GLYPHOSATE (addendum)

First draft prepared by
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Explanation

Glyphosate (*N*-(phosphonomethyl)glycine) is a non-selective systemic herbicide that was last evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2004, when a group acceptable daily intake (ADI) for glyphosate and aminomethylphosphonic acid (AMPA), the main metabolite of glyphosate, of 0–1 mg/kg body weight (bw) was established based on a no-observed-adverse-effect level (NOAEL) of 100 mg/kg bw per day for salivary gland alterations in a long-term study of toxicity and carcinogenicity in rats and a safety factor of 100. The 2004 JMPR concluded that it was not necessary to establish an acute reference dose (ARfD) for glyphosate.

Metabolism studies in genetically modified soya beans and maize containing the glyphosate-*N*-acetyltransferase (*GAT*) gene demonstrated that new metabolites are formed that were not observed in conventional crops. The major metabolite in the new maize and soya bean varieties was *N*-acetyl-glyphosate (which may be degraded to glyphosate in the rat), whereas glyphosate, *N*-acetyl-AMPA and AMPA were found in low concentrations in the edible parts of the crops. The present Meeting was asked by the Codex Committee on Pesticide Residues to evaluate newly submitted studies on toxicokinetics and metabolism, acute oral toxicity, subchronic toxicity and genotoxicity for *N*-acetyl-glyphosate and on acute oral toxicity and genotoxicity for *N*-acetyl-AMPA.

All pivotal studies were certified as complying with good laboratory practice or an approved quality assurance programme.

Evaluation for acceptable daily intake

1. *N*-Acetyl-glyphosate (metabolite of glyphosate in genetically modified plants)

In a study on toxicokinetics and metabolism conducted according to United States Environmental Protection Agency test guideline OPPTS 870.7485, 45 male Sprague-Dawley rats (Crl:CD(SD)IGS BR) received [^{14}C]*N*-acetyl-glyphosate, sodium salt (purity 84.3%; radiochemical purity 99.2%), as a solution in water by oral gavage at a single dose of 15 mg free acid equivalent per kilogram body weight; the dose volume was 5 ml/kg bw. A correction factor of 0.674 was used to derive the free acid equivalent from the weight of the test substance. Blood was collected from four animals pre-dosing and from four animals per time point at 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hours post-dosing. Excreta were collected from five animals at specified intervals through 168 hours post-dosing. Plasma, excreta and carcass were analysed for content of radioactivity using liquid scintillation counting. Selected samples of plasma, urine and faeces were analysed for unchanged parent compound and metabolites.

The mean total recovery of radioactivity was 95.5%, with 66.1% in urine, 26.4% in faeces, 2.79% in cage wash and wipe and 0.23% in residual carcass (the values do not include data for one animal [C16498] that had suspected urine contamination of faeces). More than 90% of the total radioactivity was eliminated by 48 hours post-dosing.

The mean maximum concentrations (C_{max}) in blood and plasma were 2.93 and 5.31 µg equivalent (eq) per gram at 1 and 2 hours post-dosing, respectively. Radioactivity was eliminated from blood and plasma with half-life values of 20.1 and 15.6 hours, respectively. Comparison of blood and plasma values for area under the curve ($AUC_{0-\infty}$ = 12.1 and 20.8 µg eq·h/g, respectively) indicates that [^{14}C]*N*-acetyl-glyphosate distributed preferentially into plasma.

Unchanged [^{14}C]*N*-acetyl-glyphosate recovered in urine and faeces represented over 99% of the administered radioactivity. A metabolite, glyphosate, was detected in faeces and represented less than 1% of the total radioactivity. Plasma radioactivity consisted entirely of unchanged [^{14}C]*N*-acetyl-glyphosate (Cheng & Howard, 2004).

In an acute oral toxicity study conducted according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 423 (Acute Toxic Class Method), five male and five female Sprague-Dawley rats (Crl:CD(SD)IGS BR) received *N*-acetyl-glyphosate, sodium salt (purity 84.3%), as a solution in water by oral gavage at a single dose of 5000 mg free acid equivalent per kilogram body weight (limit test); the dose volume was 10 ml/kg bw. A correction factor of 0.674 was used to derive the free acid equivalent from the weight of the test substance. Surviving animals were observed for clinical signs of toxicity, body weight effects and mortality for up to 14 days after dosing. All rats were examined for gross pathological changes.

Mortality was observed in one female at the 4-hour post-dosing observation and in one male and one female on the day after dosing. Clinical signs of toxicity observed in male and female rats included hypoactivity, irregular respiration, liquid or soft faeces, light-brown perineal staining, brown nasal crust and/or squinted eyes. All clinical signs of toxicity were resolved in all surviving animals by day 3 (2 days after dosing). All surviving animals gained weight from the initiation of dosing to study termination. At necropsy, findings were noted in the one male and two females that were found dead prior to the terminal sacrifice; however, no abnormal findings were noted in the remaining animals that survived to terminal sacrifice. Findings involved the lungs (mottled or discoloured bright red), liver (discoloured black), stomach (soft and/or with yellow fluid or gel-like clear liquid and red walls), abdominal cavity (clear fluid or reddish liquid) and duodenum, jejunum and ileum (fluid).

Under the conditions of this study, the oral median lethal dose (LD_{50}) of *N*-acetyl-glyphosate in rats was greater than 5000 mg/kg bw. The mortality rate was 20% in males and 40% in females dosed at 5000 mg/kg bw (Vegarra, 2004).

In a study of toxicity conducted according to OECD Test Guideline 408, groups of 10 male and 10 female Crl:CD(SD) rats were fed diets containing *N*-acetyl-glyphosate, sodium salt (purity 81.8%), at a concentration of 0, 180, 900, 4500 or 18 000 parts per million (ppm) (expressed as free acid equivalent), equal to 0, 11.3, 55.7, 283 and 1157 mg free acid equivalent per kilogram body weight per day in males and 0, 13.9, 67.8, 360 and 1461 mg free acid equivalent per kilogram body weight per day in females, for 90 days. A correction factor of 0.63 was used to derive the free acid equivalent from the weight of the test substance. Parameters evaluated included body weight, body weight gain, feed consumption, feed efficiency, clinical signs, ophthalmological evaluations, neurobehavioural evaluations (abbreviated functional observational battery [FOB], including forelimb and hindlimb grip strength, motor activity evaluation), haematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic pathology. At test days 82 and 83 for male and female rats, respectively, blood and urine samples were collected and processed for the analyses of *N*-acetyl-glyphosate (IN-MCX20) and its metabolites, glyphosate (DPX-B2856) and *N*-acetyl-AMPA (IN-EY252). Urine samples were pooled prior to analysis, and individual blood samples were processed to produce plasma for subsequent analyses. Pooled urine and individual plasma samples were stored frozen until analysed. The analytes were quantified by high-performance liquid chromatography with detection by tandem mass spectrometry (LC-MS/MS).

No test substance–related mortality or clinical signs of toxicity were observed for any dietary concentration in either males or females. The death of one male rat at 4500 ppm on study day 42 was considered accidental, based on necropsy findings.

There were no adverse test substance–related effects on body weights or body weight gains in females, whereas in males at 18 000 ppm, body weights and body weight gains were decreased (92% and 86% of control, respectively) (Table 1). Feed efficiency was slightly reduced in males and females at 18 000 ppm (92% of control).

No test substance–related effects were observed on ophthalmology or neurobehavioural parameters (FOB, including forelimb and hindlimb grip strength, motor activity evaluation).

No test substance–related effects were observed on clinical pathology, organ weights, or gross or microscopic pathology.

The urinary concentrations of *N*-acetyl-glyphosate (IN-MCX20) increased with the increasing dietary levels of this test substance (Table 1). Concentrations of glyphosate (DPX-B2856) and *N*-acetyl-AMPA (IN-EY252) were detected above the limit of detection at higher dietary levels (900–18 000 ppm), but at or below the limit of detection in urine samples from the 180 ppm dietary group. Further, the concentrations of these metabolites were much higher in urine samples from male rats than from corresponding female rats at 4500 and 18 000 ppm. Neither *N*-acetyl-glyphosate nor either of its metabolites was detected in urine from control rats.

The plasma concentrations of *N*-acetyl-glyphosate also increased with the increasing dietary levels of this test substance (Table 1). Concentrations of *N*-acetyl-glyphosate were less than 1.0 µg/ml for males and females at 180 ppm and increased from a mean of approximately 2 µg/ml up to approximately 15 µg/ml for the other dietary groups. In contrast to urine samples, little to no glyphosate or *N*-acetyl-AMPA was detected in plasma for all dietary levels. Neither *N*-acetyl-glyphosate nor either of its metabolites was detected in plasma from control rats.

The proposed pathway for the metabolism of *N*-acetyl-glyphosate in rats is outlined in Figure 1.

In conclusion, the analytical results from urine and plasma samples demonstrated that *N*-acetyl-glyphosate was metabolized in rats to small quantities of glyphosate and *N*-acetyl-AMPA.

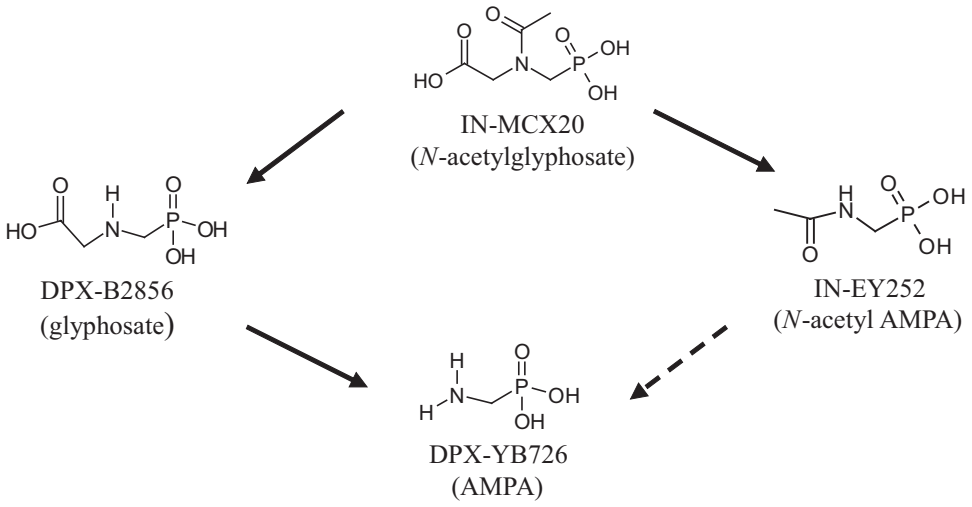
The NOAEL for the subchronic toxicity of *N*-acetyl-glyphosate was 4500 ppm (equal to 283 mg/kg bw per day), based on slightly decreased body weight gain in male rats at 18 000 ppm (equal to 1157 mg/kg bw per day) (MacKenzie, 2007; Shen, 2007).

Table 1. Summary of selected findings

	Males					Females				
	Dietary concentration (ppm)									
	0	180	900	4500	18 000	0	180	900	4500	18 000
Dose (mg/kg bw per day)	0	11.3	55.7	283	1157	0	13.9	67.8	360	1461
Body weight, day 91 (g)	583	563	575	559	534	287	303	294	303	283
Body weight gain, days 0–91 (g)	347	328	338	324	299*	111	127	121	127	107
Feed consumption, days 0–91 (g/animal per day)	29.8	28.3	28.3	28.2	27.8	19.4	20.2	19.4	21.2	20.3
Daily feed efficiency, days 0–91 (g body weight gain/g feed consumed)	0.128	0.127	0.131	0.126	0.118	0.063	0.069	0.068	0.066	0.058
Urine										
N-Acetyl-glyphosate (µg/ml)	ND	53.8	361	1150	2220	ND	71.5	360	1110	2020
Glyphosate (µg/ml)	ND	< 0.05	0.165	27.1	64.4	ND	< 0.05	0.360	2.92	4.02
N-Acetyl-AMPA (µg/ml)	ND	< 0.05	0.127	1.50	5.38	ND	< 0.05	0.179	< 0.5	2.89
Plasma										
N-Acetyl-glyphosate (µg/ml)	ND	0.33	1.85	4.77	14.83	ND	0.44	2.32	8.35	13.46
Glyphosate (µg/ml)	ND	ND	ND	ND	ND	ND	ND	ND	0.32	< 0.1
N-Acetyl-AMPA (µg/ml)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

From MacKenzie (2007); Shen (2007)
ND, not detected; * $P > 0.05$

Figure 1. Proposed metabolism of N-acetyl-glyphosate (IN-MCX20) in rats



Note: The metabolite AMPA (DPX-YB726) was not detected in the submitted rat studies with *N*-acetyl-glyphosate.
Source: Shen (2007)

In a reverse gene mutation assay in bacteria conducted according to OECD Test Guideline 471, *Salmonella typhimurium* (strains TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to *N*-acetyl-glyphosate, sodium salt (purity 84.3%), using deionized water as solvent, in the presence and absence of S9 metabolic activation. A correction factor of 0.674 was used to derive the free acid equivalent from the weight of the test substance, and all test substance

concentrations are expressed in terms of the free acid. Nominal concentrations of 100, 333, 1000, 3330 and 5000 µg/plate were evaluated in two trials using standard plate incorporation methods. The highest dose level was set based on the results of a dose range–finding study using tester strains TA100 and WP2*uvr*A and 10 doses of test substance ranging from 6.67 to 5000 µg/plate.

There was no evidence of cytotoxicity by the test compound. The number of revertants at all concentrations of the test substance was similar to that for concurrent controls in trials both with and without activation, with the exception of a 3.3-fold increase observed with tester stain TA1537 treated with 333 µg test substance per plate in the absence of S9 mix. However, this increase was not dose-responsive and did not meet the criteria for a positive evaluation. As the vehicle control value for tester strain TA98 in the absence of S9 mix was not in the acceptable range in one of the earlier trials, a third trial with TA98 was run in the absence of S9, with no increase in the number of revertants per plate. Under the conditions of this study, *N*-acetyl-glyphosate was considered to be negative for mutagenic activity in non-activated and S9-activated bacterial test systems (Mecchi, 2004).

In an in vitro mammalian cell gene mutation test conducted according to OECD Test Guideline 476, *N*-acetyl-glyphosate, sodium salt (purity 81.8%), dissolved in water was tested for its ability to induce forward mutations at the *HPRT* locus in Chinese hamster ovary (CHO) cells. A correction factor of 0.63 was used to derive the free acid equivalent from the weight of the test substance, and all test substance concentrations are expressed in terms of the free acid. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the results from a preliminary cytotoxicity assay, concentrations of 250–2091 µg/ml were used in the mutagenesis assay both with and without metabolic activation, and the same concentrations were used for the independent repeats. Ethyl methanesulfonate (EMS) and benzo(*a*)pyrene (BaP) served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 5 hours in both experiments, without and with metabolic activation. At the end of the exposure period, cells were washed and subcultured at 2- to 3-day intervals for a 7-day expression period. This was followed by incubation of the cells for 9 days in selection medium containing 6-thioguanine.

No visible precipitate in the treatment medium and no substantial toxicity were observed at any concentration in either non-activated or activated test systems. No increases in mutant frequencies were observed in any test substance treatment group, whereas the positive control substances EMS and BaP resulted in a marked increase in mutant frequency. Under the conditions of this study, *N*-acetyl-glyphosate was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Glatt, 2006).

In an in vitro mammalian chromosomal aberration test conducted according to OECD Test Guideline 473, the clastogenic potential of *N*-acetyl-glyphosate, sodium salt (purity 84.3%), using deionized water as solvent, was tested in CHO cells in the presence and absence of S9 metabolic activation. A correction factor of 0.674 was used to derive the free acid equivalent from the weight of the test substance, and all test substance concentrations are expressed in terms of the free acid. Negative and vehicle controls and positive controls (cyclophosphamide and mitomycin C for the tests with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. The treatment period was for 3 hours with and without metabolic activation, and cultures were harvested approximately 20 hours from the initiation of treatment. In addition, a set of cultures was treated for approximately 20 hours without metabolic activation and harvested approximately 20 hours from the initiation of treatment. Replicate cultures were used at each concentration (19.0, 27.1, 38.8, 55.4, 79.1, 113, 161, 231, 329, 471, 672, 960, 1370, 1960 and 2800 µg/ml) with and without metabolic activation, for the negative and vehicle controls, and for each of the two concentrations of the positive control substances. Cultures treated with 960, 1370, 1960 and 2800 µg/ml without

metabolic activation (3- and 20-hour treatments) and with metabolic activation were analysed for chromosomal aberrations. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

All dose levels tested with and without metabolic activation were non-toxic. No significant increase in cells with chromosomal aberrations, polyploidy or endoreduplication was observed in the cultures analysed. The positive and vehicle controls fulfilled the requirements for a valid test. Under the conditions of this study, *N*-acetyl-glyphosate was concluded to be negative for the induction of structural and numerical chromosomal aberrations in cultured CHO cells with and without an exogenous metabolic activation system (Murli, 2004).

In a mammalian erythrocyte micronucleus test conducted according to OECD Test Guideline 474, groups of male and female Crl:CD1(ICR) mice received *N*-acetyl-glyphosate, sodium salt (purity 81.8%), dissolved in water at a single oral (gavage) dose of 500, 1000 or 2000 mg/kg bw in a volume of 10 ml/kg bw. A correction factor of 0.63 was used to derive the free acid equivalent from the weight of the test substance. Concurrent control groups were administered water (vehicle control) or cyclophosphamide at 30 mg/kg bw (positive control). The vehicle control and the low-dose and intermediate-dose groups contained 10 animals of each sex, the high-dose group contained 14 animals of each sex and the positive control group consisted of 5 animals of each sex. Half of the animals in each test substance and vehicle control group were sacrificed at each time point, approximately 24 and 48 hours post-dosing, respectively. The positive control group was sacrificed approximately 24 hours post-dosing. Bone marrow smears were prepared immediately after the sacrifices, 2000 polychromatic erythrocytes (PCEs) per animal were evaluated for micronuclei and 1000 total erythrocytes per animal were evaluated for bone marrow toxicity.

No clinical signs of toxicity or mortality were observed in the range-finding experiment at a single oral dose of 1500 or 2000 mg/kg bw. In the main study, there were no significant changes in body weight or body weight gain in either male or female animals administered the test substance or in the vehicle or positive control groups. No clinical signs of toxicity were observed at any dose level in male or female mice exposed to the test substance. No abnormalities were detected in the vehicle or positive control groups. No mortality occurred during the study.

No statistically significant increases in micronucleated PCE frequency were observed in any evaluated test substance–treated group of male or female animals at either time point. There were no statistically significant decreases in PCEs among 1000 erythrocytes. A statistically significant increase in micronucleated PCE frequency was found in positive control animals of both sexes. Under the conditions of this study, *N*-acetyl-glyphosate was considered not to be clastogenic or aneugenic in vivo in mice (Donner, 2006).

Genotoxicity studies with *N*-acetyl-glyphosate are summarized in Table 2.

2. *N*-Acetyl-AMPA (metabolite of glyphosate in genetically modified plants)

In an acute oral toxicity study conducted according to OECD Test Guideline 425 (Up-and-Down Procedure), three fasted female Crl:CD(SD) rats received *N*-acetyl-AMPA (purity 79%), suspended in deionized water, by oral gavage at a single dose of 5000 mg/kg bw; the dose volume was 20 ml/kg bw. The animals were dosed one at a time at a minimum of 48-hour intervals. Animals were observed for clinical signs of toxicity, body weight effects and mortality for 14 days after dosing. All animals were examined to detect grossly observable evidence of organ or tissue damage or dysfunction.

No mortalities were observed. Clinical signs of toxicity were observed in all rats and included diarrhoea, dark eyes, lethargy, high posture, stained fur/skin, wet fur, ataxia and/or hyper-reactivity. All animals appeared normal by day 3 or earlier and throughout the remainder of the study. There was

Table 2. Summary of genotoxicity studies with N-acetyl-glyphosate

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537); *Escherichia coli* (WP2*uvr*A)	±S9 mix: 0–5000 µg/plate	84.3	Negative	Mecchi (2004)
Gene mutation, *HPRT* locus	CHO cells	±S9 mix: 0–2091 µg/ml	81.8	Negative	Glatt (2006)
Chromosomal aberration	CHO cells	±S9 mix: 0–2800 µg/ml	84.3	Negative	Murli (2004)
In vivo					
Micronucleus induction	Male and female Crl:CD1(ICR) mouse bone marrow erythroblasts	0, 500, 1000 and 2000 mg/kg bw; single oral (gavage) administration	81.8	Negative	Donner (2006)

CHO, Chinese hamster ovary; S9, 9000 × *g* rat liver supernatant

no effect on body weights, and no test substance–related gross lesions were observed at necropsy. Under the conditions of this study, the oral LD_{50} for *N*-acetyl-AMPA was greater than 5000 mg/kg bw for female rats (Carpenter, 2007).

In a reverse gene mutation assay in bacteria conducted according to OECD Test Guideline 471, *Salmonella typhimurium* (strains TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to *N*-acetyl-AMPA (purity 76%), using deionized water as solvent, in the presence or absence of S9 metabolic activation. The dosing solutions were adjusted to compensate for the purity of the test substance using a correction factor of 1.45. In the initial toxicity–mutation test, dose levels of 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/plate were evaluated using standard plate incorporation methods. In the confirmatory mutagenicity test, dose levels of 50, 150, 500, 1500 and 5000 µg/plate were evaluated. The highest dose level was set based on findings of the initial toxicity–mutation test and the guideline limit dose for this test system. The test substance was administered to the test system as a solution in water at a concentration of 50 mg/ml. Neither precipitate nor appreciable toxicity was observed. The positive and solvent controls fulfilled the requirements for a valid test.

The number of revertants at all concentrations of the test substance was similar to that for concurrent controls in trials both with and without activation. Under the conditions of this study, *N*-acetyl-AMPA was negative for mutagenic activity in non-activated and S9-activated bacterial test systems (Wagner & Klug, 2007).

In an in vitro mammalian cell gene mutation test conducted according to OECD Test Guideline 476, *N*-acetyl-AMPA (purity 72%) dissolved in water was tested for its ability to induce forward mutations at the *HPRT* locus in CHO cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. The dosing solutions were adjusted to compensate for the purity of the test substance using a correction factor of 1.389. Based on the results from a preliminary cytotoxicity assay, concentrations of 100–1531 µg/ml were used in the mutagenesis assay both with and without metabolic activation, and the same concentrations were used for the independent repeats. EMS and BaP served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 5 hours in both experiments, without and with metabolic activation. At the end of the exposure period, cells were washed and subcultured at 2- to 3-day intervals for a 7-day expression period. This was followed by incubation of the cells for 10 days in selection medium containing 6-thioguanine.

No visible precipitate in the treatment medium and no substantial toxicity were observed at any concentration in either non-activated or activated test systems. In trial 1, a high spontaneous mutant frequency was observed in both test conditions, resulting in an invalid test. The test was repeated (trial 2) with the same concentrations, and no positive responses were observed. The positive control substances EMS and BaP resulted in a marked increase in mutant frequency. Under the conditions of this study, *N*-acetyl-AMPA was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Glatt, 2007).

In an in vitro mammalian chromosomal aberration test conducted according to OECD Test Guideline 473, the clastogenic potential of *N*-acetyl-AMPA (purity 76%), using deionized water as solvent, was tested in human peripheral blood lymphocytes in the presence or absence of S9 metabolic activation. The dosing solutions were adjusted to compensate for the purity of the test substance using a correction factor of 1.45. A vehicle control and positive controls (cyclophosphamide and mitomycin C for the tests with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. In the preliminary toxicity assay, the maximum concentration tested was 1530 µg/ml (10 mmol/l). In the chromosomal aberration assay, the concentrations tested were 191.25, 382.5, 765 and 1530 µg/ml, and the upper three concentrations were selected for evaluation. Cells were treated for 4 and 20 hours (non-activated test system) and 4 hours (activated test system). After exposure to colcemid, metaphase cells were harvested 20 hours following the initiation of treatment. Cells were evaluated for toxicity (mitotic inhibition), then structural and numerical chromosomal aberrations. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

There was no precipitation of the test substance in the treatment medium, and no substantial toxicity was observed at any concentration in all treatment groups. There were no statistically significant increases in structural chromosomal aberrations or in polyploidy at any of the concentrations evaluated. Positive controls induced the appropriate response. Under the conditions of this study, *N*-acetyl-AMPA was concluded to be negative for the induction of structural and numerical chromosomal aberrations in both the non-activated and S9-activated test systems in the in vitro mammalian chromosomal aberration test using human peripheral blood lymphocytes (Gudi & Rao, 2007).

In a mammalian erythrocyte micronucleus test conducted according to OECD Test Guideline 474, groups of male and female Crl:CD1(ICR) mice received *N*-acetyl-AMPA (purity 72%) dissolved in water at a single oral (gavage) dose of 500, 1000 or 2000 mg/kg bw in a volume of 10 ml/kg bw. A correction factor based on the percentage of active ingredient was used for preparation of the dosing solutions. Concurrent control groups were administered water (vehicle control) or cyclophosphamide (positive control) at 30 mg/kg bw. The vehicle control and the low-lose and intermediate-dose groups contained 10 animals of each sex, the high-dose group contained 14 animals of each sex and the positive control group consisted of 5 animals of each sex. Half of the animals in each test substance and vehicle control group were sacrificed at each time point, approximately 24 and 48 hours post-dosing, respectively. The positive control group was sacrificed approximately 24 hours post-dosing. Bone marrow smears were prepared immediately after the sacrifices; 2000 PCEs per animal were evaluated for micronuclei, and 1000 total erythrocytes per animal were evaluated for bone marrow toxicity.

No clinical signs of toxicity or mortality were observed in the range-finding experiment at a single oral dose of 2000 mg/kg bw. In the main study, there were no significant changes in body weight or body weight gain in either male or female animals administered the test substance or in the vehicle or positive control groups. No clinical signs of toxicity were observed at any dose level in male or female mice exposed to the test substance. No abnormalities were detected in the vehicle or positive control groups. No mortality occurred during the study.

Table 3. Summary of genotoxicity studies with N-acetyl-AMPA

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537); *Escherichia coli* (WP2*uvr*A)	±S9 mix: 0–5000 µg/plate	76	Negative	Wagner & Klug (2007)
Gene mutation, *HPRT* locus	CHO cells	±S9 mix: 0–1531 µg/ml	72	Negative	Glatt (2007)
Chromosomal aberration	Human peripheral blood lymphocytes	±S9 mix: 0–1530 µg/ml	76	Negative	Gudi & Rao (2007)
In vivo					
Micronucleus induction	Male and female Crl:CD1(ICR) mouse bone marrow erythroblasts	0, 500, 1000 and 2000 mg/kg bw; single oral (gavage) administration	72	Negative	Donner (2007)

CHO, Chinese hamster ovary; S9, 9000 × *g* rat liver supernatant

No statistically significant increases in micronucleated PCE frequency were observed in any evaluated test substance–treated group of male or female animals at either time point. There were no statistically significant decreases in PCEs among 1000 erythrocytes. A statistically significant increase in micronucleated PCE frequency was found in positive control animals of both sexes. Under the conditions of this study, *N*-acetyl-AMPA was considered not to be clastogenic or aneugenic in vivo in mice (Donner, 2007).

Genotoxicity studies with *N*-acetyl-AMPA are summarized in Table 3.

3. Comparison of toxicological properties of glyphosate and its plant metabolites, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA

The comparison of key data on the toxicokinetics and metabolism of glyphosate and *N*-acetyl-glyphosate (Table 4) shows that both compounds have similar properties regarding the rate and extent of excretion, the lack of potential for accumulation and the very limited metabolism in animals. Oral absorption was approximately 2-fold higher for *N*-acetyl-glyphosate compared with glyphosate, but did not result in a higher acute or short-term toxicity (Table 5).

No study on toxicokinetics and metabolism has been performed with *N*-acetyl-AMPA. However, it was detected as a minor metabolite formed following oral administration of *N*-acetyl-glyphosate. It is not expected to be absorbed quickly from the gastrointestinal tract, as it is a charged molecule at physiological pH.

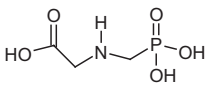
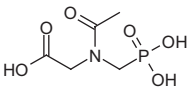
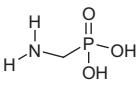
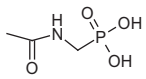
For *N*-acetyl-glyphosate, the available toxicological data package is almost complete except for studies on reproductive toxicity and long-term toxicity and carcinogenicity. A comparison with the toxicological studies performed with glyphosate showed that *N*-acetyl-glyphosate is of no greater toxicity than glyphosate (Table 5). In particular, despite a similar NOAEL for *N*-acetyl-glyphosate and glyphosate in short-term toxicity assays, the only effect occurring with *N*-acetyl-glyphosate was a slightly reduced body weight gain in male rats at a high dose of 1157 mg/kg bw per day, whereas no effects were observed in females at doses up to 1461 mg/kg bw per day. With regard to reproductive toxicity potential and carcinogenicity, it is not expected that *N*-acetyl-glyphosate can cause such effects, also taking into account chemical structural similarity with glyphosate. This assumption is supported by a structure–activity relationship (SAR) analysis of *N*-acetyl-glyphosate, with a lack of structural alerts for carcinogenicity, mutagenicity and endocrine effects.

Table 4. Comparison of toxicokinetics and metabolism of glyphosate and N-acetyl-glyphosate

	Glyphosate	*N*-Acetyl-glyphosate
Rate and extent of absorption	Rapid, approximately 30–36%	Rapid, approximately 66%
Distribution	Widely distributed	No information available
Potential for accumulation	No evidence, < 1% after 7 days (< 0.4% in residual carcass)	No evidence, 0.23% in residual carcass after 7 days
Rate and extent of excretion	Largely complete within 48 h, about 30% in urine and 70% in faeces	More than 90% within 48 h, about 66% in urine and 26% in faeces
Metabolism in rats	Very limited (< 0.7%), one metabolite (AMPA) detected in urine	Very limited (< 1%), two metabolites (glyphosate, N-acetyl-AMPA) detected in urine
Toxicologically significant compounds	Parent compound, AMPA	Parent compound, glyphosate

From Cheng & Howard (2004); Annex 1, reference *103*; O'Neal (2010)

Table 5. Summary of toxicological data for glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA

	Glyphosate	*N*-Acetyl-glyphosate	AMPA	*N*-Acetyl-AMPA
Chemical structure				
Acute oral toxicity, rat, LD_{50}	> 5000 mg/kg bw	> 5000 mg/kg bw	> 5000 mg/kg bw	> 5000 mg/kg bw
90-day oral toxicity, rat				No study submitted
- NOAEL	300 mg/kg bw	283 mg/kg bw	1000 mg/kg bw	
- LOAEL	811 mg/kg bw	1157 mg/kg bw	> 1000 mg/kg bw	
- critical effect	Salivary gland changes	Decreased body weight gain	No effects observed	
Gene mutation (Ames)	Negative	Negative	Negative	Negative
Gene mutation (*HPRT*)	Negative	Negative	Negative	Negative
Chromosomal aberration	Negative	Negative	Negative	Negative
Micronucleus test, in vivo	Negative	Negative	Negative	Negative

LD_{50}, median lethal dose; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level
Source of data on glyphosate and AMPA: Annex 1, reference *103*

For *N*-acetyl-AMPA, the available toxicological data package is limited (there are only an acute toxicity study and a complete genotoxicity data package), but a wide database is available for the structurally similar metabolite, AMPA. Comparison of toxicological studies among *N*-acetyl-AMPA, AMPA and glyphosate leads to the conclusion that *N*-acetyl-AMPA is of no greater toxicity than glyphosate (Table 5). This assumption is supported by a SAR analysis of *N*-acetyl-AMPA, with a lack of structural alerts for carcinogenicity, mutagenicity and endocrine effects.

Comments

Biochemical aspects

[^{14}C]*N*-Acetyl-glyphosate was rapidly and incompletely (approximately 66%) absorbed in rats following a single oral dose of 15 mg/kg bw. The maximum concentration of radioactivity in plasma was reached after 2 hours, and the half-life for elimination from plasma was 15.6 hours. Elimination was mainly via urine (66.1%) and, to a lesser extent, faeces (26.4%); more than 90% of the total radi-

oactivity was eliminated by 48 hours post-dosing. *N*-Acetyl-glyphosate was metabolized to a very limited extent. One metabolite, glyphosate (< 1% of the total radioactivity), was detected in faeces after a single oral dose of 15 mg/kg bw, whereas glyphosate and *N*-acetyl-AMPA were found in urine following subchronic exposure at dose levels of 56 mg/kg bw per day and above.

Toxicological data

N-Acetyl-glyphosate

N-Acetyl-glyphosate was of low acute oral toxicity; the LD_{50} was greater than 5000 mg/kg bw in rats.

In a 90-day study of toxicity with *N*-acetyl-glyphosate in rats, the NOAEL was 4500 ppm (equal to 283 mg/kg bw per day), based on slightly decreased body weight gains in male rats at 18 000 ppm (equal to 1157 mg/kg bw per day).

N-Acetyl-glyphosate was tested for genotoxicity in vitro and in vivo in an adequate range of assays; it was not found to be genotoxic in mammalian and microbial test systems.

The Meeting concluded that *N*-acetyl-glyphosate was unlikely to be genotoxic.

The Meeting concluded that *N*-acetyl-glyphosate is of no greater toxicological concern than the parent glyphosate, based on the structural similarity of *N*-acetyl-glyphosate with glyphosate and supported by the following considerations: 1) *N*-acetylation is a common detoxification pathway of xenobiotic compounds in mammals; therefore, *N*-acetyl-glyphosate is expected to be of similar toxicity to or lower toxicity than glyphosate; 2) a SAR analysis indicates that the *N*-acetylated group is not a structural alert for carcinogenicity, mutagenicity or endocrine effects; and 3) the toxicological data for *N*-acetyl-glyphosate show low acute toxicity, low subchronic toxicity (with no organ toxicity in rats at doses up to 1157 mg/kg bw per day) and a lack of genotoxicity.

N-Acetyl-AMPA

N-Acetyl-AMPA was of low acute oral toxicity; the LD_{50} was greater than 5000 mg/kg bw in rats.

N-Acetyl-AMPA was tested for genotoxicity in vitro and in vivo in an adequate range of assays; it was not found to be genotoxic in mammalian or microbial test systems.

The Meeting concluded that *N*-acetyl-AMPA was unlikely to be genotoxic.

The Meeting concluded that the toxicity of *N*-acetyl-AMPA is low and of limited concern, based on the structural similarity of *N*-acetyl-AMPA with AMPA and supported by the following considerations: 1) *N*-acetyl-AMPA is a charged molecule at physiological pH and is expected to be poorly absorbed from the gastrointestinal tract; 2) *N*-acetylation is a common detoxification pathway of xenobiotic compounds in mammals; therefore, *N*-acetyl-AMPA is expected to be of similar toxicity to or lower toxicity than AMPA or glyphosate; and 3) a SAR analysis indicates that the *N*-acetylated group is not a structural alert for carcinogenicity, mutagenicity or endocrine effects.

Toxicological evaluation

The Meeting concluded that the group ADI of 0–1 mg/kg bw established by the 2004 JMPR for glyphosate and AMPA may also be applied to *N*-acetyl-glyphosate and *N*-acetyl-AMPA, as the available toxicological data showed that these plant metabolites have no greater toxicity than the parent glyphosate.

The 2004 JMPR decided that an ARfD for glyphosate was unnecessary. The present Meeting confirmed that it is not necessary to establish an ARfD for *N*-acetyl-glyphosate or *N*-acetyl-AMPA

in view of their low acute toxicity and the absence of any toxicological effects that would be likely to be elicited by a single dose.

Estimate of acceptable daily intake for humans

0–1 mg/kg bw (for the sum of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA)

Estimate of acute reference dose

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

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[1] Study sponsored by Pioneer Hi-Bred, which is a company owned by E.I. du Pont de Nemours and Company.

[2] Study sponsored by Verdia, Inc. Data purchased from Verdia by E.I. du Pont de Nemours and Company.

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