouse fibroblast cells were treated for 1 hour at various concentrations of chlorothalonil solution at 37 ± 1 °C and for a further 50 minutes in the presence and absence of a non-toxic dose of ultraviolet A (UVA) light. One day after treatment, cytotoxicity was analysed as a measure of reduction of neutral red uptake and compared with the controls. DMSO was used as the negative control, and chlorpromazine (2-chloro-10-[3-dimethylaminopropyl] phenothiazine) was used as the positive control.

Chlorothalonil showed a cytotoxic effect with and without irradiation. The following median effective concentration (EC_{50}) values and photoirritation factor (PIF) were calculated:

−UVA: EC_{50} = 0.292 µg/mL

+UVA: EC_{50} = 0.149 µg/mL

PIF = 1.96

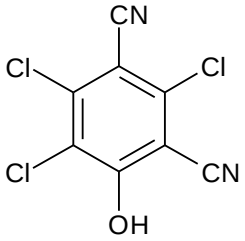
Based on the criterion in Organisation for Economic Co-operation and Development (OECD) Test Guideline 432 (i.e. PIF < 2), chlorothalonil is considered to produce no phototoxic effects (Gehrke, 2014).

3. Toxicological studies on metabolites and/or degradates

3.1 SDS-3701 (2,5,6-trichloro-4-hydroxyisophthalonitrile)

SDS-3701 (company codes R182281, DAC3701 and CNIL/02; Fig. 3) is found in soil (aerobic, anaerobic, photolysis), water (hydrolysis), crops (lettuce, tomato, carrot, wheat, rotated crops), livestock (hen, goat) and rats.

Fig. 3. Chemical structure of SDS-3701



(a) Acute toxicity

Lethal doses

In an acute oral toxicity study, SDS-3701 (purity 99.9%) was administered by oral gavage in propylene glycol as the vehicle to three female Wistar rats at 2000 mg/kg bw. Additional groups of three females were dosed at 300, 50 or 50 mg/kg bw. All animals were observed twice daily for clinical

signs and mortality for 2 weeks. Body weights were measured on days 1 (prior to administration), 8 and 15. All animals were subjected to macroscopic examination at termination.

At 2000 and 300 mg/kg bw, 3/3 and 2/3 animals died, while at 50 mg/kg bw, all animals survived. Clinical signs were observed at all doses and included hunched posture, uncoordinated movements, piloerection, lethargy and/or hyperthermia, as well as spasm and tremors at lethal doses. There were no effects of treatment on body weight. Macroscopic examination at termination revealed no test substance–related findings.

The oral median lethal dose (LD_{50} value) of SDS-3701 in female rats was identified to be between 50 and 300 mg/kg bw (Beerens-Heijnen, 2005a).

Dermal irritation

In a primary dermal irritation study, three adult male New Zealand white rabbits were each exposed via the dermal route to 0.5 g SDS-3701 (purity 99.9%). The test substance was moistened with 0.6 mL water and applied for 4 hours to the clipped skin of one flank using a semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours after exposure.

No dermal response was observed at the test site of any animal following exposure to SDS-3701, and no symptoms of systemic toxicity were found.

Based on the results of this study, it was concluded that SDS-3701 is not irritating to rabbit skin (Beerens-Heijnen, 2005b).

(b) Short-term studies of toxicity

Mice

In a repeated-dose toxicity study (performed pre-GLP), CD-1 mice (10 of each sex per dose) were administered SDS-3701 (purity 99.6%) in the diet at 0, 50, 125, 250, 500, 750 or 1500 ppm (equivalent to 0, 7.5, 18.75, 37.5, 75.0, 112.5 and 225.0 mg/kg bw per day, respectively) for 4 weeks. However, because of the lack of clearly defined effects after 2 weeks, doses of 3000 and 6000 ppm (equivalent to 450 and 900 mg/kg bw per day, respectively) were initiated at the beginning of the third week; this was accomplished by raising the doses of the 50 and 125 ppm groups, respectively. In order to evaluate the effects of the higher doses, the study was extended 1 additional week for a total duration of 5 weeks.

No treatment-related clinical signs of toxicity were observed. Four animals died spontaneously: one male at 50 ppm, one male and one female at 1500 ppm and one female at 3000 ppm. The deaths were not attributed to the treatment. In addition, three animals escaped from the cages. After dosing at 6000 ppm at week 3, animals of both sexes exhibited weight loss. Body weights were statistically significantly lower than control weights (>10%) at weeks 3 and 4 in females and at weeks 3, 4 and 5 in males. Body weights of the 3000 and 1500 ppm groups were reduced compared with those of controls (>10%) at some observation intervals in both sexes. Gross macroscopic examination did not reveal any treatment-related lesions (Ford & Killeen, 1980).

This study is not adequate to establish a NOAEL, as limited parameters were evaluated.

Rats

In a 13-week dietary toxicity study, SDS-3701 (purity 99.9%) was administered to Wistar Crl: (WI) BR (SPF) rats (10 of each sex per dose) at a concentration of 0, 15, 50 or 250 ppm (equal to 0, 1.0, 3.0 and 16 mg/kg bw per day for males and 0, 1.0, 4.0 and 17 mg/kg bw per day for females, respectively). The following parameters were evaluated: clinical signs daily; FOB and motor activity in week 12 or 13; body weight weekly; feed consumption twice weekly (weekly from week 6 onwards); ophthalmoscopy pretest and in week 12; and clinical pathology (haematology, clinical biochemistry and

urine analysis), macroscopy, selected organ weights and histopathology on a selection of tissues at termination.

Analytical measurements of the stability, homogeneity and accuracy of the target concentrations were made. Dietary analyses confirmed that diets were prepared accurately and homogeneously and were stable over at least 2 weeks at room temperature under normal laboratory light conditions.

No mortality occurred during the study. There were no treatment-related clinical signs of toxicity noted during the observation period. There were no treatment-related effects on FOB or motor activity parameters. No ophthalmological findings were noted in the treatment groups or controls.

Statistically significantly reduced body weights and body weight gains were observed in females at 250 ppm throughout the study starting from day 8 of the treatment. The body weights were reduced by about 12% in females compared with those of controls. The total difference in body weight gain over the treatment period compared with control body weight gain was approximately 35% in females at 250 ppm. The statistically significantly lower body weight gains of males at 50 and 250 ppm in the first week of treatment and of females at 15 ppm in week 12 were of a temporary and slight nature. Feed consumption was slightly reduced in females at 250 ppm throughout the treatment period. In females at 250 ppm, statistically significantly reduced red blood cell counts, haemoglobin and haematocrit levels and increased red cell distribution width were noted (Table 2). Red cell distribution width was also increased in males at 250 ppm, without concurrent changes in the other haematological parameters. Statistically significantly reduced total bilirubin levels were noted in males at 50 and 250 ppm and in females at 15 ppm and higher; the Meeting noted that decreased bilirubin is not considered to be an adverse effect in isolation. Increased creatinine levels were observed in males at 50 and 250 ppm, and reduced sodium levels were observed in males and females at 250 ppm (Table 3). Minor changes in these parameters at 50 ppm are not considered to be toxicologically relevant. No toxicologically relevant changes occurred in urine analysis parameters of treated rats. Macroscopic examination revealed no treatment-related abnormal findings. Slightly increased liver weights (absolute and relative to body weight) were observed in males at the high dose. In the absence of any changes in liver enzymes and histopathological findings, these increases in liver weights were considered adaptive and not adverse. Microscopic examination did not reveal any treatment-related lesions.

Table 2. Intergroup comparison of selected haematological parameters

	Males				**Females**			
Parameter	**0 ppm**	**15 ppm**	**50 ppm**	**250 ppm**	**0 ppm**	**15 ppm**	**50 ppm**	**250 ppm**
Red blood cells (10^{12}/L)	8.90	9.04	8.63	8.73	8.19	7.82	8.00	7.63*
Haemoglobin (mmol/L)	9.7	9.9	9.6	9.4	9.4	9.0*	9.2	8.3**
Haematocrit (L/L)	0.456	0.470	0.448	0.442	0.437	0.429	0.430	0.389**
RDW (%)	13.3	12.8	13.1	14.4**	12.3	13.5	12.4	14.6**

ppm: parts per million; RDW: red cell distribution width; *: $P < 0.05$; **: $P < 0.01$
Source: Van Otterdijk (2007a)

Table 3. Intergroup comparison of selected clinical chemistry parameters

	Males				Females			
Parameter	**0 ppm**	**15 ppm**	**50 ppm**	**250 ppm**	**0 ppm**	**15 ppm**	**50 ppm**	**250 ppm**
Total bilirubin (µmol/L)	2.5	2.2	1.9**	1.4**	3.3	2.2**	1.8**	1.5**
Creatinine (µmol/L)	39.1	41.7	44.9**	47.4**	47.2	49.7	51.1*	49.8
Sodium (mmol/L)	143.9	143.3	144.3	142.6*	142.6	141.8	142.4	140.8**

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$
Source: Van Otterdijk (2007a)

The NOAEL for SDS-3701 in this 90-day toxicity study in rats was 50 ppm (equal to 3.0 mg/kg bw per day), based on reduced body weight, body weight gain and feed consumption in females and changes in haematological and clinical chemistry parameters in both sexes at 250 ppm (equal to 16 mg/kg bw per day) (Van Otterdijk, 2007a).

In a 120-day dietary toxicity study (performed pre-GLP), SDS-3701 (purity assumed to be 100%) was administered to Sprague Dawley rats (10 males and 20 females per dose) at a concentration of 0, 10, 50, 100 or 200 ppm (equivalent to 0, 1, 5, 10 and 20 mg/kg bw per day, respectively). After 70 days, two female rats were housed with one male rat from the same dietary group for a 2-week period. These rats served as the parental generation in a (previously evaluated) three-generation reproductive toxicity study (Annex 1, reference *118*) conducted simultaneously with the 120-day feeding study.

Soft stool was observed in the 100 and 200 ppm groups. The 200 ppm group animals were observed to be nervous and irritable. The survival was 100% at all doses. The growth of males and females in the 10, 50 and 100 ppm groups and of males in the 200 ppm group was comparable to that of the control group. At the high dose, females displayed a decrease in growth rate from initiation up to mating. Body weight gain of the females at 200 ppm was statistically significantly decreased (>40%) in weeks 5–11 inclusive. Feed consumption was comparable in all dose groups. All clinical chemistry values were within the normal biological range, including a statistically significant elevation of alanine aminotransferase in high-dose animals. Haematological parameters were comparable in all groups. The results of urine analyses were comparable among all groups. Gross macroscopic findings were comparable among the groups. Liver weights (absolute and relative to body weight) were statistically significantly ($P < 0.05$) increased in the 200 ppm males compared with control values (adaptive and non-adverse response). No treatment-related histopathological findings were observed.

The NOAEL was 100 ppm (equivalent to 10 mg/kg bw per day), based on decreased body weight gains in females seen at 200 ppm (equivalent to 20 mg/kg bw per day) (Hastings & Jessup, 1975).

(c) Genotoxicity

The results of studies of the genotoxicity of SDS-3701 are summarized in Table 4. In vitro, SDS-3701 gave a positive response in only the mouse lymphoma assay, with and without metabolic activation. It was negative for genotoxicity in vivo in the mouse micronucleus test and for unscheduled DNA synthesis.

Table 4. Results of assays for the genotoxicity of SDS-3701

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 *Escherichia coli* WP2uvrA	3–5 000 µg/plate in DMSO (±S9)	99.9%	Negative	Verspeek-Rip (2004a)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	1–80 µg/mL in ethanol (−S9) 1–350 µg/mL in ethanol (+S9)	99.9%	Positive (±S9)[a]	Verspeek-Rip (2005a)
Mammalian cell gene mutation	Chinese hamster lung V79 cells; *Hprt* locus	3.9–31.3 µg/mL in DMSO (−S9) 7.8–125.0 µg/mL in DMSO (+S9)	98.7%	Negative	Chang (2017)
In vivo					
Micronucleus formation[b]	Male and female NMRI BR mice	500, 250 and 115 mg/kg bw (single oral gavage)	99.9%	Negative	Buskens (2004a)
Unscheduled DNA synthesis	Wistar Hanlbm:WIST (SPF)	250 and 500 mg/kg bw (gavage in corn oil)	99.9%	Negative	Honarvar (2005)

bw: body weight; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; *Hprt*: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

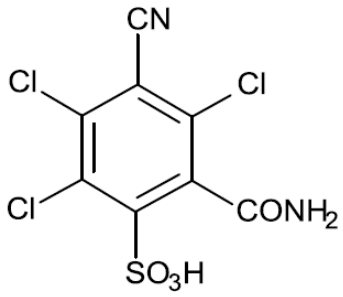
[a] Mutagenicity was observed only at highly cytotoxic concentrations.

[b] Proof of bone marrow exposure was demonstrated by Dunton (2016a).

3.2 R417888 (2-carbamyl-3,5,6-trichloro-4-cyanobenzenesulfonic acid)

R417888 (Fig. 4; company codes M12, VIS01, R6, Compound 10, U6 and CSCC890840) is found in soil (aerobic, anaerobic), crops (rotated crops) and rats.

Fig. 4. Chemical structure of R417888



(a) Acute toxicity

In an acute toxicity study, R417888 (purity 99.5%) was administered by gavage in water as vehicle to two groups of three female Wistar rats at 2000 mg/kg bw. All animals were observed twice daily for clinical signs and mortality for 2 weeks. Body weights were measured on days 1 (prior to administration), 8 and 15. All animals were subjected to macroscopic examination at termination.

No mortality occurred. Hunched posture was noted in all animals on days 1 and/or 2. There were no effects of treatment on body weight. No abnormalities were found at macroscopic examination of the animals.

In conclusion, the oral LD_{50} of R417888 in female Wistar rats was greater than 2000 mg/kg bw (Van Hygevoort, 2005).

(b) Short-term studies of toxicity

In a 90-day dietary toxicity study, R417888 (purity 100.0%) was administered to Wistar Crl: (WI) BR (SPF) rats (10 of each sex per dose) at a concentration of 0, 150, 600 or 2400 ppm (equal to 0, 13.0, 54.0 and 192 mg/kg bw per day for males and 0, 15.0, 56.0 and 218 mg/kg bw per day for females, respectively). The following parameters were evaluated: clinical signs daily; functional observational tests and motor activity in week 12; body weight weekly; feed consumption weekly (except weeks 4–9, when twice daily); ophthalmoscopy pretest (all animals) and in week 13 (controls and 2400 ppm); and clinical pathology (haematology, clinical biochemistry and urine analysis), macroscopy, selected organ weights and histopathology on a selection of tissues at termination.

Analytical measurements of stability, homogeneity and accuracy of the target concentrations were made. Dietary analyses confirmed that diets were prepared accurately and homogeneously and were stable over at least 2 weeks at room temperature under normal laboratory light conditions.

No mortality occurred during the study period. There were no clinical signs of toxicity noted during the observation period. Hearing ability, pupillary reflex, static righting reflex and grip strength were normal in all animals. The variation in motor activity did not indicate a relationship with treatment. There were no toxicologically relevant ophthalmology findings pre-dosing or in week 13. No toxicologically relevant changes in body weight or body weight gain were noted over the study period. Feed consumption before and after allowance for body weight was similar between treated and control animals. No toxicologically relevant changes occurred in haematological, clinical biochemistry or urine analysis parameters of treated rats. Necropsy did not reveal any toxicologically relevant alterations. No toxicologically relevant changes were noted in organ weights or organ to body weight ratios. There were no microscopic findings recorded that could be attributed to treatment with the test substance.

The NOAEL in the 90-day toxicity study in rats with R417888 was 2400 ppm (equal to 192 mg/kg bw per day), the highest dose tested (Van Otterdijk, 2007b).

(c) Genotoxicity

The results of genotoxicity tests with R417888 are shown in Table 5. R417888 gave a negative response for genotoxicity in several in vivo and in vitro assays, except for a positive response in the chromosomal aberration assay using human lymphocytes in the absence of metabolic activation and one mouse lymphoma assay with metabolic activation.

3.3 SYN548764 (4-carbamyl-2,3,5-trichloro-6-cyanobenzenesulfonic acid)

SYN548764 is a structural isomer of R417888 (see section 3.2 above). The sponsor noted that, historically, there had been some confusion between the identities of these two metabolites. The Ames, in vitro cytogenetics, mouse lymphoma and in vivo micronucleus study results originally conducted on what was thought to be SYN548764 were actually conducted with R417888. The new genotoxicity studies described below are reported to have been conducted with the correct metabolite (SYN548764). The actual chemical structure of SYN548764 is shown in Fig. 5.

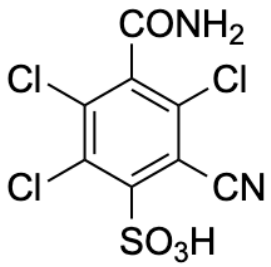
Table 5. Results of assays for the genotoxicity of R417888

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA	100–5 000 µg/plate in DMSO (±S9)	99.5%	Negative	Verspeek-Rip (2005b)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA	3.0–5 000 µg/plate in DMSO (±S9)	95.0%	Negative	Sokolowski (2007)
Chromosomal aberrations	Human lymphocytes	14.3–2 200 µg/mL in DMSO (±S9)	95.0%	Positive (−S9) Negative (+S9)	Kunz (2007)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	333–3 000 µg/mL in ethanol (−S9) 3–1 000 µg/mL in ethanol (+S9)	99.5%	Positive (+S9)	Verspeek-Rip (2006)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	8.8–280.0 µg/mL in DMSO (−S9) 75–2 300 µg/mL in DMSO (+S9)	95.0%	Negative	Wollny (2007)
In vivo					
Micronucleus formation[a]	Five male NMRI BR mice	2000 mg/kg bw (single oral gavage in water)	99.5%	Negative	Meerts (2005)
Unscheduled DNA synthesis	Wistar Hanlbm:WIST (SPF) hepatocytes	1 000 and 2 000 mg/kg bw (gavage in PEG 400)	99.5%	Negative	Honarvar (2006)

bw: body weight; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; PEG: polyethylene glycol; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

[a] Proof of bone marrow exposure was demonstrated by Dunton (2016b).

Fig. 5. Chemical structure of SYN548764



SYN548764 (company code SYN548581) is found in soil (aerobic, anaerobic), crops (rotated crops) and rats.

(a) Genotoxicity

The results of genotoxicity assays with SYN548764 are shown in Table 6. SYN548764 gave a negative response for genotoxicity in in vivo and in vitro assays except for an equivocal response in the chromosomal aberration test in the absence of metabolic activation.

Table 6. Results of assays for the genotoxicity of SYN548764

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA pKM101 and WP2pKM101	3.0–5 000 µg/plate in DMSO (±S9)	99.5%	Negative	Sokolowski (2015a)
Chromosomal aberrations	Human lymphocytes	24.0–3 700 µg/mL in DMSO (±S9)	95.0%	Equivocal (−S9) Negative (+S9)	Sokolowski (2015b)[a]
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	200.0–1 400.0 µg/mL in deionized water (−S9) 231.3–3 700 µg/mL in deionized water (+S9)	95.0%	Negative	Wollny (2015a)
In vivo					
Micronucleus formation[b]	Male CD-1 mice	500, 1 000 and 2 000 mg/kg bw (single oral gavage in aqueous CMC with Tween 80)	95.0%	Negative	Dunton (2015a)

bw: body weight; CMC: 0.5% (w/v) aqueous carboxymethylcellulose; DMSO: dimethyl sulfoxide; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

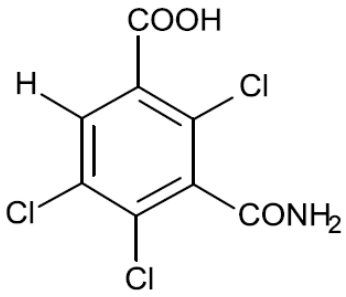
[a] Cytotoxicity at high dose.

[b] Proof of bone marrow exposure was demonstrated by Dunton (2015a).

3.4 R611965 (3-carbamyl-2,4,5-trichlorobenzoic acid)

R611965 (Fig. 6; company codes SDS-46851, M5, R14 and Compound 4) is found in soil (aerobic, anaerobic), crops (snap beans, rotated crops) and rats.

Fig. 6. Chemical structure of R611965



(a) Acute toxicity

Lethal doses

In an acute toxicity study, R611965 (purity 99.0%) was administered by oral gavage in 1% aqueous carboxymethylcellulose to two groups of three female Wistar Crl: (WI) BR (SPF) rats at 2000 mg/kg bw. All animals were observed twice daily for clinical signs and mortality for 2 weeks. Body weights were measured on days 1 (prior to administration), 8 and 15. All animals were subjected to macroscopic examination at termination.

No mortality occurred. Hunched posture was noted in all animals on days 1 and/or 2. There were no effects of treatment on body weight. No macroscopic abnormalities were observed.

In conclusion, the oral LD_{50} of R611965 in female Wistar rats was greater than 2000 mg/kg bw (Beerens-Heijnen, 2005c).

Dermal irritation

In a primary dermal irritation study, three adult male New Zealand white rabbits were each exposed via the dermal route to 0.5 g of R611965 (purity 99.0%). The test substance was moistened with water and applied for 4 hours to the clipped skin of one flank using a semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours after exposure.

No dermal response was observed at the test site of any animal following exposure to R611965, and no symptoms of systemic toxicity were observed.

Based on the results of this study, it was concluded that R611965 is not irritating to rabbit skin (Beerens-Heijnen, 2005d).

(b) Short-term studies of toxicity

In a 90-day toxicity study, R611965 (purity 99.1%) was administered to Wistar Crl: (WI) BR (SPF) rats (10 of each sex per dose) at a dietary concentration of 0, 150, 600 or 2400 ppm (equal to 0, 14.0, 57.0 and 197 mg/kg bw per day for males and 0, 14.0, 60.0 and 223 mg/kg bw per day for females, respectively). The following parameters were evaluated: clinical signs daily; FOB tests and motor activity in weeks 12–13; body weight weekly; feed consumption weekly (except weeks 3–9, when twice weekly); ophthalmoscopy pretest and in week 13; and clinical pathology (haematology, clinical biochemistry and urine analysis), macroscopy, selected organ weights and histopathology on a selection of tissues at termination.

Analytical measurements of stability, homogeneity and accuracy of the target concentrations were made. Dietary analyses confirmed that diets were prepared accurately and homogeneously and were stable over at least 2 weeks at room temperature under normal laboratory light conditions.

No toxicologically relevant changes were noted in any of the parameters investigated in this study at doses up to 2400 ppm.

The NOAEL was 2400 ppm (equal to 197 mg/kg bw per day), the highest dose tested (Van Otterdijk, 2007c).

(c) Genotoxicity

The results of genotoxicity assays with R611965 are shown in Table 7. R611965 gave a negative response for genotoxicity in all in vivo and in vitro assays conducted.

Table 7. Results of assays for the genotoxicity of R611965

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA	100.0–5 000 µg/plate in DMSO (±S9)	99.5%	Negative	Verspeek-Rip (2004b)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	1.0–2 660 µg/mL in DMSO (±S9)	99.0%	Negative	Verspeek-Rip (2005c)
In vivo					
Micronucleus formation	Male NMRI mice	2 000 mg/kg bw (single oral gavage in corn oil)	99.0%	Negative	Buskens (2004b)

bw: body weight; DMSO: dimethyl sulfoxide; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

(d) Relative potencies of chlorothalonil and R611965

In order to compare the potencies of chlorothalonil metabolite R611965 and chlorothalonil, the results of toxicological studies reviewed by this Meeting and by previous Meetings were compared (Table 8).

Table 8. Comparative toxicities of chlorothalonil and its metabolite R611965

	Chlorothalonil		**R611965**	
Study type	**NOAEL (mg/kg bw per day)**	**LOAEL (mg/kg bw per day)**	**NOAEL (mg/kg bw per day)**	**LOAEL (mg/kg bw per day)**
Acute oral toxicity (rat)	>5 000 mg/kg bw (LD_{50})	–	>5 000 mg/kg bw (LD_{50})	–
Ninety-day toxicity (mouse)	47.7	123.6	1 270	–
Thirty-day toxicity (rat)	–	80	2 000	–
Ninety-day toxicity (rat)	–	40	2 000	–
Ninety-day toxicity (rat)	10	40	197	–
Ninety-day toxicity (dog)	15	150	50	500
One-year toxicity (dog)	150	500	–	–
Two-year toxicity (dog)	3	–	–	–
Chronic toxicity (mouse)	–	40	1 022	–
Carcinogenicity (mouse)	–	40	1 022	–

Study type	Chlorothalonil		R611965	
	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Chronic toxicity (rat)	1.8	3.8	200	500
Carcinogenicity (rat)	2.7	10.6	1 000	–
Two-generation reproductive toxicity (rat)				
Parental toxicity	–	21.7	911	–
Reproductive toxicity	138	–	911	–
Offspring toxicity	68	138	911	–
Developmental toxicity (rat)				
Maternal toxicity	100	400	2 000	–
Embryo/fetal toxicity	100	400	2 000	–
Developmental toxicity (rabbit)				
Maternal toxicity	10	20	–	250
Embryo/fetal toxicity	20	–	500	1 000

bw: body weight; LD_{50}: median lethal dose; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level
Source: Current monograph; Annex 1, references *118* and *121*

The Meeting noted that in all studies except for the 90-day dog study, metabolite R611965 was at least 10 times less toxic than chlorothalonil. The three dog studies with chlorothalonil gave variable responses and showed that the dog was not the most sensitive species for chlorothalonil toxicity. The ratio between the lowest NOAEL for chlorothalonil (1.8 mg/kg bw per day) in the database and that for R611965 (50 mg/kg bw per day) was approximately 25.

3.5 R611968 (2,4,5-trichloro-3-cyano-6-hydroxybenzamide)

R611968 (Fig. 7; company codes Compound 5, SDS-47523 and R5) is found in soil (aerobic, anaerobic) and rats.

(a) Genotoxicity

The results of genotoxicity assays using R611968 are summarized in Table 8. R611968 was negative for genotoxicity in vitro in a bacterial gene mutation test and a mouse lymphoma assay, but gave a positive response in the chromosomal aberration assay in human lymphocytes, both with and without metabolic activation. It was negative for genotoxicity in an in vivo mouse micronucleus test.

Fig. 7. Chemical structure of R611968

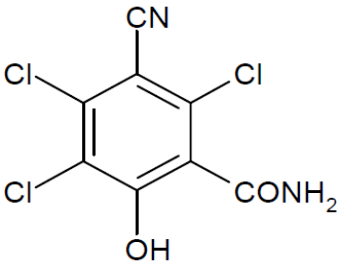


Table 8. Results of assays for the genotoxicity of R611968

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA pKM101 and WP2pKM101	3.0–5 000 µg/plate in DMSO (±S9)	99.5%	Negative	Sokolowski (2015c)
Chromosomal aberrations	Human lymphocytes	121.9–2 000 µg/mL in DMSO (−S9) 653.1–2 000 µg/mL in DMSO (+S9)	99.5%	Positive	Sokolowski (2015d)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	83.1–997.5 µg/mL in DMSO (−S9) 83.1–1 330 µg/mL in DMSO (+S9)	99.0%	Negative	Wollny (2015b)
In vivo					
Micronucleus formation[a]	Male CD-1 mice	125, 250 and 500 mg/kg bw (single oral gavage in CMC in Tween 80)	99.0%	Negative	Dunton (2015b)

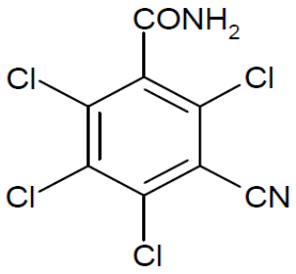
bw: body weight; CMC: 0.5% (w/v) aqueous carboxymethylcellulose; DMSO: dimethyl sulfoxide; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

[a] Proof of bone marrow exposure was demonstrated by Dunton (2015b).

3.6 R613636 (2,4,5,6-tetrachloro-3-cyanobenzamide)

R613636 (Fig. 8; company codes SDS-47525, M14, SDS-19221, R2, Compound 3 and CSCC548417) is a metabolite in soil, water and crops (rotated crops).

Fig. 8. Chemical structure of R613636



(a) Genotoxicity

The results of assays for the genotoxicity of R613636 are summarized in Table 9. R613636 was negative for genotoxicity in vitro in a bacterial gene mutation test, a gene mutation test in Chinese hamster lung cells and a mouse lymphoma assay, but gave a positive response in the chromosomal aberration test in human lymphocytes, both with and without metabolic activation. It was negative for genotoxicity in an in vivo mouse micronucleus test.

Table 9. Results of assays for the genotoxicity of R613636

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA pKM101 and WP2pKM101	10.0–5 000 µg/plate in DMSO (±S9)	98.6%	Negative	Sokolowski (2015e)
Chromosomal aberrations	Human lymphocytes	13.2–40.3 µg/mL in DMSO (−S9) 13.2–59.6 µg/mL in DMSO (+S9)	98.6%	Positive	Sokolowski (2015f)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	2.5–30.0 µg/mL in DMSO (−S9) 2.5–40.0 µg/mL in DMSO (+S9)	99.6%	Negative	Wollny (2015c)
Mammalian cell gene mutation	Chinese hamster lung V79 cells, *Hprt* locus	0.1–2.5 µg/mL in DMSO (−S9) 5.0–40.0 µg/mL in DMSO (+S9)	98.6%	Negative	Wollny (2015d)
In vivo					
Micronucleus formation[a]	Male CD-1 mice	312.5, 625 and 1 250 mg/kg bw (single oral gavage in CMC in Tween 80)	98.6%	Negative	Dunton (2015c)

bw: body weight; CMC: 0.5% (w/v) aqueous carboxymethylcellulose; DMSO: dimethyl sulfoxide; *Hprt*: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

[a] Proof of bone marrow exposure was demonstrated by Dunton (2015c).

Comments

Biochemical aspects

Following a single oral gavage administration of [^{14}C]chlorothalonil at 5 mg/kg bw to rats, the major route of elimination was via the faeces, with 85–91% of the administered dose excreted by 168 hours post-dosing. Urinary excretion accounted for 5.5–7.0% of the administered dose. Following a single oral administration of [^{14}C]chlorothalonil at 200 mg/kg bw to rats, the major route of elimination was via the faeces, with 99–115% of the administered radioactivity recovered by 168 hours post-dosing. Urinary excretion accounted for less than 3.0% of the administered dose. Following a single oral administration of [^{14}C]chlorothalonil at 5 mg/kg bw to bile duct–cannulated rats, the major route of elimination was via the faeces, with 75–80% of the administered radioactivity recovered by 72 hours post-dosing. Biliary elimination accounted for 12% of the administered dose, and urinary excretion accounted for 5.8–10% of the administered dose. Following a single oral administration of [^{14}C]chlorothalonil at 200 mg/kg bw to bile duct–cannulated rats, the major route of elimination was via the faeces, with 81–95% of the administered radioactivity recovered by 72 hours post-dosing. Biliary elimination accounted for 4.9–7.5% and urinary excretion accounted for 2.9–4.3% of the administered dose (Punler, 2013).

Following administration of a single oral gavage dose of [^{14}C]chlorothalonil at 5 mg/kg bw to rats, the C_{max} of radioactivity in blood was 0.21 µg equiv/g in males and 0.31 µg equiv/g in females, with a T_{max} of 8 hours post-dosing in males and 4 hours post-dosing in females. Following administration of a single oral gavage dose of 200 mg/kg bw, the C_{max} values in blood were 3.2 and 6.0 µg equiv/g in males and females, respectively, with a T_{max} of 12 hours post-dosing in both males and females (Punler, 2013).

Chlorothalonil was metabolized in the rat, forming up to seven metabolites through oxidation, hydroxylation and conjugation. The majority of the dose was excreted as unabsorbed parent in faeces. The most abundant component excreted in urine was R613823 (≤3.3% of the dose). The most abundant metabolite in plasma (29–38% of the total radioactivity AUC) was SDS-3701 (Punler, 2013). In an in vitro metabolism study using rat and human liver microsomes, no qualitative differences in the metabolite profiles of [^{14}C]chlorothalonil were noted, and no unique or disproportionate human in vitro metabolites were formed (Sayer, 2017).

Toxicological data

In a study of acute neurotoxicity, chlorothalonil was administered by gavage to groups of rats at a dose of 0, 100, 500 or 2000 mg/kg bw. The NOAEL for systemic toxicity and neurotoxicity was 2000 mg/kg bw, the highest dose tested (Herberth, 2014b).

In a 90-day neurotoxicity study, groups of rats were fed diets containing chlorothalonil at a concentration of 0, 30, 300 or 3000 ppm (equal to 0, 2.1, 22.0 and 232.1 mg/kg bw per day for males and 0, 2.4, 24.2 and 243.2 mg/kg bw per day for females, respectively). The NOAEL for systemic toxicity was 300 ppm (equal to 22.0 mg/kg bw per day), based on decreased body weights and reduced feed consumption at 3000 ppm (equal to 232.1 mg/kg bw per day). The NOAEL for neurotoxicity was 3000 ppm (equal to 232.1 mg/kg bw per day), the highest dose tested (Brammer, 2004).

The Meeting concluded that chlorothalonil is not neurotoxic.

In an immunotoxicity study, groups of female mice were fed diets containing chlorothalonil at a concentration of 0, 50, 750 or 2000 ppm (equal to 0, 8.2, 120.9 and 345.1 mg/kg bw per day, respectively) for a period of 28 days. The NOAEL for immunotoxicity was 2000 ppm (equal to 345.1 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity was 50 ppm (equal to 8.2 mg/kg bw per day), based on increased kidney weights at 750 ppm (equal to 120.9 mg/kg bw per day) (Donald, 2012).

The Meeting concluded that chlorothalonil is not immunotoxic.

No evidence of chlorothalonil-mediated estrogenic or androgenic activity was observed in a battery of in vivo and in vitro tests (Wilga, 2011; Willoughby, 2011a,b, 2012; Wagner, 2012; Sawhney Coder, 2012a,b).

Chlorothalonil is considered to be non-phototoxic in vitro (Gehrke, 2014).

Toxicological data on metabolites and/or degradates

SDS-3701 (2,5,6-trichloro-4-hydroxyisophthalonitrile; plant, ruminant and rat metabolite and soil and water degradate)

The oral LD_{50} of SDS-3701 in rats was between 50 and 300 mg/kg bw (Beerens-Heijnen, 2005a). SDS-3701 was not irritating to rabbit skin (Beerens-Heijnen, 2005b).

In a 13-week dietary toxicity study, SDS-3701 was administered to rats at a concentration of 0, 15, 50 or 250 ppm (equal to 0, 1.0, 3.0 and 16 mg/kg bw per day for males and 0, 1.0, 4.0 and 17 mg/kg bw per day for females, respectively). The NOAEL was 50 ppm (equal to 3.0 mg/kg bw per day), based on reduced body weights, body weight gains and feed consumption in females and changes in haematological and clinical chemistry parameters seen in both sexes at 250 ppm (equal to 16 mg/kg bw per day) (Van Otterdijk, 2007a).

In a 120-day dietary toxicity study (pre-GLP), SDS-3701 was administered to rats at a concentration of 0, 10, 50, 100 or 200 ppm (equivalent to 0, 1, 5, 10 and 20 mg/kg bw per day, respectively). The NOAEL was 100 ppm (equivalent to 10 mg/kg bw per day), based on decreased body weight gains in females at 200 ppm (equivalent to 20 mg/kg bw per day) (Hastings & Jessup, 1975).

SDS-3701 was tested for genotoxicity in vitro in a bacterial gene mutation test (Verspeek-Rip, 2004a), a forward mutation test in Chinese hamster lung cells (Chang, 2017) and a mouse lymphoma assay (Verspeek-Rip, 2005a), with and without metabolic activation. It gave a positive response in the in vitro mouse lymphoma assay only, with and without metabolic activation. It was negative for genotoxicity in vivo in the mouse micronucleus test (Buskens, 2004a) and for unscheduled DNA synthesis (Honarvar, 2005). The Meeting confirmed the previous conclusion of the 2009 Meeting that SDS-3701 is unlikely to exhibit mutagenic activity in vivo.

R417888 (2-carbamyl-3,5,6-trichloro-4-cyanobenzenesulfonic acid; crop and rat metabolite and soil degradate)

The acute oral LD_{50} of R417888 in rats was greater than 2000 mg/kg bw (Van Hygevoort, 2005).

In a 90-day dietary toxicity study, R417888 was administered to rats at a concentration of 0, 150, 600 or 2400 ppm (equal to 0, 13.0, 54.0 and 192 mg/kg bw per day for males and 0, 15.0, 56.0 and 218 mg/kg bw per day for females, respectively). The NOAEL was 2400 ppm (equal to 192 mg/kg bw per day), the highest dose tested (Van Otterdijk, 2007b).

R417888 was tested for genotoxicity in vitro in bacterial gene mutation tests (Verspeek-Rip, 2005b; Sokolowski, 2007), a chromosomal aberration test in human lymphocytes (Kunz, 2007) and mouse lymphoma assays (Verspeek-Rip, 2006; Wollny, 2007), with and without metabolic activation. It gave a positive response in the chromosomal aberration assay without metabolic activation (Kunz, 2007) and one mouse lymphoma assay with metabolic activation (Verspeek-Rip, 2006), but it was negative in a repeat mouse lymphoma assay at higher concentrations (Wollny, 2007). It was negative for genotoxicity in vivo in a mouse micronucleus test (Meerts, 2005) and for unscheduled DNA synthesis (Honarvar, 2006).

The Meeting concluded that R417888 is unlikely to be genotoxic in vivo.

SYN548764 (4-carbamyl-2,3,5-trichloro-6-cyanobenzenesulfonic acid; crop and rat metabolite and soil degradate)

SYN548764 was tested for genotoxicity in vitro in a bacterial gene mutation test (Sokolowski, 2015a), a mouse lymphoma assay (Wollny, 2015a) and a chromosomal aberration test in human lymphocytes (Sokolowski, 2015b), with and without metabolic activation. It gave an equivocal response in the chromosomal aberration test only, in the absence of metabolic activation. It was negative for genotoxicity in the in vivo mouse micronucleus test (Dunton, 2015a).

The Meeting concluded that SYN548764 is unlikely to be genotoxic in vivo.

R611965 (3-carbamyl-2,4,5-trichlorobenzoic acid; crop and rat metabolite and soil degradate)

The acute oral LD_{50} of R611965 in rats was greater than 2000 mg/kg bw (Beerens-Heijnen, 2005c). R611965 was not irritating to rabbit skin (Beerens-Heijnen, 2005d).

In a 90-day toxicity study, R611965 was administered to rats at a dietary concentration of 0, 150, 600 or 2400 ppm (equal to 0, 14.0, 57.0 and 197 mg/kg bw per day for males and 0, 14.0, 60.0 and 223 mg/kg bw per day for females, respectively). The NOAEL was 2400 ppm (equal to 197 mg/kg bw per day), the highest dose tested (Van Otterdijk, 2007c).

R611965 was negative in vitro in a bacterial gene mutation test (Verspeek-Rip, 2004b) and a mouse lymphoma assay (Verspeek-Rip, 2005c), with and without metabolic activation. It was negative for genotoxicity in the in vivo mouse micronucleus test (Buskens, 2004b). The Meeting confirmed the conclusion of the 2010 Meeting that R611965 is unlikely to be genotoxic.

R611968 (2,4,5-trichloro-3-cyano-6-hydroxybenzamide; rat metabolite and soil degradate)

R611968 was tested for genotoxicity in vitro in a bacterial gene mutation test (Sokolowski, 2015c), a chromosomal aberration test in human lymphocytes (Sokolowski, 2015d) and a mouse lymphoma assay (Wollny, 2015b), with and without metabolic activation. It gave a positive response in the chromosomal aberration assay (Sokolowski, 2015d) only, both with and without metabolic activation. It was negative for genotoxicity in an in vivo mouse micronucleus test (Dunton, 2015b).

The Meeting concluded that R611968 is unlikely to be genotoxic in vivo.

R613636 (2,4,5,6-tetrachloro-3-cyanobenzamide; crop metabolite and soil and water degradate)

R613636 was tested for genotoxicity in vitro in a bacterial gene mutation test (Sokolowski, 2015e), a gene mutation test in Chinese hamster lung cells (Wollny, 2015d), a chromosomal aberration test in human lymphocytes (Sokolowski, 2015f) and a mouse lymphoma assay (Wollny, 2015c), with and without metabolic activation. It gave a positive response in the chromosomal aberration test only, both with and without metabolic activation. It was negative for genotoxicity in an in vivo mouse micronucleus test (Dunton, 2015c).

The Meeting concluded that R613636 is unlikely to be genotoxic in vivo.

Toxicological evaluation

The Meeting concluded that no revision of the ADI or ARfD for chlorothalonil was necessary.

The Meeting concluded that no revision of the ADI or ARfD for metabolite SDS-3701 was necessary.

For metabolite R611965, the Meeting concluded that it would be covered by the ADI and ARfD of chlorothalonil, but noted that it is at least 10 times less potent than chlorothalonil in a wide range of studies.

The Meeting concluded that metabolite R417888 would be covered by the ADI and ARfD of chlorothalonil because of its lower acute and repeated-dose toxicity in comparison with the parent compound.

The Meeting concluded that SYN548764, R611968 and R613636 are unlikely to be genotoxic in vivo. Following the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015), the Meeting concluded that, for chronic toxicity, these three metabolites could be assessed using the threshold of toxicological concern (TTC) approach. All three metabolites are categorized in Cramer class III, and therefore a TTC of 1.5 µg/kg bw per day applies.

The ADI for chlorothalonil is 0–0.02 mg/kg bw. This ADI applies to chlorothalonil plus the metabolites R611965 and R417888, expressed as chlorothalonil.

The ARfD for chlorothalonil is 0.6 mg/kg bw. This ARfD applies to chlorothalonil plus the metabolites R611965 and R417888, expressed as chlorothalonil.

Acceptable daily intake (ADI) (applies to chlorothalonil plus R611965 and R417888, expressed as chlorothalonil)

0–0.02 mg/kg bw

Acute reference dose (ARfD) (applies to chlorothalonil plus R611965 and R417888, expressed as chlorothalonil)

0.6 mg/kg bw

Critical end-points for setting guidance values for exposure to chlorothalonil and metabolites

Short-term studies of toxicity	
Target/critical effect	Body weight
Lowest relevant inhalation NOAEC	0.001 mg/L (rat)
Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw, highest dose tested (rat)
Subchronic neurotoxicity NOAEL	232.1 mg/kg bw per day, highest dose tested (rat)
Other toxicological studies	
Immunotoxicity NOAEL	345 mg/kg bw per day, highest dose tested (mouse)
Endocrine effects	No evidence in in vitro or in vivo tests
Phototoxicity	Not phototoxic in vitro
Studies on metabolites	
SDS-3701	
Acute toxicity	
Rat, LD_{50}, oral	$50 < LD_{50} < 300$ mg/kg bw
Rabbit, dermal irritation	Not irritating
Short-term studies of toxicity	
Target/critical effect	Body weight, body weight gain, changes in haematology and clinical chemistry parameters
Lowest relevant oral NOAEL	3 mg/kg bw per day (rat)
Genotoxicity	

	No evidence of genotoxicity in vivo
R417888	
Acute toxicity	
Rat, LD_{50}, oral	>2 000 mg/kg bw (rat)
Short-term studies of toxicity	
Target/critical effect	None
Lowest relevant oral NOAEL	192 mg/kg bw per day, highest dose tested (rat)
Genotoxicity	
	No evidence of genotoxicity in vivo
SYN548764	
Genotoxicity	
	No evidence of genotoxicity in vivo
R611965	
Acute toxicity	
Rat, LD_{50}, oral	>2 000 mg/kg bw
Rabbit, dermal irritation	Not irritating
Short-term studies of toxicity	
Target/critical effect	None
Lowest relevant oral NOAEL	197 mg/kg bw per day, highest dose tested (rat)
Genotoxicity	
	No evidence of genotoxicity
R611968	
Genotoxicity	
	No evidence of genotoxicity in vivo
R613636	
Genotoxicity	
	No evidence of genotoxicity in vivo

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