**Supplemental Information – Genotoxicity Expert Panel Review**

**Appendix A**

**Summary tables of studies included in Williams et al. (2000) or Kier and Kirkland (2013) but not included in the IARC Monograph**

Table 1. Bacterial reverse mutation

| Test systema | High doseb (µg/plate) | Maxc | Statd | Resulte | Reff |
| --- | --- | --- | --- | --- | --- |
| **Glyphosate and Salts** |  |  |  |  |  |
| *Regulatory Studies* |  |  |  |  |  |
| 0,9,5,7 | 2500-5000 | T | S | neg | Jensen (1991a) |
| 0,9,5,7,8 | 1000 | T | N | negea | Suresh (1993a) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Akanuma (1995) |
| 0,9,5,7,PK,PUK | 5000 | L, T | S | negea | Callander (1996) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Thompson (1996) |
| 0,9,5,7,PK,PUK | 5000 | L, T | S | negea | Callander (1999) |
| 0,9,5,7a | 5000 | L, T | S | negea | Ranzani (2000) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Sokolowski (2007a) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Sokolowski (2007b) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Sokolowski (2007c) |
| 0,9,5,7,2 | 5000 | L | S | negea | Ribeiro do Val (2007) |
| 0,9, 5,7a,2 | 1000 | T | S | negea | Miyaji (2008) |
| 0,9,5,7,2 | 3160 | T | Nda | neg | Flugge (2009a) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Sokolowski (2009a) |
| 0,9,5.7,PK,PUK | 5000 | L, T | N | neg | Sokolowski (2009b) |
| 0,9,5,7,2 | 3160 | T | Nda | neg | Flugge (2010b) |
| 0,9,5,7, PU | 5000 | L, T | N | neg | Schreib (2010) |
| 0,9,5,7,2 | 5000 | L, T | N | neg | Wallner (2010) |
| *Published/Public Studies* |  |  |  |  |  |
| 0,9,5,7 | 3333-10000 | T |  | neg | \*Chan and Mahler (1992) |
| ***GBF’s*** |  |  |  |  |  |
| *Regulatory Studies* |  |  |  |  |  |
| 0,9,5,7 | 500-1500 | T | S | neg | \*Kier et al. (1992a) |
| 0,9,5,7 | 5000 | L, T | S | neg | \*Kier et al. (1992b) |
| 0,9,5,7 | 500-1500 | T | S | neg | \*Kier et al. (1992c) |
| 0,9,5,7,PU | 3330-5000 | T | N | negeb | Mecchi (2003a) |
| 0,9,5,7,PU | 3330 | T | N | neg | Mecchi (2003b) |
| 0,9,5,7,2 | 100-316 | T | N | neg | Uhde (2004) |
| 0,9,5,7,PU | 3330-5000 | T | N | neg | Xu (2006) |
| 0,9,5,7,2 | 2000 | T | N | neg | Lope (2008) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Mecchi (2008a) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Mecchi (2008b) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Mecchi (2008c) |
| 0,9,5,7,2 | 200 | Tca | N | neg | Camolesi (2009) |
| 0,9,5,7,2 | 2000 | T | N | neg | Catoyra (2009) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Mecchi (2009a) |
| 0,9,5,7,PU | 5000 | L, T | N | neg | Mecchi (2009b) |
| 0,9,5,7,2 | 200 | Tca | N | neg | Camolesi (2010) |
| 0,9,5,7,2 | 31.6-1000 | T | N | neg | Flugge (2010a) |
| 0,9,5,7,2 | 10-100 | T | N | neg | Flugge (2010d) |
| *Published/Public Studies* |  |  |  |  |  |
| 0,9,5,7,8,P | 5000ba | ?cb | N | negec | \*Moriya et al. (1983) |
| 0,9, 7a,2 | 0.2 | Tca | N | negec | Chruscielska et al. (2000) |
| **AMPA** |  |  |  |  |  |
| *Regulatory Studies* |  |  |  |  |  |
| 0,9,5,7,8,P | 5000 | L | N | neg | \*Shirasu et al. (1980) |

a Bacterial reverse mutation test strains used: 0, TA100; 9, TA98; 5, TA1535; 7, TA1537;7a, TA97a; 2, TA102; 8, TA1538; P, *Escherichia coli* WP2 hcr; PU, *E. coli* WP2 (uvrA); PUK, *E. coli* WP2 (uvrA) [pKM101]; PK, *E. coli* WP2 [pKM101]

b Highest dose level used. Range indicates different maximum dose levels depending on experimental conditions such as presence or absence of exogenous mammalian metabolic activation, preincubation or plate incorporation methodology.

ba Publication indicates pesticides were tested up to 5000 µg/plate or toxic levels but amounts tested for specific pesticides not indicated.

c Observations relevant to maximum dose level tested

L Meets or exceeds maximum of 5000 µg/plate recommended for soluble, non-cytotoxic substances by OECD Test Guideline (OECD, 1997)

T Toxicity observed for one or more strain/metabolic activation combinations as indicated by reduction in revertants/plate and/or reduction in background lawn.

ca Cytotoxicity observed at higher concentrations in rangefinder experiment.

cb Publication indicates testing to 5000 µg/plate or toxic levels but conditions for specific pesticides not indicated.

d Statistical analysis method and results indication in supplement, publication or publicly available report.

N Statistical analysis not indicated

S Statistical analysis method and results presented

da Statistical analysis suggested in text but not clearly evident in data tables.

e Assay result

neg—negative

pos—positive

ea Statistically significant increases in revertants/plate observed for some strain/S9 combinations but increases were judged not to be treatment related because they were less than 2-fold and, in most cases, not reproducible or consistent with a dose response.

eb Several dose levels exceeded control revertants/plate by more than three fold in one experiment for TA98 -S9 and TA1535 -S9. There was no dose response and the result was not observed in a second experiment. The >3-fold response was considered due to low control values rather than a treatment related response.

ec Results presented as “-“.

f References listed in Table 1 that are not found in Kier and Kirkland (2013) are marked with an \* and listed within the bibliography

Table 2. *In vitro* mammalian cell studies.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Endpointa | Test systemb | High dosec | Maxd | State | Resultf | Refg |
| **Glyphosate and Salts** | | | | | | |
| *Regulatory Studies* | | | | | | |
| Tk | ML | 4200-5000 µg/mL | L | N | neg | Jensen (1991b) |
| Tk | ML | 1000 µg/mL | P | N | neg | Clay (1996) |
| CA | HL | 333 µg/mL | T | S | neg | \*van de Waart (1995) |
| CA | HL | 1250 µg/mL | P | S | neg | Wright (1996) |
| CA | HL | 1250 µg/mL | T | S | neg | Fox (1998) |
| CA | CHL | 1000 µg/mL | Nda | N | neg | Matsumoto (1995) |
|  |  |  |  |  |  |  |
| UDS | PRH | 111.69 mM | T | N | neg | Rossberger (1994) |
| *Published/Public Studies* | | | | | | |
| CA | HL | 51 µM | Nda | S | pos | \*Lioi et al. (1998) |
| CB MN | BL | 0.56 mM | N | S | incfa | Piesova (2004) |
| CB MN | BL | 0.56 mM | N | S | incfa | Piesova (2005) |
| CA (1) | BL | 1.12 mM | Tdb | S | negfb | \*Holeckova (2006) |
| CB MN | HL | 580 µg/mL | T | S | pos?fc | Mladinic et al. (2009)h |
| CB MN | TR146 | 20 mg/L | T | S | pos | Koller et al. (2012)h |
| ***GBF’s*** | | | | | | |
| *Published/Public Studies* | | | | | | |
| CA | MS | 50 mMca | L, T | S | pos | Amer et al. (2006) |
| CB MN | TR146 | 20 mg/L | T | S | pos | Koller et al. (2012)h |
| SCE | MS | 50 mMca | L, T | S | pos | Amer et al. (2006) |
| ***AMPA*** | | | | | | |
| *Regulatory Studies* | | | | | | |
| UDS | PRH | 5000 µg/mL | L, T | N | neg | \*Bakke (1991) |

a Assay endpoint: Tk, gene mutation at the Tk locus; CA, chromosomal aberration; CA (1), chromosomal aberration (FISH analysis of chromosome 1 for acentric fragments); CB MN, cytokinesis block micronucleus; SCE, sister chromatid exchange; UDS, unscheduled DNA synthesis.

b ML, L5178Y mouse lymphoma cell line; HL, human peripheral blood lymphocytes; CHL, Chinese hamster lung cell line; PRH, primary rat hepatocyte; BL, bovine peripheral blood lymphocytes; TR146, human buccal epithelial cell line; MS, mouse spleen cells.

c Highest analyzable dose level used in publication in reported units. A range indicates different highest dose levels for different experimental conditions (e.g. with or without exogenous mammalian metabolic activation or different exposure times).

ca Calculated from the stated concentration of 5 x 10-5 M glyphosate/mL.

d Observations relevant to maximum dose level tested

L Meets or exceeds current OECD guideline maximum recommended concentration. For relatively non-cytotoxic compounds the recommend maximum concentration is 10 mM, 2 mg/mL or 2 µl/mL, whichever is lower, for the *in vitro* mammalian cell Tk gene mutation test (OECD, 2015), the *in vitro* mammalian cell chromosomal aberration test (OECD, 2014a) and the *in vitro* mammalian cell micronucleus test (OECD, 2014b). For glyphosate (MW 169.1) the maximum is 10 mM or 1690 µg/mL. For test materials of unknown or variable composition a higher top concentration such as 5000 µg/mL is suggested in these guidelines. No specific maximum concentration is recommended for relatively non-cytotoxic compounds in the *in vitro* mammalian cell UDS test guideline (OECD, 1986).

T Toxicity observed at maximum concentration. In some cases, as indicated by footnote, toxicity was observed at higher concentrations in a rangefinder experiment.

P Top dose level selected to avoid excessive changes in pH.

N No significant toxicity observed.

da Higher doses caused excessive toxicity in rangefinder experiments.

db Highest dose reported to cause reduction in mitotic index >50% but data not presented.

e Status of statistical analysis method and results indication in supplement, publication or publicly available report:

N Statistical analysis not indicated in report or publication.

S Statistical analysis method and results presented.

f Assay result:

neg—negative

pos—positive

inc—inconclusive

fa Statistically significant increases observed at a single different dose level for each of two donors for 48 hours treatment without S9 metabolic activation. Publications indicate dose responses were not observed and effects were very weak or minimal with 48 hours treatment.

fb No positive control reported.

fc Small increases in MN frequency in binucleate cells observed for a wide range of dose levels (3.5-580 µg/mL) without S9 but not statistically significant. Statistically significant increase in MN frequency only observed at highest dose level (580 µg/mL) with S9 and was interpreted in the publication as possibly an aneugenic effect exhibited only above a threshold.

g References listed in Table 2 not found in Kier and Kirkland (2013) are marked with an \* and listed within the bibliography

h IARC monograph only reports comet results but not MN results for Mladinic et al. (2009) and Koller et al. (2012).

Table 3. *In vivo* mammalian studies

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Endpointa | Test system | Rteb | High dosec | Maxd | State | Resultf | Refg |
| **Glyphosate and Salts** | | | | | | | |
| *Regulatory Studies* | | | | | | | |
| BM CA | mouse | p.o. | 5000 | L, T | S | neg | Suresh (1994) |
| BM MN | mouse | p.o. | 5000 | L | S | neg | Jensen (1991c) |
| BM MN | mouse | p.o. | 5000 | L, T | S | incfa | Suresh (1993b) |
| BM MN | mouse | p.o. | 5000 | L | S | neg | Fox & Mackay (1996) |
| BM MN | mouse | p.o. | 2000 | L | S | negfb | Jones (1999) |
| BM MN | mouse | i.p. | 562.5 | Nda | S | neg | Marques (1999) |
| BM MN | mouse | i.p. | 3024 | L, T | S | neg | Gava (2000) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Honarvar (2005) |
| BM MN | mouse | i.p. | 600 | T | S | negfc | Durward (2006) |
| BM MN | mouse | p.o. | 30 | N | S | negfd | Zoriki Hosomi (2007) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Honarvar (2008) |
| BM MN | mouse | i.p. | 62.5 | N | S | neg | Costa (2008) |
| BM MN | rat | p.o. | 2000 | L | S | neg | Flugge (2009b) |
| *Published/Public Studies* | | | | | | | |
| PB MN | mouse | diet ba | 3393 | L | S | neg | \*Chan and Mahler (1992) |
| BM MN | mouse | i.p | 300 | Ndb | S | neg | Chruscielska et al. (2000) |
| ***GBF’s*** | | | | | | | |
| *Regulatory Studies* | | | | | | | |
| BM MN | mouse | i.p. | 555 | T | S | neg | \*Kier et al. (1992d) |
| BM MN | mouse | i.p. | 3400 | T | S | neg | \*Kier et al. (1992e) |
| BM MN | mouse | i.p. | 365 | T | S | neg | \*Kier et al. (1992f) |
| BM MN | mouse | p.o. | 2000 | L | S | negfe | Erexson (2003a) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Erexson (2003b) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Erexson (2006) |
| BM MN | mouse | p.o. | 2000 | L, T | S | negfe | Xu (2008a) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Xu (2008b) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Xu (2009a) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Xu (2009b) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Xu (2009c) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Negro Silva (2009) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Flugge (2010c) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Flugge (2010e) |
| BM MN | mouse | p.o. | 2000 | L | S | neg | Negro Silva (2011) |
| *Published/Public Studies* | | | | | | | |
| BM MN | mouse | i.p. | 90 | Ndc | S | neg | Chruscielska et al. (2000) |
| BM MN | mouse | i.p. | 200 | T?dd | S | neg | \*Coutinho do Nascimento A (2000) |
| BM CA | rabbit | d.w.bb | 750 ppm | N | S | pos | \*Helal and Moussa (2005) |
| BM, SC CA | mouse | i.p., p.o.bc | 50 glyca | Nde | S | incff, pos | \*Amer et al. (2006) |
| BM SCE | mouse | p.o. | 200 glyca | N | S | pos | \*Amer et al. (2006) |
| ***AMPA*** | | | | | | | |
| *Regulatory Studies* | | | | | | | |
| BM MN | mouse | i.p. | 1000 | T | S | negfg | \*Kier and Stegeman (1993) |

a Endpoint: BM MN, bone marrow polychromatic erythrocyte micronucleus; BM CA, bone marrow chromosomal aberration; PB MN, normochromatic erythrocyte micronucleus in peripheral blood; SC CA, spermatocyte chromosomal aberration; BM SCE, bone marrow sister chromatid exchange.

b Rte--Route of administration: p.o. oral (gavage); i.p., intraperitoneal injection; d.w., drinking water. Except as noted by footnote acute dosing (single or two doses 24 hours apart were used)

ba 13 week feeding study.

bb 60 days drinking water study.

bc 1, 3 and 5 days i.p.; 1, 7, 14 and 21 days p.o.

c Maximum glyphosate, GBF or AMPA treatment dose level in mg/kg body weight except for ppm which indicates amount in drinking water.

ca dose units were reported as mg/kg body weight of glyphosate (gly)

d Observations relevant to maximum dose level tested

L Meets or exceeds current OECD guideline maximum recommended dose (OECD, 2014c).

T Signs of general or target organ toxicity observed at highest dose level.

da Maximum concentration close to reported LD50 of 750 mg/kg

db Indicated as “maximal dose succeeded in administration”

dc Indicated as 70% of the LD50

dd Reduction in PCE/NCE ratio observed but not indicated as statistically significant.

de Statistically significant increases in abnormal sperm observed at p.o. doses of 100 and 200 mg/kg gly

e Statistical analysis method and results indication in supplement, publication or publicly available report.

N Statistical analysis not indicated

S Statistical analysis method and results presented

f Assay result

neg—negative

pos—positive

inc—inconclusive

fa Statistically significant increase in MN erythrocytes for high dose females. Control MN frequencies were unusually high and historical control data not presented.

fb Statistically significant increase in MN PCE frequency at 24 h only, within historical control, not judged to be treatment-related.

fc Statistically significant increase in MN PCE frequency only for 24 h high dose, within historical control , not judged to be treatment-related.

fd Statistically significant increase for high dose MN PCE frequency, within historical control , not judged to be treatment-related.

fe Statistically significant increase for high dose at 48 hours, within historical control, but judged to be due to a low control group value and not treatment-related.

ff For BM CA by p.o route increases in abnormal metaphases not statistically significant excluding gaps from aberrant cells. Authors conclude positive result based on statistically significant increases in abnormal metaphases including gaps.

fg Statistically significant increase in MN PCE for low dose females at 72 h. Increase was within historical control and statistically significant increases were not observed at higher dose levels, not judged to be treatment-related.

g References listed in Table 3 that are not found in Kier and Kirkland (2013) are marked with an \* and listed within the bibliography.

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