An earlier review of the toxicity of glyphosate and the original Roundup™ formulation concluded that neither glyphosate nor the formulation pose a risk for the production of heritable/somatic mutations in humans (Williams et al., 2000, ASB2012-12053). This review of subsequent glyphosate genotoxicity publications includes analysis of study methodology and incorporation of all the findings into a weight of evidence for genotoxicity. Two publications provided limited additional support for the conclusion that glyphosate and glyphosate based formulations (GBFs) are not active in the gene mutation assay category. The weight of evidence from in vitro and in vivo mammalian chromosome effects studies supports the earlier conclusion that glyphosate and GBFs are predominantly negative for this end point category. Exceptions are mostly for unusual test systems but there are also some unexplained discordant positive results in mammalian systems. Several reports of positive results for the SCE and comet DNA damage endpoints have been published for glyphosate and GBFs. The data suggest that these DNA damage effects are likely due to cytotoxic effects rather than DNA reactivity. This weight of evidence review concludes that there is no significant in vivo genotoxicity and mutagenicity potential of glyphosate or GBFs that would be expected under normal exposure scenarios.

B.6.4.8.2 General review and analysis considerations

The published studies for review consideration were identified by literature searches for published reports containing references to glyphosate or glyphosate based formulations (GBFs) that also contained searchable terms which indicated that genotoxicity studies were performed. Literature search utilised Chemical Abstracts (provided by Chemical Abstracts Service, a division of the American Chemical Society) and Web of Knowledge (Thompson Reuters), using the following modules: Web of ScienceSM, BIOSIS Previews®, MEDLINE®, and CAB Abstracts® (CABI) abstracting services. Search criteria were as follows (glyphosate acid and the various salts): glyphosat* OR glifosat* OR glyfosat* OR 1071-83-6 OR 38641- 94-0 OR 70901-12-1 OR 39600-42-5 OR 69200-57-3 OR 34494-04-7 OR 114370-14-8 OR
40465-66-5 OR 69254-40-6 OR (aminomethyl w phosphonic*) OR 1066-51-9. Each identified publication was evaluated to verify that it contained original results of one or more genotoxicity studies on glyphosate or GBFs. Emphasis was placed on publications in peer- reviewed journals and abstracts or other sources with incomplete information were not considered. Reviews without original data were not considered for evaluation; however, these reviews were examined to determine if there were any cited publications that had not been detected in the literature searches. Each relevant publication was examined using several criteria to characterize the scientific quality of the reported genetic toxicology studies. Useful, objective criteria for this purpose were international guidelines for genetic toxicology studies developed by expert groups. These include principles for conducting studies, reporting results and analyzing and interpreting data. Some of the principles of the guidelines are generally applicable to categories of studies or all studies while others are specific for a particular type of test system and end point. Some of the specific types of studies encountered in the review do not yet have international guidelines; however, some of the guideline elements should be generically applicable to these studies. The guidelines for genetic toxicology tests developed for the Organisation for Economic Cooperation and Development (OECD) are a pre-eminent source of internationally agreed and expert guidelines. Other regulatory international and national regulatory genetic toxicology testing guidance are usually concordant with the OECD guidelines. Table B.6.4-28 presents some key OECD guideline criteria that were found to be relevant to analysis of the studies considered in this review.

Comparison of the published studies to the criteria in guidelines used for regulatory purposes does not represent an absolute judgment standard but it does serve to provide one means of

characterization of the various published studies. Some of the criteria are rarely met in scientific publications. For example, data for individual cultures and individual animals are not commonly included in publications in scientific journals. These data are presumably collected but are usually summarised as means with a measure of variance for the treatment and control groups. This is not considered to be a significant omission in a scientific publication. However, other guideline features are more essential in demonstrating scientific quality standards and should be considered as having greater weight in evaluating a study. For example, there are consistent recommendations that assays involving visual scoring (e.g. chromosome aberration, micronucleus and sister chromatid exchange) should use slides that are independently coded so that scoring is performed without knowledge of the treatment or control group being scored. This guidance is good scientific practice and studies that do not include a description of coding or "blind" scoring in the methodology would appear to have a deficiency either in the methodology or the description of the methodology used. Other examples of guideline features that have clear experimental scientific value are the use of concurrent negative and positive controls and concurrent measurement and reporting of toxicity endpoints in main experiments, especially in in vitro mammalian cell assays.

Test materials, as described in the publications, were reviewed by industry experts to identify any publicly available and useful information on composition for the reported formulations to assist in interpreting the relevance of findings to glyphosate and/or formulation components. It should be noted that a common problem encountered in the published literature is the use of the terms "glyphosate", "glyphosate salt" or "Roundup" to indicate what may be any GBF that contains additional components such as surfactants. Published results from studies with different formulations have sometimes been incorrectly or inappropriately attributed to the active ingredient. The original Roundup formulation (MON 2139), containing 41 % isopropyl amine glyphosate salt and 15.4 % MON 0818 (a polyethoxylated based surfactant blend), is no longer sold in many markets. However, other glyphosate based formulations are sold under the Roundup brand name with varying glyphosate forms, concentrations and surfactant systems. Clear identification of the test material is very important in toxicology studies because toxicity of formulations can be dramatically different than the active ingredient. The fact that test materials identified as Roundup formulations may actually have different compositions should be considered when comparing results of different studies. A major consideration, especially for DNA damage endpoints and for in vitro mammalian cell assays, is an assessment of whether observed effects might be due to toxicity or extreme culture conditions rather than indicating DNA-reactive mediated processes. Relevant considerations include control of medium pH and osmolality for in vitro mammalian cell studies and whether effects are observed only at cytotoxic doses or in association with severe toxicity to the test system. Other important generic considerations in evaluating experimental results of each published study are evidence of experimental reproducibility and whether a biologically plausible dose response has been demonstrated.

Table B.6.4-28: Genetic Toxicology Test Guideline Criteria

Area	Guidance	Reference
All studies	Test material purity and stability should be reported	
	OECD 471 (1997)	
	OECD 473 (1997)	
	Concurrent negative and positive controls should be included	

with each assay
Assays with visual
scoring
All slides should be independently coded before analysis
(i.e. scored without knowledge of the treatment or control group)
OECD 473 (1997)
OECD 479 (1986)
In vitro mammalian
cell assays
Assay should be usually be conducted in the presence and
absence of an appropriate exogenous metabolic activation system
OECD 473 (1997)
Cytotoxicity should be determined in the main experiment
At least three analyzable concentrations should be used
Maximum dose determined by toxicity or 5 µg/ml, 5 mg/ml or
10 mM for soluble non-toxic test materials
Individual culture data should be provided
In vivo mammalian assays
Five analyzable animals per group. Single sex may be used if there are no substantial difference in toxicity between sexes
OECD 475 (1997)
OECD 474 (1997)
Limit dose for non-toxic substances of 2000 mg/kg for treatments up to 14 days and 1000 mg/kg for treatments longer
than 14 days
In vitro chromosome aberration
Treatment for 3-6 hours in one experiment and harvest at 1.5 cell cycles. If negative a second experiment with continuous
treatment for 1.5 cell cycles
OECD 473 (1997)
Scoring of at least 200 metaphases ideally divided between duplicate cultures
In vitro sister
chromatid exchange
Treatment for 1-2 hours up to two cell cycles with harvest after
two cell cycles in the presence of bromodeoxyuridine
OECD 479 (1986)
Scoring of 25 metaphases per culture (50 per treatment group)
In vitro micronucleus
Most active agents detected by treatment for 3-6 hours with harvest at 1.5-2 cell cycles after treatment. An extended treatment for 1.5-2 cycles in the absence of metabolic activation
is also used
OECD 487 (2010)
Scoring of at least 2000 binucleated cells or cells for micronuclei
for each treatment or control group
In vivo bone marrow chromosome
aberration

Single treatment with first harvest at 1.5 cell cycles after treatment and second harvest 24 hour later or single harvest 1.5
cycles after last treatment for multiple daily treatments
OECD 475 (1997)
Three dose levels usually recommended except when limit dose
produces no toxicity
Concurrent measures of animal toxicity and toxicity to target
cells
At least 100 cells analyzed per animal
Individual animal data should be reported
In vivo erythrocyte
micronucleus
Three dose levels for first sampling time
OECD 474 (1997)
Treatment once with at least 2 harvests usually at 24 and 48 h after treatment or one harvest 18-24 h after final treatment if two
or more daily treatments are used
Scoring of 2000 immature erythrocytes per animal or 2000
mature erythrocytes for treatments of 4 weeks or longer
Table B.6.4-29 presents a summary of genotoxicity test results for glyphosate and GBFs published subsequent to Williams et al. (2000, ASB2012-12053). Test results are organised by the major genotoxicity assay categories of gene mutation, chromosome effects and DNA damage and other end points. Major features presented for each publication are the assay endpoint, the test system, the test material, the maximum dose tested and comments relevant to the reported conduct and results of the assay. For brevity, earlier reviewed individual publications of genotoxicity study results are referred to by citation of (Williams et al., 2000, ASB2012-12053) rather than the original references reviewed in (Williams et al., 2000, ASB2012-12053).
Table B.6.4-29: Genetic toxicology studies of glyphosate and glyphosate formulations published on or after 2000
End point
Test System
Test Material
Maximum
Dose
Result
Commenta
Reference
In Vitro Gene Mutation
End point
Test System
Test Material
Maximum
Dose
Result
Commenta
Reference

Point
Ames strains
Perzocyd 10
2 µg/plate
Negative
TA1535 not
Chrusciels
mutation
SL
(toxic)
used
ka et al.,
formulation
2000,
(ASB2013-
9830)
Wing spot test
Drosophila
glyphosate
10 mM in
Negative/
Negative or
Kaya et al.,
(96%)
larval stage
inconclusive
c
inconclusive in crosses not
2000, (ASB2013-
sensitive to
9832)
recombination
events
In Vitro Chromosome Effects—Mammalian Systems
Cytokinesis
Bovine
Glyphosate
560 µM
Positive?
PH, MA, SC,
Piesova,
block
lymphocytes
formulation
48 h –S9
TO

2004
micronucleus
(62%
(ASB2012-
glyphosate
12001)
Monsanto
source)
Cytokinesis
Bovine
Glyphosate
560 µM
Positive?
PH, SC, TO
Piesova,
block
lymphocytes
formulation
48 h –S9
Negative
2005
micronucleus
(62%
2 h –S9
Negative
(ASB2012-
glyphosate
2 h +S9
12000)
Monsanto
source)
Chromosome
Mouse spleen
herbazed
50 µM?
Positive
Concentrations
Amer et
aberration
cells
formulation
used not clear.
al., 2006
PH, MA, SC,
(ASB2012-
TO, RE

11539)
Chromosome
Bovine
Glyphosate
1.12 mM
Negative
Chromosome 1
Holeckova,
aberration
lymphocytes
formulation
(toxic)
FISH analysis.
2006
(62%
(24 h)
PH, MA, PC,
(ASB2012-
glyphosate)
SC, TO, RE
11847)
Monsanto
source
Chromosome
Bovine
Glyphosate
1.12 mM
Negative
PH, MA, SC,
Sivikova
aberration
lymphocytes
formulation
(toxic)
RE
and
(62%
(24 h)
Dianovsky,
glyphosate)
2006
Monsanto
(ASB2012-
source
12029)
Chromosome

Human
Glyphosate
6 mM (not
Negative
MA, IC, RE
Manas et
aberration
lymphocytes
(96%)
toxic)
al., (2009
ASB2012-
11892)
Cytokinesis
Human
Glyphosate
580 µg/mL
Negative
SC, RE
Mladinic et
block
lymphocytes
(technical,
(toxic)
(-S9)
al., 2009
micronucleus
96%)
(est. 3.43
Positive
(ASB2012-
mM)
(+S9)
11906)
Cytokinesis
Human
Glyphosate
580 µg/mL
Negative
SC, RE
Mladinic et
block
lymphocytes
(technical,
(toxic)
(-S9)

al., 2009
micronucleus
96%)
(est. 3.43
Positive
(ASB2012-
mM)
(+S9)
11907)
In Vitro Chromosome Effects— Non Mammalian Systems
Chromosome aberration
Onion root tip meristem
Roundup formulation (Bulgaria)
1% active ingredient (estimated
Negative
TO, IC, RE
Dimitrov et al., 2006 (SB2012-
End point
Test System
Test Material
Maximum
Dose
Result
Commenta
Reference
4.4-5.9
mM)
11607)
Micronucleus
Onion root
Roundup
1% active
Negative
TO, RE
Dimitrov et
tip meristem
formulation
ingredient
al., 2006
(Bulgaria)
(estimated
(SB2012-
4.4-5.9
11607)
mM)
In Vivo Chromosome Effects—Mammalian Systems

Bone marrow erythrocyte micronucleus
Mouse
Glyphosate
300 mg/kg i.p.
Perzocyd 10 SL
formulatio n
Negative Negative
DL, TO, SC, IM, RE
DL, TO, SC, IM, RE
2000, (ASB2013- 9830)
Bone marrow
Mouse
Roundup 69
2 x 200
Negative
TO, SC, IE, RE
erythrocyte
formulation
mg/kg i.p.
micronucleus
2000
(ASB2013-
11477)
Bone marrow
Mouse
Roundup™
2 x 200
Negative
TO, SC, IE, RE
,
erythrocyte
formulation
mg/kg i.p.
2002
micronucleus
(Monsanto)
(SB2012-
11834)
Bone marrow
Rabbit
Roundup™
750 ppm in
Positive?
DL, PC, TO,
Chromosome
formulation

drinking
SC, IC
aberration
water
2005
(ASB2012-
11841)
Bone marrow Chromosome aberration
Mouse
Herbazed formulation (84%
glyphosate)
50 mg/kg
i.p. (1,3, 5 days)
Negative
TO, SC, RE
2006 (ASB2012- 11539)
100 mg/kg
oral (1,7,
14, and 21 days)
Positive
Spermatocyte Chromosome aberration
Mouse
Herbazed formulation (84%
glyphosate)
50 mg/kg
i.p. (1,3, 5 days)
Negative
TO, SC, RE
2006
(ASB2012- 11539)
100 mg/kg
oral (1,7,
14, and 21 days)
Positive
Bone marrow
Mouse
Roundup
1080 mg/kg
Negative
DL, TO, IC,
Chromosome
formulation
p.o. (1/2
RE
2006
aberration

(Bulgaria)
LD50)
(ASB2012-
11607)
Bone marrow
Mouse
Analytical
2 x 200
Positive
Erythrocytes
erythrocyte
glyphosate
mg/kg i.p.
scored?
2009
micronucleus
(96%)
TO, SC, IC, RE
(ASB2012-
11892)
Bone marrow
Mouse
Roundup™
50 mg/kg
Positive
DL, SC, IC, RE
End point
Test System
Test Material
Maximum
Dose
Result
Commenta
Reference
Chromosome
formulation
i.p.
, 2009
aberration
(Monsanto)
(ASB2012-
12005)
In Vivo Chromosome Effects—Non-Mammalian Systems
Erythrocyte
Oreochromis
Roundup 69

170 mg/kg
Negative?c
TO, RE
micronucleus
niloticus
i.p.
(Tilapia)
(maximum
tolerated)
2000
(ASB2013-
11477)
Wing spot test
Drosophila
Glyphosate (96%)
10 mM in larval stage
Positive/inco nclusiveb
2000
(ASB2013-
9832)
Erythrocyte
Tilapia
Roundup™
170 mg/kg
Positive
TO, RE
micronucleus
formulation
(abdominal
2002
(Monsanto)
injection)
(ASB2012-
11834)
Erythrocyte
Crasseus
Roundup
15 ppm
Positive
TO, IE, RE
micronucleus
auratus
formulation
glyphosate
(goldfish)
in water (2,

2007
4 and 6
(ASB2012-
days)
11587)
Prochilodus
Roundup™
10 mg/l (6,
Negative
DL, TO, SC,
lineatus
formulation
12 and 24
RE
2008
(tropical fish)
(75% of 96 h
h) in water
(ASB2012-
LC50)
11586)
Erythrocyte
Caiman eggs
Roundup®
1750
Positive
RE
micronucleus
Full II
ug/egg
2009
formulation
(ASB2012-
12002)
Erythrocyte
Caiman eggs
Roundup®
Sprayed 2x
Positive
DL, TO, RE
micronucleus
Full II
with 100
2009
formulation
litres of

(ASB2012-
3%/ha 30
12002)
days apart
Micronucleus
Fish (Guppy)
Roundup®
5.65 µg/l
Positive
(and alkaline
Transorb
SCGE)
2013
(ASB2014-
7617)
In Vitro DNA Damage Mammalian Systems
Alkaline
GM38 human
Glyphosate
6.5 mM
Positive
MA, PH, TO,
Monroy et
SCGE
fibroblasts
(technical
SC, RE
al., 2005
and
grade)
(ASB2012-
HT1090
11910)
human
fibrosarcoma
Sister
mouse spleen
herbazed
50 µM?
Positive
Concentrations
Amer et
chromatid
cells
formulation
used not clear

al., 2006
exchange
MA, PH, TO,
(ASB2012-
SC, RE
11539)
Sister chromatid exchange
bovine lymphocytes
Glyphosate formulation (62%
1.12 mM (toxic)
Positive
PH, SC, RE
Sivikova and Dianovsky,
End point
Test System
Test Material
Maximum
Dose
Result
Commenta
Reference
glyphosate, Monsanto)
2006 (ASB2012-
12029)
Alkaline single cell gel electrophoresi s (SCGE,
comet)
Hep-2 cells
Glyphosate (analytical, 96%)
7.5 mM (limited by toxicity)
Positive
MA, PH, RE
Manas et al., 2009 (ASB2012- 11892)
Alkaline SCGE
Human lymphocytes
Glyphosate (technical, 96%)
580 µg/ml (toxic) (est. 3.43
mM)
Positive (- S9)
Positive (+S9)
Mladinic et al., 2009 (ASB2012-
11906)
SCGE
Human lymphocytes (compared with Tilapia erythrocytes and Tradescantia
nuclei)
Glyphosate (96%)
700 µM

Positive (according to authors)
Inconsitent and not clear dose dependent
Alvarez- Moya et al., 2014 (ASB2014- 6902)
SCGE
Human buccal epithelial cells
Glyphosate (95%) and Roundup
Ultra Max
200 mg/l
Positive
Higher activity of formulation than pure a. s.
Koller et al., 2012 (ASB2014-
7618)
In Vitro DNA Damage Non-Mammalian Systems
SOS
E. coli
Roundup BIO
formulation
2.5
ug/sample
Positive
Raipulis et al. 2009 (ASB2012- 12008)
Alkaline SCGE
Tradescantia flowers and nuclei
Glyphosate(technical, 96%)
700 µM
Positive
PH, SC
Alvarez- Moya et al., 2011 (ASB2012-
11538)
In Vivo DNA Damage Mammalian Systems
Spermatocytes and bone marrow
Mouse
herbazed formulation (84%
glyphosate)
200 mg/kg p.o.
Positive
TO, SC, RE
Amer et al., 2006
(ASB2012- 11539)
SCGE
blood cells, liver cells,
Mouse
Glyphosate (96%) and AMPA
400 mg/kg bw/day Glyphosate or 100 mg/kg
bw/day AMPA
Glyphosate and AMPA positive

Manas et al., 2013 (ASB2014- 6909)
In Vivo DNA Damage Non-Mammalian Systems
Erythrocyte alkaline SCGE
Crasseus auratus (goldfish)
Roundup formulation
15 ppm glyphosate in water (2,
4 and 6 days)
Positive
TO, RE
2007 (ASB2012- 11587)
Erythrocyte and gill cell alkaline
SCGE
Prochilodus lineatus (tropical fish)
Roundup™ formulation (75% of 96 h
LC50)
10 mg/l (6,
12 and 24
h) in water
Positive
DL, TO, RE
2008 (ASB2012- 11586)
Erythrocyte
Caiman
Roundup®
1750
Positive
RE
End point
Test System
Test Material
Maximum Dose
Result
Commenta
Reference
alkaline SCGE
eggs/hatchlin gs
Full II
formulation
µg/egg
., 2009 (ASB2012-
12002)
Erythrocyte alkaline SCGE
European eel
Roundup formulation
166 µg/liter
Positive

DL, SC, RE
2010 (ASB2012- 11836)
Erythrocyte alkaline SCGE
Caiman eggs/hatchlin gs
Roundup® Full
II
formulation
Sprayed 2x with 100 l of 3%/ha 30 days
apart
Positive
DL, RE
2009 (ASB2012- 12002)
SCGE
blood cells
European eel
Roundup Ultra and
Glyphosate and
POAE
116 µg/l
35.7 µg/l
18.6 µg/l
positive
No increased effect of glyphosate in combination with POAE
Guilherme et al., 2012 (ASB2014- 7619)
SCGE
Fish (Prochilidus)
Roundup Transorb and Glyphosate
5 mg/l
2.4 mg/l
positive
Inconsistent and not clearly dose dependent
Moreno et al., 2014 (ASB2014- 7522)
a MA, Mammalian metabolic activation system not used and short exposure not used; PH, no indication of pH or osmolality control;
DL, less than three dose levels used; PC, no concurrent positive control;
TO, no concurrent measurement of toxicity reported or toxicity not observed for highest dose level;
SC, independent coding of slides for scoring not indicated for visually scored slides;
IC, less than 200 cells scored per treatment or less than 100 metaphases scored per animal for chromosome aberrations.;
IE, less than 2000 erythrocytes scored per animal;
RE, results not reported separately for replicate cultures or individual animals;.
b Positive for small wing spots only in one cross. Negative or inconclusive for all spot categories for three other crosses.
c Statistically significant increase in micronucleated PCE frequency only at mid dose level but overall result judged negative.
B.6.4.8.3 Structure Activity Analysis

Glyphosate was evaluated using Derek for Windows (Llhasa Ltd., Leeds, UK, Version 11.0.0, October 24, 2009). No structural alerts were identified for chromosome damage, genotoxicity, mutagenicity or carcinogenicity. This small molecule consists of the amino acid, glycine, joined with a phosphonomethyl group. These moieties are not known to be genotoxic; therefore, the lack of structure activity alerts for glyphosate is expected.

B.6.4.8.4 Gene Mutation

As reviewed by Williams et al., (2000, ASB2012-12053), most gene mutation studies for glyphosate and GBFs were negative. Gene mutation assays included numerous Ames/Salmonella and E. coli WP2 bacterial reversion assays, Drosophila sex-linked recessive lethal assays and a CHO/HGPRT in vitro mammalian cell assay. Of fifteen gene mutation assays reported, there were only two positive observations. A reported positive Ames/Salmonella result for Roundup formulation was not replicated in numerous other studies. There was one report of a positive result for a GBF in the Drosophila sex-linked recessive lethal assay but this was contradicted by a negative result for the same GBF in this assay reported by another laboratory. Further, the positive study had some features that hampered interpretation, including the lack of concurrent negative controls (Williams et al., 2000).

Subsequent to the Williams et al. (2000, ASB2012-12053) review only two gene mutation studies have been reported (Table B.6.4-29). One negative Ames/Salmonella assay result was reported for a GBF of undefined composition, Percozyd 10 SL (Chruscielska et al., 2000, ASB2013-9820). Although this result is consistent with a large number of negative Ames/Salmonella results for glyphosate and GBFs, the reported study results have significant limitations. One of the recommended test strains, TA1535, was not used and results were only presented as "-" without presentation of revertant/plate data. A positive result for glyphosate was reported in the Drosophila wing spot assay which can indicate both gene mutation and mitotic recombination endpoints (Kaya et al., 2000, ASB2013-9832). Small increases in small wing spot frequencies were observed in one of four crosses of larvae treated with up to 10 mM glyphosate. The lack of a positive response in the balancer-heterozygous cross offspring, which are insensitive to mitotic recombination events, suggests that there is no evidence for effects on gene mutation endpoint events such as intragenic mutations or deletions in this publication.

These gene-mutation publications add very limited data to the weight of evidence conclusion that glyphosate and GBFs do not pose significant risk for gene mutation.

B.6.4.8.5 Chromosome effects

Assays to detect chromosome effects such as structural chromosome aberrations and micronucleus incidence constitute a second major genotoxicity end point category. A large number of publications with chromosome effects endpoints have been reported since the Williams et al. (2000, ASB2012-12053) review. These are described in Table B.6.4-29 and are separated into various test system categories which include in vitro cultured mammalian cell assays, in vitro tests in non-mammalian systems, in vivo mammalian assays and in vivo assays in non-mammalian systems. A Drosophila wing spot test (discussed previously) is also included in this category because results are relevant to somatic recombination.

B.6.4.8.5.1 In vitro chromosome effects

Two human and one bovine in vitro peripheral lymphocyte chromosome aberration studies of glyphosate were considered in the earlier review (Williams et al., 2000, ASB2012-12053). One human lymphocyte in vitro study had negative results for glyphosate tested up to approximately 2-3 mM (calculated from reported mg/ml) in the absence and presence of an exogenous mammalian activation system. The other two studies with human and bovine lymphocytes and no metabolic

activation system reported positive results at concentrations more than two orders of magnitude lower. The earlier review noted several other unusual features about the positive result studies including an unusual exposure protocol and discordant positive results for another chemical found negative in other laboratories.

As indicated in Table B.6.4-29 both positive and negative results have been reported for glyphosate and GBFs in the nine in vitro chromosome effects assays published after the Williams et al. (2000, ASB2012-12053) review. It is noteworthy that many of these studies have various deficiencies in conduct or reporting compared to internationally accepted guidelines for conduct of in vitro chromosome aberration or micronucleus studies (see Table B.6.4-28). Perhaps the most significant deficiency was that coding and scoring of slides without knowledge of the treatment or control group was not indicated in seven of nine publications. This could be a deficiency in conducting the studies or perhaps a deficiency in describing methodology in the publications. Other common deficiencies included failure to indicate control of exposure medium pH, no use of exogenous metabolic activation and no reporting of concurrent measures of toxicity.

Results for glyphosate active ingredient

Three publications reported testing of technical glyphosate for micronucleus or chromosome aberration endpoints in cultured human lymphocytes (Manas et al., 2009, ASB2012-11892; Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012-11907). Negative results for the micronucleus or chromosome aberration end points were observed in the absence of exogenous metabolic activation (S9) in all three publications. The maximum exposure concentration in the absence of S9 was in the range of 3-6 mM in these studies.

Two publications by one author reported cytokinesis block micronucleus results for cultured bovine lymphocytes treated with what was reported as 62 % by weight isopropyl amine salt of glyphosate from a Monsanto Belgium source (Piesova, 2004, ASB2012-12001; Piesova, 2005, ASB2012-12000). This test material appears to be a manufacturing batch of the isopropylamine salt of glyphosate in water without surfactants, which is not sold as a GBF. In one publication no statistically significant increases in binucleated cell micronucleus frequency were observed with 24 hours of treatment (Piesova, 2004, ASB2012-12001). For

48 hours of treatment a statistically significant increase in micronucleus frequency was observed in one donor at 280 µM but not at 560 µM and in a second donor at 560 µM but not

280 µM. The second publication reported negative results for the cytokinesis block micronucleus assay in bovine lymphocytes incubated with glyphosate formulation up to 560 µM for two hours in the absence and presence of a mammalian metabolic activation system (Piesova, 2005, ASB2012-12000). This publication also reported positive results for 48 hours of treatment without S9. Curiously, in this second publication the same inconsistent dose response pattern was observed in which a statistically significant increase in micronucleus frequency was observed in one donor at 280 µM but not at 560 µM and in a second donor at 560 µM but not 280 µM. The lack of a consistent dose response pattern between donors suggests that the results with 48 hours of treatment are questionably positive. Two other publications found negative results for the chromosome aberration endpoint in cultured bovine lymphocytes treated with what appears to be the same test material of 62 %

by weight isopropylamine salt of glyphosate from a Monsanto Belgium source, (Holeckova, 2006, ASB2012-11847; Sivikova and Dianovsky, 2006, ASB2012-12029). Both the studies used a maximum concentration of 1.12 mM which was reported to cause a decrease in mitotic inhibition of >50 %. These two studies have several limitations including that an exogenous mammalian metabolic activation system was not used for chromosome aberration and scoring was not reported to be on

coded slides. In addition, Holeckova (2006, ASB2012-11847) only examined effects detectable by staining of chromosome 1 and did not report positive control results (Holeckova, 2006, ASB2012-11847). Despite these limitations and the variable donor results, the results from these two studies are generally consistent with a lack of chromosome aberration effects of the isopropylamine salt of glyphosate on in vitro cultured mammalian cells in several experiments using high, toxic dose levels and exposures of 2-24 hours in the absence of S9.

One laboratory reported increases in cytokinesis-blocked micronucleus frequency in cultured human lymphocytes exposed to glyphosate for 4 hours in the presence of an exogenous human liver metabolic activation system (S9) in two publications (Mladinic et al., 2009a; Mladinic et al., 2009b). In both publications a statistically significant increase in micronuclei was observed with S9 at the highest dose level of glyphosate tested (580 µg/mL, ≈ 3.4 mM). Increased proportions of centromere- and DAPI-positive micronuclei were observed for the high dose with S9 suggesting that the induced micronuclei were derived from chromosomes rather than chromosome fragments. Statistically significant increases in the frequency of nuclear abnormalities (buds and bridges) and DNA strand breakage were also observed at the highest dose tested in both publications. In parallel experiments cytotoxic effects such as early apoptosis, late apoptosis and necrosis were observed and these effects were uniquely or preferentially observed in the presence of S9 and at the highest dose level tested (Mladinic et al., 2009, ASB2012-11906). Also, the negative control level of such end points as necrosis and alkaline SCGE tail moment was significantly increased in the presence of S9 (Mladinic et al., 2009, ASB2012-11906). It should be noted that glyphosate is mostly excreted unmetabolised in vivo in mammals with only very small levels of aminomethylphosphonic acid (AMPA) or an AMPA-related structure observed (, 2009, ASB2012-11542; 1991, TOX9551791). These observations suggest that the observations of S9

mediated effects by Mladinic et al. are not likely to be due to in vivo relevant metabolites. The preponderance of in vitro genotoxicity studies conducted with exogenous mammalian metabolic activation systems has been negative, including a previously reviewed chromosome aberration study in human lymphocytes conducted up to a similar dose level (Williams et al., 2000, ASB2012-12053) and a bovine lymphocyte cytokinesis block micronucleus study (Piesova, 2005, ASB2012-12000). Overall these results suggest the possibility of a weak aneugenic rather than clastogenic (chromosome breaking) effect occurring in the presence of S9 at high dose levels of glyphosate. The pattern of activity as well as the failure to observe activity in several other in vitro genotoxicity assays conducted with S9 suggests that the activity observed in the Mladinic et al. studies does not have a significant weight of evidence for in vitro genotoxicity and is not likely to be relevant to in vivo genotoxicity.

The recently published results for mammalian in vitro chromosome aberration and micronucleus assays demonstrate a weight of evidence that technical glyphosate and glyphosate salt concentrates are negative for these end points in cultured mammalian cells in the absence of an exogenous mammalian metabolic activation system. Five publications from four laboratories report negative in vitro mammalian cell chromosome or micronucleus results in the absence of exogenous activation while three publications from two laboratories report positive results. These results reinforce the Williams et al. (2000, ASB2012-12053) conclusion that positive chromosome aberration results reported for glyphosate in cultured human lymphocytes in the absence of an exogenous metabolic activation system are aberrant.

Recent reports of positive chromosome aberration and micronucleus results for glyphosate in the presence of an exogenous mammalian activation system in cultured human lymphocytes in one laboratory (Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012- 11907) have no

substantial reproducibility verification from other laboratories in the recent in vitro chromosome effects studies considered in this review because most of the studies performed by other laboratories (Table B.6.4-29) did not employ an exogenous mammalian activation system. These results are discordant with one previously reviewed result demonstrating a negative result for glyphosate in cultured human lymphocytes with mammalian metabolic activation using the chromosome aberration endpoint (Williams et al 2000, ASB2012-12053) and a negative result in the presence of S9 for the micronucleus endpoint in bovine lymphocytes (Piesova, 2005, ASB2012-12000). The numerous consistent negative results for glyphosate and GBFs in gene mutation studies which employed exogenous mammalian metabolic activation and careful examination of the data suggests that the positive results indicate a possible threshold aneugenic effect associated with cytotoxicity rather than a DNA-reactive mechanism resulting in chromosome breakage. Thus, the weight evidence for the in vitro chromosome effect assays indicates a lack of DNA-reactive clastogenic chromosome effects.

Results for GBFs

Amer et al. (2006, ASB2012-11539) reported positive in vitro chromosome aberration effects in mouse spleen cells for a formulation described as herbazed, which was reported to contain 84 % glyphosate and 16 % solvent, an unusually high glyphosate concentration for a formulation. The test material is not further characterised, lacking description of the glyphosate salt form and inert ingredients. The glyphosate concentrations used in the study are not clear because there are different descriptions of the concentration units (M or M glyphosate/ml medium) in the publication. Thus, the maximum concentration might have been 5 x 10-5 M (50 µM) or 5 x 10-5 M glyphosate/mL medium (50 mM). The former concentration, which was reported as toxic, would indicate effects at concentrations well below those typically found toxic for GBFs in cultured mammalian cells. The latter level of 50 mM would be well in excess of the limit level of 10 mM recommended in OECD guidelines (OECD473, 1997). In addition to a question about the concentration used there are several other limitations to the reported study including no indication that pH of treatment solutions was controlled, no use of a mammalian metabolic activation system, no reported concurrent toxicity measurements and no reported use of coded slides for scoring. Given these limitations, the uncertainty about the concentrations used and the nature of the test material, these results should not be considered to have significant relevance or reliability with respect to glyphosate or GBFs.

In addition to in vitro mammalian cell studies there is also a report of negative results for the chromosome aberration and micronucleus endpoints in onion root tips incubated with a Roundup formulation (Dimitrov et al., 2006, ASB2012-11607). The maximum exposure concentration (stated as 1 % active ingredient) is estimated to be on the order of 4-6 mM. This study did not employ an exogenous mammalian metabolic activation system; however, it does provide evidence for a lack of chromosome effects for glyphosate and a GBF in a non- mammalian in vitro system. The result agrees with earlier reported negative onion root tip chromosome aberration results for glyphosate but is discordant with earlier reported positive results for a Roundup GBF in this system (Williams et al., 2000, ASB2012-12053).

B.6.4.8.5.2 In vivo Chromosome Effects—Mammalian Systems

The Williams et al. (2000, ASB2012-12053) glyphosate toxicity review presented results from in vivo mammalian chromosome effect assays. Results from several mouse bone marrow erythrocyte micronucleus studies of glyphosate and GBFs (e.g. Roundup, Rodeo and Direct) were negative for micronucleus induction. These included studies from different laboratories mostly following modern guidelines. The intraperitoneal (i.p.) route was used for most of the negative studies and maximum doses for many of the studies were toxic or appropriately close to LD50 values. In addition to i.p.

studies a 13 week mouse feeding study was also negative for the micronucleus endpoint with an estimated maximum daily glyphosate dose of over 11,000 mg/kg/day. There was one published report of a weak positive mouse bone marrow micronucleus response observed for glyphosate and Roundup GBF. This study, which employed a smaller number of animals per group than other negative studies, was clearly aberrant from the numerous other negative studies not only in micronucleated cell frequency finding but also the finding of altered polychromatic erythrocyte to normochromatic erythrocyte (PCE/NCE) ratios. The overall weight of evidence from the earlier reviewed studies was that glyphosate and GBFs were negative in the mouse bone marrow erythrocyte micronucleus assay. The earlier review also noted a negative mouse dominant lethal result for glyphosate administered by gavage at a maximum dose level of 2000 mg/kg.

As indicated in Table B.6.4-29, there are numerous subsequent publications of in vivo mammalian chromosome effects assays. With one exception, all of the in vivo mammalian studies were conducted in the mouse using either the bone marrow chromosome aberration or micronucleus endpoints. It should be noted that there are some fairly consistent limitations in the reported conduct of these studies compared to OECD guidelines. In most studies concurrent indications of toxicity (other than effects on the bone marrow) are not reported, coding of slides for scoring is not reported, individual animal data are not reported and fewer than recommended cells or metaphases per animal were scored. Other limitations encountered include use of only a single or two dose levels rather than three dose levels.

Results for glyphosate active ingredient

Two publications reported results for glyphosate in the mouse bone marrow erythrocyte micronucleus assay. Negative results were reported in one study which used a dose of 300 mg/kg of glyphosate administered once i.p. with sacrifices at 24, 48 and 74 hours after dosing (2000, ASB2013-9820). This study had some limitations including the use of only one dose level, no reporting of toxicity other than PCE/NCE ratio, no reported coding of slides for scoring and scoring of 1000 PCE's per animal (scoring of 2000 PCE's per animal is recommended by OECD guidelines). A second publication reported positive results for glyphosate administered at 50, 100 and 200 mg/kg via i.p. injections repeated at 24 hours apart with sacrifice 24 hours after the second dose (., 2009, ASB2012-11892). A statistically significant increase in micronucleated erythrocytes was observed in the high dose group. This study had limitations comparable to the negative study. A more significant potential difficulty with this second publication is that "erythrocytes" rather than polychromatic erythrocytes were indicated as scored for micronuclei. This does not appear to be a case of using "erythrocytes" to mean polychromatic erythrocytes because the term "polychromatic erythrocytes" is used elsewhere in the publication describing measurements of PCE/NCE ratios. Scoring of total erythrocytes instead of immature polychromatic erythrocytes for micronuclei would be inappropriate in an assay with the stated treatment and harvest times because of the transient nature of micronucleated PCE's in bone marrow (OECD474, 1997).

There is no definitive explanation for the discrepancy between the two publications. Although one study used a single dose with multiple harvest times and the second used two doses and a single harvest time, both are acceptable protocols and would not be expected to lead to such discordant results (OECD474, 1997). The negative result reported for the 13 week feeding study in the earlier review (Williams et al., 2000, ASB2012-12053) confirms that positive results are not simply due to repeat dosing. The reported negative result (., 2000, ASB2013-9820) seems to be in accord with a majority of earlier reviewed mouse bone marrow micronucleus studies of glyphosate using similar doses and the

i.p. or feeding routes (Williams et al., 2000, ASB2012-12053). Also, the apparent scoring of

micronuclei in erythrocytes rather than just polychromatic erythrocytes raises a significant methodological question for the reported positive study.

Results for GBFs

There are several publications reporting in vivo mammalian bone marrow chromosome aberration and micronucleus endpoint results for Roundup GBFs. Three publications report negative results for Roundup branded GBF in mouse chromosome aberration or micronucleus assays. Negative results were reported for two different Roundup branded GBFs administered at 2 x 200 mg/kg i.p. in mouse bone marrow erythrocyte micronucleus assays (
2000, ASB2013-11477; 2002, ASB2012-11834). The
second study did not report coding of slides for scoring. Another publication reported negative results in mouse bone marrow studies for both the chromosome aberration and erythrocyte micronucleus endpoints (2006, ASB2012-11607) using a dose of 1080 mg/kg administered orally (p.o.). In contrast, one publication reported positive results for Roundup GBF in mouse bone marrow for the chromosome aberration and erythrocyte micronucleus endpoints using a single maximum dose of 50 mg/kg i.p. (2009, ASB2012-12005). Both the positive results and the magnitude of the increases in the chromosome aberration and micronucleus endpoint reported in this study are remarkably discordant with other reported results for Roundup and other GBFs in mouse bone marrow chromosome aberration and erythrocyte studies in a number of laboratories and publications (Table B.6.4-29 and Williams et al., 2000, ASB2012-12053). The reasons for this discordance are not clear. One unusual feature of the positive study is that the Roundup GBF was administered in dimethylsulfoxide. This is an unusual vehicle to use in in vivo genotoxicity studies, particularly for glyphosate which is water soluble and especially so in a formulated product. A published toxicity study found that use of a dimethylsulfoxide/olive oil vehicle by the i.p. route produced dramatically enhanced toxicity of glyphosate formulation or the formulation without glyphosate compared to saline vehicle and that the enhanced toxicity observed with this vehicle was not observed when the oral route was used (., 2008, ASB2012-11845). These observations suggest that use of DMSO as a vehicle for administration of formulation components by the i.p. route might produce unusual toxic effects that are not relevant to normally encountered exposures. Regardless of the reasons for the discordant positive results it is clear that a large preponderance of evidence indicates that GBFs are typically negative in mouse bone marrow chromosome aberration and erythrocyte assays.

One publication reported positive results for bone marrow chromosome aberration in rabbits administered Roundup GBF in drinking water at 750 ppm for 60 days (, 2005, ASB2012-11841). This study is relatively unique in terms of species and route of administration. The results do not report water intake in the test and control groups. Given the potential for water palatability issues with a formulated product, this is a significant shortcoming, as any effects noted may be attributable to dehydration. This study had further limitations including the use of only a single dose level and not coding slides for scoring.

Examination of the chromosome aberration scoring results showed that large increases for the treated group were observed for gaps and “centromeric attenuation” which were included in the summation and evaluation of structural chromosome aberration effects. Ordinarily gaps are scored but are not recommended for inclusion in total aberration frequency and centromeric attenuation is not included in ordinary structural aberrations (OECD475, 1997). These unusual scoring and interpretive features raise significant questions about using this study to make conclusions about clastogenicity of the GBF tested.

Two other publications report in vivo mammalian chromosome aberration or micronucleus results

for GBFs. An uncharacterisd GBF, Percozyd 10L, was reported to be negative in a mouse bone marrow erythrocyte micronucleus assay (2000, ASB2013- 8929 and ASB2013-8931). The maximum dose level tested, 90 mg/kg i.p., was reported to be 70 of the i.p LD50 as determined experimentally by the authors. This study had several limitations including use of less than three dose levels and no reported coding of slides for scoring. Positive results were reported for another uncharacterized GBF, herbazed, in mouse bone marrow and spermatocyte chromosome aberration studies (2006, ASB2012- 11539). No statistically significant increases in aberrant cells were observed in bone marrow cells for i.p. treatment of 50 mg/kg for 1, 3 or 5 days or in spermatocytes for 1 or 3 days treatment. Statistically significant increases in frequency of spermatocytes with aberrations were reported for 5 days of treatment with 50 mg/kg (i.p.). Oral treatment of 50 mg/kg and 100 mg/kg were reported to produce increases in aberrant cell frequency in bone marrow cells after extended treatments (14 and 21 days) but not after shorter 1 and 7 day treatments. Similarly, significant increases in aberrant cell frequencies of spermatocytes were reported at 14 and 21 days of 50 mg/kg oral treatment (negative for 1 and 7 days treatment) and at 7, 14 and 21 days of 100 mg/kg treatment (negative for 1 day treatment). Although not a genotoxic endpoint per se, it should be noted that statistically significant increases in frequency of sperm with abnormal morphology were also observed in mice treated with 100 and 200 mg/kg p.o. for 5 days. The positive results for the uncharacterized herbazed GBF were only observed after extended oral treatments (bone marrow and spermatocytes) and extended i.p. treatments (spermatocytes). The fact that positive results were not observed in an erythrocyte micronucleus test of mice treated with glyphosate up to 50,000 ppm in feed for 13 weeks (Williams et al., 2000, ASB2012-12053) provides direct evidence that extended glyphosate treatment by the oral route does not induce detectable chromosome effects. This treatment was longer and up to much higher glyphosate exposures than those used for the . (2006, ASB2012-11539) studies. Thus, it appears likely that these effects were due to some component(s) of the specific herbazed GBF tested rather than glyphosate.

In vivo mammalian assays for chromosome effects are an important category for characterisng genotoxicity that complements the gene mutation category. While some positive results have been reported the preponderance of evidence and published results are negative for glyphosate and GBFs.

B.6.4.8.5.3 In vivo Chromosome Effects—Non-Mammalian Systems

The Williams et al. (2000, ASB2012-12053) review reported a few in vivo plant assays for chromosome effects in non-mammalian systems. These included negative results for glyphosate and positive results for Roundup GBFs for chromosome aberrations in an onion root tip assay and negative results for glyphosate with the micronucleus end point in a Vicia faba root tip assay. Subsequent to the earlier review a number of publications reported results for erythrocyte micronucleus assays conducted on GBFs in several non-mammalian fish and reptile species with discordant results. One publication reported apparently negative results for the erythrocyte micronucleus test in Oreochromis niloticus (Nile tilapia) administered a test material described as Roundup 69 GBF, at an upper dose of 170 mg/kg i.p. (
2000, ASB2013-11477). Although there was an increase in micronucleated erythrocyte frequency at the mid-dose level this was not observed at the high dose level and considerable variability in frequencies in different groups was noted. Negative results were also reported in another fish species (Prochilodus lineatus) exposed to 10 mg/liter Roundup branded GBF for 6, 24 and 96 hours (2008, ASB2012- 11586). This concentration was reported to be 96 % of a 96 hour LC50. Positive results were reported for the erythrocyte micronucleus assay conducted in the fish Tilapia rendalii exposed to 170 mg/kg i.p. of another Roundup GBF (2002, ASB2012-11834). Examination of the micronucleus frequencies in this publication indicated that the negative control micronucleus

frequency was considerably lower than the frequencies for all but one of 21 treatment groups for 7 different test materials. This suggests an unusually low control frequency and at least one treatment group was statistically significantly elevated for each of the 7 test materials, including many instances where the statistically significant increases were not consistent with a biologically plausible dose response. The possibility that the apparently significant increases were due to a low negative control value should be considered for this publication. Another publication reported positive erythrocyte micronucleus results in goldfish (Carassius auratus) exposed to 5 to 15 ppm of a Roundup GBF for 2 to 6 days (, 2007, ASB2012-11587). The reasons for the discordant results are not clear for these fish erythrocyte micronucleus assays of Roundup GBFs. Although different species and GBF's were used in the different studies there were pairs of studies with positive and negative results that used similar treatment conditions (170 mg/kg i.p. or 10-15 mg/litre in water).

Results for an unusual test system of exposed caiman eggs are reported by 2009, ASB2012-12002. Eggs were topically exposed in a laboratory setting to Roundup Full II GBF, and erythrocyte micronucleus formation was measured in hatchlings (., 2009, ASB2012-12002). The GBF tested was reported to contain the potassium salt of glyphosate and alkoxylated alkylamine derivatives as surfactants. Statistically significant increases in micronucleated erythrocytes were observed in hatchlings from eggs treated with 500-1750 µg/egg. This system is quite unusual in the species tested and even more so in using an egg application with measurement of effects in hatchlings. Although there is some experience with a hen's egg erythrocyte micronucleus assay using in ovo exposure the erythrocytes are evaluated in embryos with only a few days between treatment and the erythrocyte micronucleus end point. In the reported caiman egg assay there was presumably a single topical exposure followed by an egg incubation period of about 10 weeks before hatching. Biological plausibility raises questions whether genotoxic events in ovo can produce elevated micronucleated erythrocyte frequencies detectable after 10 weeks, given the number of cell divisions occurring in development of a hatchling.

One published study reported a weak positive result in a Drosophila wing spot assay (Kaya et al., 2000, ASB2013-9832). Statistically significant positive increases were only in one of four crosses for small twin spots and not for the two other wing spot categories (large wing spots and twin wing spots). As discussed above, only negative or inconclusive results were observed for crosses that were not subject to mitotic recombination effects. If the result was actually treatment related it only would indicate an increase in recombination events and not in somatic mutations.

The above in vivo chromosome effect assays in non-mammalian systems give discordant results for reasons that aren't precisely defined. Typically these results would be given lower weight than mammalian systems in being predictive of mammalian effects, especially since
there is little or practically no assay experience with these systems in comparison with in vivo mammalian chromosome effects assays, such as the rat or mouse bone marrow chromosome aberration or erythrocyte micronucleus assays.

B.6.4.8.6 DNA damage and other end points

A number of studies of glyphosate and GBFs have been published since 2000 which used various DNA damage end points in a variety of in vitro and in vivo systems. The DNA damage category includes end points such as sister chromatid exchange and DNA repair response in bacteria, but the most common DNA damage end point encountered was the alkaline single cell gel electrophoresis end point (alkaline SCGE) also commonly referred to as the "comet" assay. The alkaline SCGE end point has been applied to both in vitro and in vivo test systems.

In addition to DNA damage there are a few reports of other types of studies which can be associated with genotoxic effects even though the end points are not specific indicators of genotoxicity per se.

These include sperm morphology and carcinogenicity studies.

In vitro DNA Damage Studies

Some positive results for glyphosate or GBFs in the SCE end point were reported in cultured human and bovine lymphocytes in the earlier review (Williams et al., 2000, ASB2012- 12053). These results tended to be weak, inconsistent and with limited evidence for dose response. A number of limitations were observed for the studies such as the failure to control pH and abnormally low control values. Additional in vitro DNA damage end point results described in the earlier review included negative results for glyphosate in the B. subtilis rec- assay and in the primary hepatocyte rat hepatocyte unscheduled DNA synthesis assay.

There are two subsequent publications using in vitro cultured mammalian cells and the SCE endpoint. Positive SCE results were reported for the uncharacterised herbazed GBF in mouse spleen cells (Amer et al., 2006, ASB2012-11539). The dose response pattern for SCE response in this study was similar to the response for chromosome aberrations in this publication. Limitations of this study are in common to those described above for the chromosome aberration end point portion of the study; no indication that pH of treatment solutions was controlled, no use of a mammalian metabolic activation system, no reported concurrent toxicity measurements and no reported use of coded slides for scoring. Positive SCE results were also reported for cultured bovine lymphocytes treated with up to 1.12 mM glyphosate for 24 and 48 hours without exogenous mammalian metabolic activation (Sivikova and Dianovsky, 2006, ASB2012-12029). The highest dose of 1.12 mM significantly delayed cell cycle progression with 48 hour treatment. These same concentrations for 24 h exposures did not induce statistically significant increases in chromosome aberrations which provides a clear example of a differential response of the SCE endpoint (Sivikova and Dianovsky, 2006, ASB2012-12029). This is an important consideration in these publications, as chromosome effects are considered more relevant to genotoxicity than DNA damage.

Positive results for glyphosate are reported for the alkaline SCGE end point in three publications. Positive SCGE results were observed for two mammalian cell lines exposed to glyphosate for 4 hours at concentrations of 4.5-6.5 mM (GM39 cells) and 4.75-6.5 mM (HT1080 cells) (Monroy et al., 2005, ASB2012-11910). These concentrations are close to the upper limit dose of 10 mM generally recommended for in vitro mammalian cell assays and control of medium pH is not indicated. Characterisation of nuclear damage was done by visual scoring without coding of slides being indicated. Positive alkaline SCGE results were also reported in Hep-2 cells exposed for 4 hours to 3.5-7.5 mM glyphosate (Manas et al.,

2009, ASB2012-11892). Higher concentrations of glyphosate were reported to result in viability of <80 % as determined by dye exclusion. As noted for the preceding publication, the concentrations employed were reasonably close to a limit dose of 10 mM and control of medium pH was not reported. This publication reported negative results for the chromosome aberration endpoint in cultured human lymphocytes exposed to up to 6 mM glyphosate for 48 hours and it should be noted that in this case an appropriate control of medium pH was reported for this human lymphocyte experiment. Positive alkaline SCGE results have also been reported for cultured human lymphocytes exposed to glyphosate at concentrations up to 580 µg/ml (estimated 3.4 mM) for 4 hours (Mladinic et al., 2009, ASB2012-11906). Effects were observed both in the presence and absence of S9 with statistically significant increases in tail intensity at 3.5, 92.8 and 580 µg/ml without S9 and at 580 µg/ml with S9. A modification of the alkaline SCGE assay employing human 8-hydroxyguanine DNA-glycosylase (hOGG1) to detect oxidative damage only indicated statistically significant effects on tail length for treatment with 580 µg/ml with S9. Increases in nuclear abnormalities (nuclear buds and/or nucleoplasmic bridges) were also observed at 580 µg/mL with and without S9 and in micronucleus

frequency at 580 µg/ml with S9. Measurements of total antioxidant capacity and thiobarbituric acid reactive substances showed statistically significant increases at 580 µg/ml in the presence or absence of S9. Interpretation of the significance of metabolic activation effects is complicated by the observation that several of the end points (alkaline SCGE tail intensity and nuclear abnormalities) tended to show increases in the presence of S9 in negative controls or at the very lowest concentrations of glyphosate. A reasonable summation of the results in this publication is that alkaline SCGE effects and other effects such as nuclear abnormalities, early apoptosis, necrosis and oxidative damage were consistently observed at 580 µg/mL.

In addition to mammalian cell studies there are publications reporting positive alkaline SCGE effects for glyphosate in Tradescantia flowers and nuclei exposed to up to 700 µM glyphosate (Alvarez-Moya et al., 2011, ASB2012-11538) and in the E. coli SOS chromotest for DNA damage conducted on a Roundup BIO GBF (Raipulis et al., 2009, ASB2012-12008). Observations of DNA damage in plants exposed to glyphosate are of questionable significance because of the herbicidal nature of glyphosate and the SOS chromotest provides only indirect evidence of DNA damage in a bacterial system.

Overall there appear to be a number of studies in which glyphosate or GBFs have been reported to produce positive responses in DNA damage endpoints of SCE or alkaline SCGE in vitro in mammalian cells. Most of these have occurred with exposures to mM concentrations of glyphosate. Although this dose level range is lower than the limit dose of 10 mM recommended for several in vitro mammalian cell culture assays (OECD473, 1997; OECD476, 1997; OECD487, 2010), an even lower limit dose of 1 mM was recently recommended for human pharmaceuticals, particularly because of concerns about relevance of positive in vitro findings observed at higher dose levels. In addition, many of the studies have limitations such as not indicating control of medium pH and not coding slides for visual scoring. Concerns over the possibility of effects induced by toxicity have led to several suggestions for experimental and interpretive criteria to distinguish between genotoxic DNA-reactive mechanisms for induction of alkaline SCGE effects and cytotoxic or apoptotic mechanisms. One recommendation for the in vitro alkaline SCGE assay is to limit toxicity to no more than a 30 % reduction in viability compared to controls. Importantly, dye exclusion measurements of cell membrane integrity, such as those reported in some of the above publications may significantly underestimate cytotoxicity that could lead to alkaline SCGE effects. Other

recommendations include conducting experiments to measure DNA double strand breaks to determine if apoptotic process might be responsible for alkaline SCGE effects. Measurement of apoptotic and necrotic incidence were only performed in one publication (Mladinic et al., 2009, ASB2012-11906) and these measurements indicated both apoptotic and necrotic processes occurring in parallel with observations of alkaline SCGE effects. These direct observations as well as the reported dose responses, consistently suggest that biological effects and cytotoxicity accompany the observations of DNA damage in vitro in mammalian cells and therefore confirm the likelihood that the observed effects are secondary to cytotoxicity and are thresholded.

In vivo DNA damage studies

In the earlier review positive results for DNA strand breakage were reported for mice treated by the i.p. route with glyphosate and GBFs and for the alkaline SCGE endpoint in tadpoles of the frog Rana catesbiana exposed to a GBF (Williams et al., 2000, ASB2012-12053).

. (2006, ASB2012-11539) reporten an increase in SCE frequency in bone marrow cells of mice treated with uncharacterised herbazed GBF. Statistically significant positive effects were only observed at the highest dose level tested (200 mg/kg administered p.o.).

Several recent publications report alkaline SCGE results for GBFs in aquatic species. Three

publications reported positive alkaline SCGE results in aquatic vertebrates exposed to Roundup GBFs in water. These publications have a common feature that alkaline SCGE results were reported as visually scored damage category incidence rather than instrumental measurements of properties such as the tail length or tail intensity. In one publication increases in nuclei exhibiting alkaline SCGE visual damage effects were observed in erythrocytes and gill cells of the tropical fish Prochilodus lineatus exposed to 10 mg/litre of a Roundup GBF in water (2008, ASB2012-11586). Results were variable with cell type and incubation; statistically significant positive responses were observed for erythrocytes at 6 hours and 96 hours, but not 24 hours or for branchial cells from the gills at 6 hours and 24 hours. Measurement of erythrocyte micronucleus frequency and nuclear abnormalities did not show statistically significant increases in these endpoints. The concentration used was reported to be 75 % of the 96 hour LC50, but trypan blue dye measurements apparently indicated >80 % viability of cells used in the alkaline SCGE assays. A second publication reported positive alkaline SCGE results in erythrocytes of the goldfish, Carasseus auratus, exposed to 5, 10 and 15 ppm of a Roundup GBF for 2, 4 or 6 days (
2007, ASB2012-11587). Similar effects were observed for other end points (micronucleus and nuclear abnormalities). In general, effects increased with concentration and time. This publication did not report toxicity measurements or, more specifically, measurements of cell viability in the population studied. Positive results were also reported in erythrocytes of the European eel, Anguilla anguilla, exposed to 58 and 116 µg/liter of a Roundup GBF in water for 1 or 3 days ., 2010, ASB2012-11836). Increases in nuclear abnormalities were also observed in erythrocytes from animals exposed for 3 days. Measurement of toxicity was not reported for the animals or erythrocytes; however, several endpoints relevant to antioxidant responses and oxidant effects were made in whole blood samples. No statistically significant effects were observed for catalase, glutathione transferase, glutathione peroxidase, glutathione reductase or reduced glutathione content. A large statistically significant increase for thiobarbituric acid reactive substances (TBARS, a measure of lipid peroxidation) was observed for the 115 µg/litre concentration group at 1 day. Statistically significant TBARS increases were not observed at 3 days, but, the 3-day negative control value appeared to be several fold higher than the 1-day value.

Significance of DNA damage end point results

DNA damage end points such as SCE or alkaline SCGE are generally regarded as supplementary to the gene mutation and chromosome effects end point categories. DNA damage endpoints do not directly measure effects on heritable mutations or events closely associated with chromosome mutations. In vitro DNA damage endpoints such as the SCE or alkaline SCGE can be induced by cytotoxicity and cell death processes rather than from DNA-reactive mechanisms.

The observation of effects of sodium dodecyl sulfate is also interesting because it suggests responses to surfactants which are typically components of GBFs. As a more specific example, polyoxyethylenealkalylmine (POEA), a surfactant component of some GBFs has been shown to elicit cytotoxic effects such as perturbation of the mitochondrial membrane and disruption of mitochondrial membrane potential in cultured mammalian cells (, 2007, ASB2009-9030). Surfactant effects provide a plausible mechanism for observations of GBFs inducing DNA damage responses. Such responses would be expected to be associated with cytotoxicity-inducing exposures and exhibit a threshold.

B.6.4.8.7 Human and environmental studies

A number of human and environmental studies have been published in or after 2000 where some exposures to GBFs in the studied populations were postulated. These publications are summarised in

Table B.6.4-30.
Table B.6.4-30: Studies of Human and Environmental Populations with Reported or Assumed Glyphosate Exposure

Exposed Population	End point	Exposures	Result	Reference
Human Studies				
Open field and fruit farmers	Bulky DNA adducts	glyphosate formulation use	No effects attributed to glyphosate (ASB2012-11543)	., 2007
farmers		reported in only 1 formulation		
of 29 fruit farmers		exposure		
Humans in areas where glyphosate spraying of but no clear relationship to applied	Lymphocyte cytokinesis block micronucleus (CB MN)	Aerial or manual glyphosate formulation for assumed or reported illicit crop control exposures and sugar cane maturation	Increase in CB MN	2009
Floriculturists	Lymphocyte CB			

Glyphosate
Increase in CB MN
MN
formulation use
but not statistically
2004
reported in 21/51
significant
(ASB2012-11572)
workers with
average of 106.5 kg
applied
Floriculturists
Lymphocyte CB
Glyphosate
Statistically
MN
formulation use
significant increase
2002
reported in 57/107
in CB MN
(ASB2012-11573)
workers. Numerous
other pesticides
reported as used by
a similar number or
more of workers
Exposed Population
End point
Exposures
Result
Reference
Agricultural workers
Buccal cell
Glyphosate
Statistically
micronucleus
formulation use
significant increase
2009
reported along with
in MN
(ASB2012-11570)
numerous other
pesticides

Fruit growers
Lymphocyte
Glyphosate use
No effects
Alkaline SCGE;
reported in 2/19 1
attributable to
2003
Ames test on urine
day before captan
glyphosate
(ASB2012-11878)
spraying and 1/19
formulation
on the day of
exposure
captan spraying
Agricultural workers
Lymphocyte CB
Glyphosate
No statistically
2003
MN; buccal cell
formulation use
significant increases
(ASB2012-11991)
micronucleus
reported in 16% of
in CB MN or buccal
one of four
cell micronucleus
populations studied
frequencies
(Hungary)
Individuals on or
Lymphocyte
Glyphosate
Statistically
near glyphosate
alkaline SCGE
formulation
significant increases
2007
spraying
aerially sprayed
in damaged cells

(ASB2012-11992)
within 3 km
Greenhouse Farmers
Lymphocyte SCE
Glyphosate
Statistically
formulation use
significant increases
2001
reported in 99/102
in SCE
(ASB2012-12025)
workers; numerous
other pesticides
used
Farmers
Lymphocyte CB
Glyphosate
Statistically
2006
MN
formulation use
significant increase
(ASB2012-12045)
reported in 3/11
in micronucleus
farmers
frequency but not in
frequency of
binucleated cells
with micronuclei
Environmental Studies
Meadow voles living
Blood cell alkaline
Glyphosate
Some effects judged
on golf cousres
SCGE; erythrocyte
formulation use
possibly related to
2004
micronucleus
reported along with
Daconil® fungicide
(ASB2012-11871)
numerous other

pesticides
Fish from dams
Erythrocyte
Glyphosate
Higher MN
2011
(various species)
micronucleus
formulation use
frequencies than
(ASB2012-12017)
reported in adjacent
normal or expected
lands along with
but no negative
other pesticides
concurrent controls
used
Many of the human studies either found no effects attributable to GBFs or the reported GBF usage by the studied population was too low to be associated with observed population effects (., 2007, ASB2012-11543; ., 2004, ASB2012-11572; ., 2003, ASB2012-11878; ., 2003, ASB2012-11991; ., 2006, ASB2012-
12045).
In some studies, incidence of GBF use by the population studied was significant but high incidence of use of other pesticides was also reported (2002, ASB2012- 11573; 2001, ASB2012-12025). Even though positive effects were observed in these populations, ascribing these effects to any particular environmental exposure is not
scientifically justifiable and such results certainly cannot be considered as definitive evidence for GBF-induced human genotoxic effects.
Two published studies focused on populations believed to be exposed to GBFs by their presence at or near aerial or manual spraying operations. One publication reported induction of alkaline SCGE effects in blood lymphocytes of populations living within 3 km of areas sprayed with glyphosate formulation for illicit crop eradication 2007, ASB2012-11992). The populations studied were relatively small (24 exposed individuals and 21 non-exposed individuals). The sprayed material was reported to be Roundup Ultra, a GBF containing 43.9 % glyphosate, polyethoxylated surfactant and a proprietary component, Cosmoflux 411F. Specific methods for collection, storage, and transport of blood samples are not described for either the exposed population or control group. The publication also does not indicate that slides were coded for scoring which consisted of visual classification into damage categories and measurement of DNA migration (tail length). There were fairly large differences in ages and sex distribution of the exposed and control populations but these did not appear to be statistically significant. The study reported increases in damaged cell categories and statistically significant increases in DNA migration (tail length) in the presumably exposed population. Interpretation of the results of this study should consider numerous reported signs of toxicity in the exposed population and the reported application rate of 24.3 litres/ha which was stated to be 20 times the maximum recommended application rate. Some of the reported human health effects described by

(2007, ASB2012-11992) appear to be consistent with severe exposures noted in clinical reports of acute poisoning incidents with GBFs and other pesticide formulations (often self- administered) rather than typical bystander exposures. Given the considerably favorable general toxicology profile of glyphosate as reported by the WHO/FAO Joint Meeting on Pesticide Residues (WHO/FAO, 2004, ASB2008-6266) and in Williams et al. (2000, ASB2012-12053), factors related to either high surfactant exposure, unusual GBF components in this formulation or other undocumented variables appear to be confounding factors in this study. It appears that the reported alkaline SCGE effects could well have been secondary to the ailments reported in this study population.

A second publication reported results for a blood lymphocyte cytokinesis-block micronucleus study of individuals in areas treated with glyphosate formulation by aerial spraying or manual application (2009, ASB2012-11570). Although the title of the publication contains the term “agricultural workers”, most of the populations studied do not appear to be agricultural workers who are involved in application of GBFs. The human lymphocyte culture and scoring methodology employed in the . (2009, ASB2012-11570) study appear to be generally consistent with commonly used and recommended practices for this assay. However, there is a significant question as to how long the blood samples used in the study were stored prior to initiating cultures and this may have affected the micronucleus numbers observed in the different sets of samples and populations. Also, the populations in the aerially sprayed regions had a second sampling a few days after the first sampling and this second sampling was not performed in the control populations. The publication reported a small increase in the frequency of binucleated cells with micronuclei and micronuclei per cell in samples collected from people living in three regions after spraying of GBFs compared with control values of samples collected just before spraying. However, the pattern of the increases did not correlate either with the application rate or with self-reported exposure. The largest post-spraying increase in binuclated cell micronucleus frequency was reported for a population with a much lower glyphosate active ingredient application rate and only 1 of 25 people in this region reported contact with sprayed glyphosate formulation. Increases in binucleated cell micronucleus frequency did not have a statistically significant relationship with self-reported exposure for two other populations. Some interpretative statements in

(2009, ASB2012-11570) suggest a small transient genotoxic effect of glyphosate formulation spraying on frequencies of binucleated cells with micronuclei, but other statements indicate that causality of the observed effects could not be determined using reasonable criteria and that lack of exposure data precluded conclusions. This study has a combination of uncontrolled or inadequately characterized variables, such as uncharacterisd exposure to ”genotoxic pesticides”, that would appear to preclude using the data to support any conclusion that exposure to GBFs affects binucleated micronucleus frequencies. Actually, the available data, while certainly limited in nature, support a conclusion that the observed effects do not appear to be attritubable to glyphosate formulation exposure. This conclusion is reinforced by (2004, ASB2012-11528), where biomonitoring of agricultural workers applying GBFs reports systemic exposures orders of magnitude below in vivo model chromosome aberration and micronucleus study doses, the majority of which were negative for glyphosate and GBFs.

There are two publications related to environmental monitoring for genotoxic endpoints. One study using blood cell alkaline SCGE and micronucleus endpoints was conducted on samples from meadow voles living on or near golf courses where pesticides had been applied (2004, ASB2012-11871). Results were significantly inconsistent between two seasons. Although some suggestions of effects were reported, glyphosate was only one of a number of applied pesticides and the effects observed were considered as possibly attributable to exposure to Daconil® fungicide. A second publication

reported results for the erythrocyte micronucleus assay applied to fish collected from several dams in Brazil (, 2011, ASB2012-12017). Glyphosate formulation was one of a number of pesticides reported to be used in the area of the dams. No efforts appear to have been made to measure glyphosate or other pesticide concentrations in any of the ten dams from which fish were sampled. This study reported what were considered to be high levels of micronucleated cell frequency but there were no concurrent negative controls. In the absence of these controls the results cannot be interpretted as indicating any effect of pesticide exposure.

Although there have been a fairly large number of human genotoxicity studies reported where there was some exposure to GBFs, the large majority of these studies do not allow any conclusions about possible effects of glyphosate or GBFs because the exposure incidence was low or because there were reported exposures to a large number of pesticides. One report found an increase in alkaline SCGE effects in humans living in or near areas where a GBF was sprayed but that study had a number of methodology reporting and conduct deficiencies and the reported effects could well have been due to toxicity reported in the study population. A second study found some increases in cytokinesis-block micronucleus frequency in humans possibly exposed to GBFs but the effects were not concordant with application rates or self- reported exposures and thus do not constitute reliable indications of effects for this endpoint in humans exposed to GBFs. Neither of the two environmental monitoring studies in meadow voles or fish provide any reliable evidence of exposures to glyphosate or GBFs or adverse effects resulting from potential exposures to glyphosate or GBFs.

As noted in the earlier review, 32P-postlabelling DNA adduct studies in mice did not indicate formation of adducts from glyphosate and questionable evidence of adducts from Roundup GBF administered as a high 600 mg/kg i.p. dose in an unusual dimethylsulfoxide/olive oil vehicle (Peluso et al., 1998, TOX1999-318; Williams et al., 2000, ASB2012-12053). Another earlier reviewed study reported DNA strand breakage in liver and kidneys of mice injected i.p with glyphosate and Roundup GBF. This study also reported an increase in 8- hydroxydeoxyguanosine (8-OHdG) residues in liver DNA from mice injected with glyphosate but not GBF. Increased 8-OHdG was found in kidney DNA from mice injected with GBF but not glyphosate (Bolognesi et al., 1997, Z59299; Williams et al., 2000, ASB2012-12053). No new direct studies of DNA reactivity of glyphosate or GBFs were encountered in publications since 2000. One publication did report on studies in mice to further investigate toxic effects and 8-OHdG levels associated with the routes, vehicles and dose levels employed in earlier 32P-postlabelling and DNA strand breakage and 8-OHdG studies (Heydens et al., 2008, ASB2012-11845). This publication reported that high i.p. dose levels of GBF induced significant liver and kidney toxicity that were not observed with oral administration. Statistically significant increases in 8-OHdG were not observed in this study under the same conditions as employed by the earlier study. The dimethylsulfoxide/olive oil vehicle dramatically enhanced toxicity of GBF administered by the i.p. route and the toxicity was also observed for formulation components without glyphosate. These results indicated that the effects reported in the earlier studies were associated with high liver and kidney toxicity that was primarily due to the non-glyphosate components of the formulation and which were produced by the i.p. route of exposure to very high dose levels. The enhancement of toxicity by the unusual dimethylsulfoxide/olive oil dosing vehicle further calls into question whether the 32P-postlabelling finding represented effects associated with unusual toxicity rather than being indicative of adducts formed from glyphosate or glyphosate formulation components.

Carcinogenicity is not a direct endpoint for genotoxicity but it is one of the possible consequences of genotoxicity and, conversely, lack of carcinogenicity in well-conducted experimental studies provides some evidence that a significant genotoxic mode of action is not operating in vivo. The earlier review of glyphosate concluded that it was not carcinogenic

in mouse or rat chronic studies and notes that glyphosate was not considered carcinogenic by numerous regulatory agencies and scientific organisations (Williams et al., 2000, ASB2012- 12053).

B.6.4.8.9 AMPA and POEA

In addition to glyphosate and GBFs, the earlier review included information on the toxicity and genotoxicity of the major environmental breakdown product of glyphosate, aminomethylphosphonic acid (AMPA), and what was at that time a common GBF surfactant mixture of polyethoxylated long chain alkylamines synthesized from animal-derived fatty acids (polyethoxylated tallow amine, ethoxylate, POEA). Today a wide variety of surfactant systems are employed by different companies for different regions and end uses.

In the earlier review, summarised genotoxicity results for AMPA included negative results in the Ames/Salmonella bacterial reversion assay, an in vitro unscheduled DNA synthesis assay in primary hepatocytes and a mouse bone marrow erythrocyte micronucleus assay (Williams et al., 2000, ASB2012-12053). One publication of AMPA genotoxicity results was observed subsequent to 2000. In this publication analytical grade AMPA was reported to have positive effects in several assays including an alkaline SCGE endpoint in cultured mammalian Hep-2 cells, a chromosome aberration endpoint in cultured human lymphocytes and in a mouse bone marrow erythrocyte micronucleus assay (Manas et al., 2009, ASB2012-11891). Experimental limitations in the conduct of the alkaline SCGE assay included no inclusion of mammalian metabolic activation and no reported control of medium pH even though relatively high concentrations of AMPA acid (2.5-10 mM for 4 hours) were used. Although nucleoid images were analyzed with software rather than visual analysis the methodology doesn't indicate that slides were coded and there may have been a visual judgment component in selection of images for analysis. The positive results were statistically significant increases in tail length,

% DNA in tail and tail moment at 4.5 to 7.5 mM AMPA. The human lymphocyte chromosome aberration assay also did not employ an exogenous mammalian metabolic activation system but control of medium pH and blind scoring of slides were reported for this assay. A small increase in chromosome aberrations per 100 metaphases was observed in cells exposed to 1.8 but not 0.9 mM AMPA for 48 hours. The increase was marginally significant ($p<0.05$) and no statistically significant increases were observed for any specific chromosome aberration category. Although number of cells with aberrations are commonly used to describe results from in vitro chromosome aberration assays (OECD473, 1997) these data were not presented. Given the marginal significance, these omissions are a significant limitation in interpreting the results. Positive results were also reported for a mouse micronucleus bone marrow assay in mice administered 2 x 100 mg/kg or 2 x 200 mg/kg i.p at 24 hour intervals. The methodology description did not indicate that slides were coded for analysis in this assay. Results were reported as a statistically significant increase from a negative control value of 3.8/1000 micronucleated erythrocytes to 10.0 and 10.4/1000 micronucleated erythrocytes in the 2 x 100 and 2 x 200 mg/kg dose groups, respectively. These data do not indicate a reasonable dose response and a third dose level was not employed as recommended for this assay (OECD474, 1997). The publication indicates micronucleus scoring results for "erythrocytes" and not polychromatic or immature erythrocytes as would be appropriate for the acute dose protocol employed. Although this might be an inadvertent error in methodology description the term polychromatic erythrocytes was used in the methods section and PCE was used in the results table to describe scoring of PCE/NCE ratio.

The reported positive effects for AMPA in the in vitro studies are not concordant with in vitro results for other endpoints or the lack of genotoxic structural alerts in the structurally similar parent molecule moieties from DEREK in silico analysis. The alkaline SCGE effect could be due to cytotoxicity,

especially considering the relatively high dose levels employed (close to the 10 mM upper limit dose) and the lack of indication of pH control. Although limited cytotoxicity (>80 % viability) was reported using the trypan blue exclusion method this endpoint may grossly underestimate cytotoxic effects observed with other end points.

The in vitro chromosome aberration assay positive result was of low magnitude and was of particularly questionable significance, considering the lack of statistical significance for any individual chromosome aberration category and that the results for number or percent of cells with chromosome aberrations were not reported.

There is a clear discordance in results for AMPA in the mouse bone marrow micronucleus assay. In the earlier review negative results were reported for AMPA in a mouse bone marrow micronucleus assay conducted with dose levels up to 1000 mg/kg i.p. (Williams et al., 2000, ASB2012-12053) The maximum dose level was much higher than those used by Manas et al. (2009, ASB2012-11891) Although Manas et al. used a protocol with two doses separated by 24 hours and a single harvest time, this protocol would not be expected to give different results than a single dose with multiple harvest times, particularly when the maximum single dose was much higher (OECD474, 1997). PCE/NCE ratio data from the Manas et al. (2009, ASB2012-11891) study do not indicate that there were detectable bone marrow toxic effects observed under the conditions of their study. It appears possible that Manas et al. may have inappropriately scored erythrocytes for micronuclei instead of polychromatic erythrocytes, but if this is the case lower sensitivity rather than higher sensitivity would be expected. These limitations suggest the possibility that the aberrant result might be that of Manas et al. (2009, ASB2012-11891) but further studies might be necessary to resolve the discordance.

The earlier review reported negative results for POEA in an Ames/Salmonella assay (Williams et al., 2000, ASB2012-12053). No other genotoxicity results were reported for POEA individually but numerous genotoxicity results were presented, as described earlier, for GBFs containing POEA. Examination of subsequent literature for this review did not produce any new publications reporting genotoxicity results for POEA as an individual test material (i.e. not as a glyphosate formulation). However, there were some publications confirming that POEA can be a significant contributor to toxicity of GBFs and that it exhibits biological effects consistent with surfactant properties. As noted earlier, experiments with a POEA- containing formulation without glyphosate administered i.p. in DMSO/olive oil vehicle to mice produced the same severe liver and kidney toxicity as a GBF indicating that the toxicity primarily resulted from the formulation components rather than glyphosate (Heydens et al., 2008, ASB2012-11845). Similarly, dose-response curves were superimposed for an in vitro system evaluating a GBF and the same formulation without glyphosate present (Levine et al., 2007, ASB2009-9030). Effects on mammalian cells consistent with membrane disruption and consequent cytotoxicity were observed for POEA (Benachour and Seralini, 2009, ASB2012- 11561).

Genotoxicity Weight of Evidence

The earlier review applied a weight of evidence analysis to the available genotoxicity data. Various weighted components included assay system validation, test system species, relevance of the endpoint to heritable mutation, reproducibility and consistency of effects and dose-response and relationship of effects to toxicity (Williams et al., 2000, ASB2012-12053). The conclusion of this analysis was that glyphosate and Roundup GBFs were not mutagenic or genotoxic as a consequence of direct chemical reaction with DNA. This was supported by a strong preponderance of results indicating no effects in in vivo mammalian assays for chromosome effects and consistently negative results in gene mutation assays. Although some DNA damage responses were noted, these were judged likely to be secondary to toxicity rather than DNA reactivity.

Since this earlier review, a large number of genotoxicity studies have been conducted with glyphosate and GBFs. For gene mutation, one of the two primary endpoint categories with direct relevance to heritable mutation, one subsequent publication contains a summary of results from a bacterial gene mutation endpoint assay (Ames/Salmonella bacterial reversion assay). Although there were very significant limitations to the information published, the negative result is consistent with the majority of negative results reported for glyphosate and GBFs in Ames/Salmonella bacterial reversion assays. Another publication reported results for a Drosophila wing spot assay of glyphosate. Results were negative or inconclusive in this assay for crosses that would have detected gene mutation as loss of heterozygosity. The new results provide some support to reinforce the earlier conclusion that glyphosate and GBFs are not active for the gene mutation endpoint category.
The second primary endpoint category with direct relevance to heritable mutation is chromosome effects. The earlier review noted mixed results for two in vitro chromosome effects assays in mammalian cells but concluded that the most reliable result was the negative assay. A number of in vitro mammalian cell chromosome aberration or micronucleus assay results have been subsequently published using bovine or human lymphocytes. These assays suffer from some technical limitations in conduct or reporting of methodology that frequently included failure to indicate control of medium for pH and failure to indicate coding of slides for visual scoring. Both positive and negative results are reported in these assays. A large preponderance of results in the absence of an exogenous mammalian metabolic activation system were negative up to high (mM) dose levels that were toxic or close to toxic levels observed in parallel experiments. The exceptions were a weak and inconsistent response reported in two publications from the same laboratory and a positive response for the uncharacterized formulation, herbazed. In addition to these findings in mammalian cells negative results were also reported for Roundup GBF in an onion root tip assay conducted without exogenous mammalian metabolic activation. Thus, the preponderance of evidence from assays not employing an exogenous mammalian metabolic activation system indicates that glyphosate and GBFs are not structural chromosome breakage inducers (clastogenic) in in vitro mammalian chromosome aberration or micronucleus assays.
Two publications from one laboratory reported an increase in micronucleus frequencies for glyphosate in in vitro cultured mammalian cells in the presence of an exogenous S9 metabolic activation system (Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012- 11907). An enrichment for centomeric-containing micronuclei suggested that the increased micronuclei observed in these studies were derived from aneugenic processes, probably mediated through toxicity, rather than chromosome breakage. Thus, these two reports of weak micronucleus responses in the presence of exogenous mammalian metabolic activation appear to result from toxicity-associated aneugenic rather than clastogenic mechanisms. A number of other gene mutation and in vitro chromosome effect genotoxicity studies are negative with exogenous metabolic activation which supports the conclusion that the weight of evidence does not indicate a DNA-reactive clastogenic activity in in vitro assays using mammalian cells.
All except one of a number of in vivo mouse bone marrow chromosome aberration or micronucleus assays of glyphosate and GBFs were reported as negative in the earlier review. In the updated review both positive and negative results were reported for glyphosate and GBFs in these types of assays. Many of these studies had limitations or deficiencies compared to international guidelines with the most common and significant being no indication of slide coding for visual scoring. Four publications from three laboratories reported negative results in mouse bone marrow erythrocyte micronucleus assays of glyphosate and GBFs which are consistent with the earlier reviewed studies. These studies used high, peri-lethal dose levels administered by the i.p. or oral routes.

Two publications from two laboratories reported positive results for glyphosate and GBFs in the mouse bone marrow erythrocyte micronucleus assay. One positive result for glyphosate was encountered using dose levels and routes that were similar to those employed in the negative glyphosate studies in the same assay system. The publication reporting this result indicates that erythrocytes rather than polychromatic erythrocytes were scored which would be inappropriate for the treatment protocol but it is possible that this is a misreporting of what types of cells were actually scored. Although there is no definitive explanation for the discordance, the preponderance of mouse bone marrow erythrocyte micronucleus studies of glyphosate are clearly negative. The reported positive result for Roundup GBF is discordant with a number of negative results for Roundup or other GBFs conducted at higher dose levels. The most unique feature of this study was the use of dimethylsulfoxide as a vehicle. The preponderance of mouse bone marrow erythrocyte micronucleus studies for Roundup and other GBF studies is negative.

Positive results were reported in an unusual test system (rabbit) and route (drinking water), but water intake was not reported and effects may therefore be attributable to dehydration. Furthermore, most of the effects were on endpoints not usually considered as indicators of clastogenicity and structural chromosome aberration. One laboratory reported positive results for chromosome aberration effects in bone marrow and spermatocytes after extended dosing. However, the herbazed formulation test material was not characterised.

While more discordant results in the important in vivo mammalian chromosome effect assay category have been reported in publications subsequent to the earlier 2000 review the preponderance of evidence continues to indicate that glyphosate and GBFs are not active in this category of end point.

Several in vivo erythrocyte micronucleus assay results for GBFs in non-mammalian systems (fish and caiman eggs) have been published since the earlier review. These test systems have relatively little experience and are largely unvalidated in comparison to the mouse bone marrow erythrocyte micronucleus assay. Two publications report negative results and two publications report positive results in different fish species and there is no definitive explanation for the discordance. Both the positive and negative studies employed maximum dose levels that were toxic or close to toxic dose levels. One possible explanation for the discordance is that the positive effects were associated with toxicity that only occurred beyond an exposure threshold and over a fairly narrow dose range. Positive results in hatchlings derived from caiman eggs exposed to Roundup formulation are given relatively

little weight because of extremely limited experience with this assay system and because of significant questions about how DNA damage effects induced in embryos can persist and be evident in cells of hatchlings after several weeks and numerous cell divisions. The reported weak and inconsistent response in one of four crosses for somatic recombination in the Drosophila wing spot assay is also accorded relatively low weight. These non-mammalian test systems are generally considered of lower weight for predicting mammalian effects than mammalian test systems. Also, the environmental significance of effects for GBFs should consider the relationship between concentrations or exposures producing effects and likely environmental concentrations or exposures. This is particularly important if the effects are produced by threshold mediated toxic processes.

There have been a significant number of publications since the earlier review of results for assays in the DNA damage category with some SCE and a large number of alkaline SCGE endpoint publications. In general, the DNA damage end point category is considered supplementary to the gene mutation and chromosome effect categories because this endpoint category does not directly measure heritable events or effects closely associated with heritable events. Regulatory genotoxicity testing

recommendations and requirements focus on gene mutation and chromosome effect end points for initial core testing, particularly for in vitro testing. This consideration is underscored by the observation of some cases of compounds where positive effects are observed in these assays that are not observed for gene mutation or chromosome effect assays. Also, there are numerous examples of responses in these endpoints that do not appear to result from mechanisms of direct or metabolite DNA-reactivity. The unique response consideration is reinforced in this data set by observations of responses in DNA damage endpoints but not in chromosome effect end points.
Many DNA damage endpoint assays of glyphosate or GBFs have produced positive results at high, toxic or peri-toxic dose levels for the SCE and alkaline SCGE endpoints in a variety of test systems including cultured mammalian cells, several aquatic species and caiman eggs. The only new report of positive in vivo mammalian DNA damage effects are for an uncharacterised formulation, herbazed. There are several examples of negative results for a chromosome aberration or micronucleus endpoint and positive results for the alkaline SCGE or SCE endpoint in the same publication (Cavalcante et al., 2008, ASB2012-11586; Manas et al., 2009, ASB2012-11892; Mladinic et al., 2009, ASB2012-11906; Sivikova and Dianovsky, 2006, ASB2012-12029). These examples confirm the impression that the DNA damage endpoints are not necessarily predictive of heritable mutation effects and are also consistent with the DNA damage endpoints reflecting toxic effect mechanisms.
While the number of reported positive responses in these endpoints does suggest that effects in these endpoints can be induced by glyphosate or GBFs, comparison with results for gene mutation and chromosome effects endpoints, examination of the dose response and association of the effects with toxic endpoints indicates that these effects are likely secondary to toxicity and are threshold mediated. Surfactants in GBFs increase toxicity compared to the active ingredient of glyphosate salts and are shown to induce effects such as membrane damage and oxidant stress which are likely capable of inducing DNA damage effects at cytotoxic doses. These factors as well as other considerations presented in Section 6.3 indicate that these DNA damage effects have negligible significance to prediction of hazard or risk at lower and more relevant exposure levels.
Most of the human studies do not provide interpretable or relevant information regarding whether there are in vivo human genotoxic effects of GBFs because the reported incidence of glyphosate formulation exposure in the population was low or because there were reported exposures to a relatively large number of pesticides. Two studies with focus on glyphosate exposure through presence in or near areas of glyphosate formulation spraying found increases in the DNA damage alkaline SCGE end point. In one study clinical signs of toxicity
were reported in the population and spraying concentrations were reported to be many times the recommended application rate. Given the nature of the end point a reasonable interpretation is that effects might well be due to the overt toxicity that was reported in the publication. This would be a threshold mediated, non-DNA reactive mechanism and is consistent with experimental system results showing alkaline SCGE effects in animals exposed to high levels of formulation components. The low weight of evidence for significant genotoxic hazard indicated by this particular endpoint in human monitoring is reinforced by findings that exercise induces alkaline SCGE effects in humans. The other study found increases in binucleated micronucleated cell frequency in population in spraying areas but the increases were not consistent with spraying levels or self-reported exposure. These latter observations are not consistent with the study presenting clear evidence of GBF effects on this endpoint. In sum, the available human data do not provide any clear indications that exposed humans are substantially different in response than mammalian animal models or that exposure to GBFs produces DNA-reactive genotoxicity.
Carcinogenicity is an adverse effect that is a possible consequence of genotoxic and mutagenic

activity. Conversely, lack of carcinogenicity in properly conducted animal models is supportive for lack of significant in vitro mammalian genotoxicity. The updated review provides one new study of glyphosate formulation which is negative for either initiator or complete carcinogenesis activity which provides additional evidence to reinforce the conclusion from earlier mammalian carcinogenicity assays that glyphosate and GBFs are non- carcinogenic. These findings support the conclusion that glyphosate and GBFs do not have in vivo mammalian genotoxicity or mutagenicity.
In addition to considering the results relevant to genotoxicity hazard assessment, an important additional perspective on risk can be provided by comparing levels used in experimental studies with expected human and environmental exposure levels. A study of farmers indicated a maximum estimated systemic glyphosate dose of 0.004 mg/kg for application without protective equipment and a geometric mean of 0.0001 mg/kg (Acquavella et al., 2004, ASB2012-11528). When compared with in vivo mammalian test systems that utilize glyphosate exposures on the order of 50-300 mg/kg, the margins of exposure between the test systems and farmers is 12,500-75,000 for the maximum farmer systemic exposure and 0.5-3 million for the geometric mean farmer systemic exposure. These margins are quite substantial, especially considering that many of the in vivo genotoxicity studies are negative. Assuming reasonable proportionality between exposure to glyphosate and GBF ingredients, similar large margins of exposure would exist for GBF components. The margins of exposure compared to in vitro mammalian cell exposures are estimated to be even larger. Assuming uniform distribution, the systemic concentration of glyphosate from the Aquavella et al. (2004, ASB2012-11528) farmer biomonitoring study would be on the order of 24nM for the maximum and 0.59 nM for the geometric mean exposure. A typical maximum in vitro mammalian exposure of 1-5 mM represents a margin of exposure of 42,000-211,000 for the maximum farmer exposure and 1.7-8.4 million for the geometric mean farmer systemic exposures, respectively.
Overall, the weight of evidence of the studies considered in the earlier review as well as the studies considered in this review indicates that glyphosate and GBFs are not genotoxic in the two general endpoint categories most directly relevant to heritable mutagenesis, gene mutation and chromosome effects. This conclusion results from a preponderance of evidence; however, there are reports of positive discordant results in both end point categories. The new studies considered in this review provide some evidence for DNA damage effects induced by high, toxic exposures, particularly for the alkaline SCGE end point and for GBFs containing surfactant. Several considerations, including the lack of response in other endpoint
categories, suggest that these effects result from toxic and not DNA-reactive mechanisms and that they do not indicate in vivo genotoxic potential under normal exposure levels.
Regulatory and authoritative reviews of glyphosate supporting registrations and registrations in all regions of the world over the last 40 years have consistently determined that glyphosate is nongenotoxic (Commission, 2002, ASB2009-4191; WHO/FAO, 2004, ASB2008-6266). Scientific publications contrary to these regulatory reviews should be evaluated using a weight of evidence approach with consideration for reliability of the assay used and data quality presented.
Abbreviations
AMPA, aminomethylphosphonic acid ; CB MN, cytokinesis block micronucleus; GBF, glyphosate based formulation; i.p., intraperitoneal ; NCE, normochromatic erythrocyte; OECD, Organization for Economic Cooperation and Development; PCE, polychromatic erythrocyte; POEA, polyethoxylated tallow amine, ethoxylate; SCE, sister chromatid exchange; SCGE, single cell gel electrophoresis (comet).
Klimisch evaluation
Reliability of study:

Not reliable
Comment:
Exposure conditions of plants (immersion) not representative for glyphosate. Inappropriate test model
as herbicides are toxic to plants. Presentation of results not sufficient for assessment. Reporting deficiencies (e.g. positive controls).
Relevance of study:
Not relevant (Due to reliability, and exposure
conditions of plants and inappropriate test model).
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Basic data given, however, the study is performed with methodological and reporting deficiencies (only data without metabolic activation generated in in vitro tests, no positive controls included in in vitro SCE and in vivo
experiments, in some experiments only two test substance concentrations tested).
Relevance of study:
Not relevant (Due to methodological and reporting deficiencies data considered to be supplemental information; i.p. exposure route is not relevant for human exposure)
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable for glyphosate
Comment:
MN-test comparable to OECD guidelines, but not equal. Exposures to multiple pesticides with no information on exposure concentrations to individual pesticides make result unreliable for glyphosate.
Relevance of study:
Not relevant (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenicity possible. Not relevant for
glyphosate).
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable for glyphosate
Comment:
Well-documented study. MN-test comparable to OECD guidelines, but not equal. No information on exposure concentrations to individual pesticides
Relevance of study:
Not relevant (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenic non-statistically significant differences possible. No statistically relevant

findings
reported for glyphosate alone).
Klimisch code:
2

Klimisch evaluation
Reliability of study:
Not Reliable
Comment:
Methodological and reporting deficiencies (e.g. test substance source, no concurrent measurement of toxicity reported, less than 2000 erythrocytes scored per animal and results not reported separately for replicates).
Relevance of study:
Relevant with restrictions (Due to reliability. Discussion confuses glyphosate with glyphosate formulated products.)
Klimisch code:

3

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
No positive controls were included, which significantly detracts from the utility of a non-validated, non- standard test method. Less than the standard of a minimum of three dose levels used, independent coding of slides for scoring and results not reported separately
for replicates.
Relevance of study:
Not relevant (Non-standard test system, no positive
controls to verify test method/study validity.)
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Comparable to 1984 OECD guideline, but with several deficiencies (no positive controls reported and thus study validity not verifiable; wild type male treatment age different than recommended, purity of test substances not reported, tested formulation other
ingredients such as surfactants not reported.)
Relevance of study:
Not relevant for glyposate (Glyphosate not tested;
formulation tested)
Klimisch code:
3
Klimisch evaluation
Reliability of study:

Not reliable
Comment:
Reporting deficiencies (purity of AMPA not specified, several parameters in the MNT not reported, only 2 dose levels used in both CA and MNT). Exposure route used in the MNT is not relevant for human exposure;
methodological deficiencies (see guideline deviations).
Relevance of study:
Not relevant (Due to reliability)
Klimisch code:

3

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Guideline deviations and reporting deficiencies. Several parameters in the MNT not reported. Blind scoring reported for the CA but not MNT. Exposure route used in the MNT is not relevant for human exposure. (see guideline deviations). No indication of pH or osmolality control for the comet assay. Results not reported separately for replicates.
Relevance of study:
Not relevant (Due to guideline deviations and reporting
deficiencies).
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Reliable with restrictions
Comment:
Non-GLP, non-guideline in vitro study, meeting scientific principles
Relevance of study:
Relevant with restrictions (Assessment of Genotoxicity in vitro at concentrations relevant to human exposure levels; authors state that no clear dose-dependent effect was observed, and results indicate that glyphosate in concentrations relevant to human exposure do not pose
significant health risk.
Klimisch code:
2
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-GLP, non-guideline study in vitro. Positive and negative control results almost indistinguishable for MN assay without metabolic activation. Negative control NB and NBP results not reported.
Relevance of study:
Not relevant (Proposed mechanism of genotoxicity (in vitro) is not relevant to human exposure levels. Authors express confidence that estimated maximum human exposure levels correspond to acceptable safety levels based on evaluated in vitro endpoints, and that their

findings need to be verified in vivo.)
Klimisch code:
3

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Documentation of Comet assay insufficient for assessment.
Relevance of study:
Not relevant (Glyphosate formulation was applied at much higher dose rates than recommended for the intended uses in the EU. In addition, the herbicide was combined with the adjuvant (Cosmoflux 411F) that can increase the biological action of the herbicide. This
adjuvant will not be used in the EU.)
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not Reliable
Comment:
A non-guideline study with confounding results based on testing a surfactant containing formulation. Reporting deficiencies (statistical methods). Toxic surfactant effects subsequently verified in Heydens et al. (2008, ASB2012-11845) reporting the same study type with a glyphosate formulated product and an appropriate control; formulation blank without
glyphosate.
Relevance of study:
Not relevant (i.p. administration of high doses of a surfactant containing formulation a relevant exposure scanario for human risk assessments. In addition, the DNA adducts observed were not related to the active ingredient (isopropylammonium salt of glyphosate), but to another, unknown component of the herbicide
mixture.)
Klimisch code:

3

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-GLP studies in a unique test model. Micronucleus assay followed guideline, Comet assay similar to guideline.
Test methods have been modified to be applied caiman species. Methodological deficiencies: housing and feeding conditions of parents not specified; sex not distinguished, stability and homogeneity assessment of test substance preparations not reported. Results not reported separately for replicate individual animals.
Relevance of study:
Not relevant. Highly artificial in ovo exposure scenario not relevant to real world environmental

exposures.
Caiman eggs are covered and not exposed to the surface. Any glyphosate in a potential herbicide overspray would sorb to sediment and organic matter. and not transport to the egg surface.
Klimisch code:

3

Klimisch evaluation
1Reliability of study:
Not reliable. Determination of the stability of human erythrocytes: Results are not surprising because surfactants are known to compromise cell membrane integrity. Doses not reflective of physiological concentrations of either glyphosate or surfactant.
Micronucleus test in vivo: Irrelevant route of exposure for surfactant containing formulated products. Results confounded by presence of surfactant toxicity; refer to Heydens et al. (2008, ASB2012-11845)
Comment:
Non-guideline, non-GLP studies
Determination of the stability of human erythrocytes Results attributable to surfactant induced cytotoxicity Micronucleus test in vivo
Major reporting deficiencies (no information on number of cells evaluated, only graphical documentation of results, no information on absolute MN frequencies).
Relevance of study:
Not relevant (Test material containing surfactant is not
appropriately evaluated in either model).
Klimisch code:

3

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Test material was a formulated product containing surfactant. Authors acknowledge cytotoxicity was a confounding factor for data interpretation; since the time of this study, around 1980, surfactant effects on in vitro test systems have been well documented. Only very minor changes in SCE were reported, with a limited data set of two donors and a lack of dose- response. Statistical analysis was not feasible with this very limited data set.
Relevance of study:
Not relevant (Limited data set, internally consistent
findings, no statistics conducted and no dose-response)
Klimisch code:
3

Based on mortality at the upper limit of the historical control range, the NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is conservatively set at 1000 ppm, corresponding to 149.7 mg/kg bw/day for males, 151.2 mg/kg bw/day for females, and
150.5 mg/kg bw/day for both sexes combined. It is concluded that Glyphosate is not carcinogenic in mice.

A number of epidemiology studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the specificity of their conclusions regarding pesticides in general, classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, few draw tenable associations with any specific cancer outcome. Publications suggesting glyphosate is associated with any cancer outcome are discussed below.

An essential consideration in both, risk assessment and interpreting the relevance of toxicology data is exposure assessment. An inherent low level of confidence exists for epidemiological studies where tenuous links to exposure exist. Suggested associations between health outcomes and any possible causative agent are merely speculation if exposures are not identifiable. Pivotal to the understanding of glyphosate exposure are data published by Acquavella et al. (2004, ASB2012-11528; 2005, ASB2012-11530), which quantified human systemic glyphosate exposure levels in farmer applicators and their families. The geometric mean systemic dose for farmers applying glyphosate, some of whom applied glyphosate to areas up to 400 acres, was 0.0001 mg/kg/day, approximately 0.03 % of the EU glyphosate acceptable operator exosure Level (AOEL) according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191). The highest systemic dose, skewed well above the geometric mean, was 0.004 mg/kg/day, which is 1.95 % EU glyphosate AOEL according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191) and 1.3 % of the current EU glyphosate attapcable daily intake (ADI) according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191). Even lower systemic doses were determined for spouses and children, 0.00004 mg/kg and 0.0008 mg/kg, respectively. Multiple carcinogenicity studies have since been conducted by numerous glyphosate registrants demonstrating NOAELs of at least ten-fold higher than the highest dose tested in the study driving the current EU ADI calculation.

The largest epidemiological study of pesticide exposure and health outcomes in the United States is the Agricultural Health Study (AHS), which included glyphosate. Dozens of publications have resulted from data generated in this study of approximately 57,000 enrolled farmer applicators. Blair et al. (2009, ASB2012-11566) provided an overview of cancer endpoints associated with different agricultural chemicals reported in earlier AHS publications. Glyphosate was not reported to be associated with leukemia, melanoma, or cancers of the prostate, lung, breast, colon or rectum. De Roos et al. (2005, ASB2012-11605) reported AHS data evaluating glyphosate use and multiple cancer endpoints; no association was noted for glyphosate with all cancers, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, non-Hodgkin's lymphoma (NHL) and leukemia. In an earlier publication based on another data set, however, De Roos et al., (2003, ASB2012-11606) reported an association between NHL and glyphosate use. McDuffie et al. (2001, ASB2011-364) reported a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study. Blair et al. (2009, ASB2012-11566) did not report an association between glyphosate use and NHL in the AHS data, but a "possible association" between glyphosate use and multiple myeloma was mentioned. The AHS publication reporting this refers to a "suggested association" between glyphosate use and multiple myeloma (De Roos et al., 2005, ASB2012- 11605), yet it did not demonstrate significant increase in relative risk for multiple myeloma. Both De Roos papers will be discussed in more detail below. Interestingly, a subsequent AHS review paper for the President's Cancer Panel (Freeman, 2009, ASB2012-11623) specifically references De Roos (2005 ASB2012-11605) as providing no observed incidents of cancers of any type being associated with glyphosate.

Lee et al. (2005, ASB2012-11882) reported a glyphosate association with gliomas, with the odds ratio differing between self-respondents (OR = 0.4) and proxy respondents (OR = 3.1). The authors

expressed concern that higher positive associations observed for proxy respondents with glyphosate and several other pesticides, and suggested perhaps more accurate reporting of proxies for cases, and underreporting by proxies for controls; proxy respondents were spouses in 62 % of cases versus 45 % of controls, lending to lower reported incidents in the control group.
Monge et al (2007, ASB2012-11909) investigated associations between parental pesticide exposures and childhood Leukaemia in Costa Rica. Results are not interpretable for glyphosate as exposure was estimated with “other pesticides”, including paraquat, chlorothalanil and “others”. No association was noted for paternal exposures, but elevated leukaemias were associated with maternal exposures to “other pesticides” during pregnancy. Similarly, glyphosate is captured under “other pesticides” being associated with NHL by Fritschi et al. (2005, ASB2012-11624) and therefore should not be interpreted as an association with glyphosate.
The following epidemiology publications report a lack of association between glyphosate and specific cancer types.
• Alavanja et al. (2003, ASB2012-11535) reported on prostate cancer associations with specific pesticide exposures in the AHS; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.
• Multigner et al, (2008, ASB2012-11917) also reported a lack of association between glyphosate use and prostate cancer. This data appears to have also been reported by Ndong et al. (2009, ASB2012-11922).
• The lack of association between glyphosate use and prostate cancer was also supported recently in an epidemiology study of Farmers in British Columbia, Canada by Band et al. (2011, ASB2012-11555).
• Lee et al. (2004, ASB2012-11883) reported a lack of association between glyphosate use and stomach and esophageal adenocarcinomas.
• Carreon et al. (2005, ASB2012-11585) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.
• Engel et al. (2005, ASB2012-11613) reported AHS data on breast cancer incidence among farmers’ wives, with no association between breast cancer and glyphosate.
• Flower et al (2004, ASB2012-11620) reported AHS data on parental use of specific pesticidesa and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate.
• Andreotti et al. (2009, ASB2012-11544) reported AHS data where glyphosate was not associated with pancreatic cancer.
• Landgren et al. (2009, ASB2012-11875) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS), showing no association with glyphosate use.
• Karunanayake et al. (2011, ASB2012-11865) reported a lack of association between glyphosate and Hodgkin’s lymphoma.
In summarizing AHS publications, Weichenthal et al. (2010, ASB2012-12048) noted that increased rates in the following cancers were not associated with glyphosate use; overall cancer incidence, lung cancer, pancreatic cancer, colon or rectal cancer, lymphohematopoietic cancers, leukemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, esophagus cancer and thyroid cancer.
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Study prone to selection and recall bias. No evidence of relevant glyphosate exposures. Medical

history was assessed, but not reported.
Relevance of study:
Not relevant (Exposure to multiple chemicals and though glyphosate exposure data were convincing (7/1145 subjects) and statistically non-significant positive associations reported.)
Klimisch code:
3
Additional comments:
Hardell and Eriksson (1999, ASB2012-11838) conducted a case control study to look for associations between reported pesticide use and non-Hodgkin's lymphoma (NHL). The study included 404 NHL cases and 741 controls. The measure of association in this study was the odds ratio (OR), a statistic that estimates of the ratio of disease rates (in this case NHL rates) for exposed and unexposed populations.
The authors reported statistically significant associations for NHL with: reported use of any herbicide (OR = 1.6), reported use of any fungicide (OR = 3.7), and reported use of 4-chloro- 2-methylphenoxyacetic acid (OR = 2.7). The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43 % of the pesticide use information, and the difficulty in controlling for potential confounding factors, given the small number of exposed subjects.
The authors also reported a moderately elevated OR of 2.3 for glyphosate. This OR was not statistically significant and was based on only four "exposed" cases and three "exposed" controls. This study has several important limitations: no exposure assessment, dependence on next-of- kin's recollections of study subjects' pesticide use for approximately 43 % of study subjects, potential recall bias, and the very small number of subjects who reported using specific herbicides. The latter leads to findings that are statistically imprecise. Due to the potential for bias and the statistical imprecision, the results of this study are not convincing.
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
This publication combines the results of two previous studies by the authors on HNL (Hardell and Eriksson, 1999, ASB2012-11838) and HCL (Nordström,et al., 1998, TOX1999-687). No information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc). Study documentation is insufficient for assessment.
Relevance of study:
Not relevant (Due to reliability of data set drawn from Hardell and Eriksson, 1999, ASB2012-11838)
Klimisch code:
3
Additional comments:
This study pools data from the previously reviewed publication by Hardell and Eriksson (1999, ASB2012-11838) with data from Nordström et al. (1998, TOX1999-687). Therefore the discussion of limitations of Hardell and Eriksson (1999, ASB2012-11838) also applies to Hardell et al. (2002, ASB2012-11839) (see above).
Klimisch evaluation

Reliability of study:
Not reliable
Comment:
No information about exposure duration, used glyphosate products, exposure duration and application
rates. Documentation is insufficient for assessment.
Relevance of study:
Not relevant (Multiple pesticide exposures. No definitive association between NHL and glyphosate can
be made.)
Klimisch code:
3
Additional comments:
No information about exposure duration, used glyphosate products, exposure duration and application rates. Only multiple pesticide exposures are reported. No association between NHL and glyphosate can be made on basis of this study.
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc were reported. Specific lymphomas are not identified (NHL captures all types of lymphoma other than Hodgkin's lymphoma). Documentation is insufficient to associate exposures with specific NHL
diseases.
Relevance of study:
Not relevant (No report of identifying various types of lymphoma under the NHL umbrella; no definite association between specific NHL diseases and
glyphosate can be made)
Klimisch code:
3
Additional comments:
No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc) were reported. Specific lymphomas are not identified. The reported hierarchical regression did not find a statistically significant odds ratio for ever use of glyphosate and NHL.
Klimisch evaluation
Reliability of study:
Reliable without restrictions
Comment:
Well documented publication. Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.
Relevance of study:
Relevant (Evaluation focussed on glyphosate, although other pesticides were also considered in the data

evaluation)
Klimisch code:
2
Additional comments:
Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Multiple avenues for bias were introduced in study design, execution and data processing. No information about exposure duration, used glyphosate products and application rates. Other factors (i.e. smoking habits, medication etc.) were assessed but not included in the
evaluation.
Relevance of study:
Relevant with reservation
Klimisch code:
3
Additional comments:
The authors (Eriksson et al. 2008, ASB2012-11614) conducted a population-based case- control study of exposure to a variety of pesticides and non-Hodgkin lymphoma (NHL), including separate analyses of histopathological categories of NHL. Study subjects were males and females, ages 18-74, living in Sweden between December 1, 1999 and April 30, 2002. The final study group included 910 cases and 1016 controls. Exposure, ascertained via an interviewer-administered questionnaire, focused on pesticide and other chemical agents, and included a total work history (although a job-exposure matrix was not used). For pesticide exposure, information on number of years, number of days per year, and approximate length of exposure per day was also obtained. A minimum of one full day of exposure was required for categorization as “exposed.”
The authors reported a statistically significant positive association between “herbicide exposure” and NHL (OR = 1.72; 95% CI: 1.18-2.51). Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02 (95% CI:
1.10-3.71). The ORs for glyphosate exposure of <10 days and >10 days were 1.69 (95% CI:
0.70-4.07) and 2.36 (1.04-5.37), respectively. The ORs for glyphosate were 1.11 (95% CI:
0.24-5.08) and 2.26 (95% CI: 1.16-4.40) for “latency” periods of 1-10 years and >10 years, respectively. In analyses of glyphosate and type of NHL, statistically significant positive associations were observed for small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) (OR = 3.35; 95% CI: 1.42-7.89) and for “unspecified NHL” (OR = 5.63; 95% CI:
1.44-22.0). Odds ratios for the other types (total B-cell lymphomas, grade I-III follicular lymphoma, diffuse large B-cell lymphoma, other specified B-cell lymphoma, unspecified B- cell lymphoma, and T-cell lymphomas) were above 1.0, but were not statistically significant (i.e., the 95% confidence intervals were relatively wide and included the null value of 1.0).
The authors concluded, “Glyphosate was associated with a statistically significant increased OR for lymphoma in our study, and the result was strengthened by a tendency to dose- response effect…” (p. 1662). The authors suggested that their findings are consistent with results of a previous case-control study (Hardell and Eriksson 1999, ASB2012-11838) and pooled analysis (Hardell et al. 2002, ASB2012-11839) that they conducted. In the case- control study, an OR of 2.3 (95% CI: 0.4-13.0),

based on 4 exposed cases and 3 exposed controls, was reported for glyphosate and NHL. In the pooled analysis of two case-control studies, which included data from Hardell and Eriksson (1999, ASB2012-11838), an OR of
3.04 (95% CI: 1.08- 8.52) was reported, based on 8 exposed cases and 8 exposed controls. The authors also cited three studies (De Roos et al. 2003, ASB2012-11606; McDuffie et al. 2001; ASB2011-364, De Roos et al. 2005, ASB2012-11605) by other groups as being consistent with their results in that they "also associate glyphosate with different B-cell malignancies such as lymphomas and myelomas." It should be noted, however, that the relative risk (RR) reported by De Roos et al. (2005, ASB2012-11605) for the highest versus lowest category of cumulative exposure days of glyphosate and NHL in the prospective Agricultural Health Study was 0.9.

Interpretation Issues
Identification of Cases and Potential Referral Bias. It is noteworthy that the cases in the current analysis were identified from some of the same hospitals as the authors' prior publication; thus, referral bias may have been an issue. In particular, the researchers approached the patients after diagnosis if the physicians deemed it appropriate. Therefore, if the physicians were concerned that their patient's NHL was associated with agricultural exposures, they may have suggested participation in the study.
Participation Rates and Potential Selection Bias. The authors report a participation rate of 91% and 92% for cases and controls, respectively; however, these figures are based on completed questionnaires out of those who had previously said they would participate in the study. The number of eligible patients (i.e., prior to physician approval to "approach") was not reported, so the computation of an exact participation rate is difficult. Based on information provided in the paper, participation among cases is estimated to be about 80%. Nonparticipation is a concern for several reasons. First, in a case-control study, an odds ratio will be an accurate representation of the exposure-disease association when the cases are representative of all cases and the controls are representative of the exposure experience of the population that gave rise to the cases. If the final study sample is not representative of this "target population" then measures of effect (e.g., the odds ratio) may not be valid. In addition, one must be concerned about selection bias. Selection bias occurs in a case-control study when the exposure distribution for cases and controls differ for those who participate in the study compared to those who are eligible but do not participate in the study. It is not possible to determine whether there is selection bias without information about nonparticipants.
Strengths and Limitations of Using Living Cases Only versus All Cases (Living + Dead).
The authors noted that 88 potential cases died before they could be interviewed and were therefore excluded from the study. It is also stated in the Discussion that restricting the study to living cases and controls was an "advantage" of the study, as interviewing cases and controls directly compared to interviewing next-of-kin was preferable. While it is generally true that this would be an advantage, the following statement by the authors, therefore, is not accurate, "The study covered all new cases of NHL during a specified time" (p. 1660). The study did not include all new cases; it included only those cases who survived until the time of the interview. Thus, while there may have been an advantage to restricting the study to living cases, there was a trade-off in that the study population did not represent all cases, specifically those cases with more aggressive disease. This disadvantage was not discussed by the authors, nor was the potential bias that could have resulted from excluding many eligible cases.
Exposure Measurement and Information Bias. Exposure was ascertained via a questionnaire oriented

towards pesticide and other chemical agents. In addition, interviewers collected information by telephone if “important” data were lacking, incomplete, or unclear. It is unknown what is meant by “important,” and the proportion of cases and controls who received phone calls was not reported. Thus, information bias may be a concern. Even though interviewers were blinded to case and/or control status, they may have been able to determine this information during the course of the interview. Furthermore, recall bias may be an issue because exposure information was based on participant response and cases and controls may recall and/or report past pesticide exposures differently. No exposure validation techniques were implemented, nor did an industrial hygienist (or any other type of personnel trained in assessing occupational exposures) independently validate/estimate the frequency and/or intensity of exposure. The authors assumed that “some misclassification regarding quantity of exposure has probably occurred, but such misclassification would most probably be nondependent of case/control status, and therefore only weaken any true risks” (p. 1660). They do not provide any explanation as to why they believe that exposure misclassification would be “most probably” nondifferential. If NHL cases believe that pesticides may be related to their disease, then it is certainly possible that they may recall and/or report pesticide exposure differently than NHL-free controls, which could result in odds ratios that are inflated as a result of bias.

Interpretation of “dose-response” analyses. The referent group in the statistical analyses consisted of participants who were unexposed to all pesticides. The dose-response analyses were based on a dichotomy of the median number of days exposed to a particular agent. It is difficult to analyze “dose-response” when only two exposure categories are considered. Furthermore, the dose-response analyses were based on median values of exposure but heterogeneity of cut-points is evident across agents. For example, glyphosate was analyzed as < 10 days and > 10 days, whereas, “other” herbicides were analyzed as < 32 days and > 32 days. Although analytical cut-points were data driven, interpretation across the wide variety of exposures is complicated by the variability in exposure cut-points. In addition, even though the OR for the higher category of exposure days was greater than the OR for the lower category, the two 95% confidence intervals were wide and overlapped considerably (0.70- 4.07 and 1.04-5.37). Thus, it is not clear whether the two point estimates reported (1.69 and 2.36) are significantly different from each other. Finally, this result cited in the “dose-response” analyses may have been confounded by exposure to other herbicides. In Table II (Eriksson et al. 2008, ASB2012- 11614), the authors observed elevated associations for other herbicides, including MCPA, 2,4,5-T and/or 2,4-D. The correlation between exposure to glyphosate and other herbicides was not provided nor were analyses of glyphosate-exposed individuals after accounting for the collinear relation between this agent and other agents. The odds ratio for “ever” exposure to glyphosate was attenuated after additional adjustment for other pesticides (Table VII, Eriksson et al. 2008, ASB2012-11614), but multi-variate -adjusted estimates for the “dose- response” odds ratios were not reported.

Unusual Pattern of Positive Associations. The authors conducted multiple comparisons, and one would expect a certain proportion of their findings to be statistically significant (whether in the positive or inverse direction) simply as a result of chance. It is somewhat surprising, therefore, that the vast majority of the ORs presented in this manuscript are greater than 1.0, regardless of the statistical significance. The authors do note that for some of the analyses (e.g., latency), only chemicals for which ORs were greater than 1.5 and for which there were at least 10 exposed cases, or for which there was a statistically significant OR were evaluated. On the other hand, dose-response was evaluated based on the number of exposed subjects and not on the strength or significance of the findings. The authors do not address this directly, but do state in their Discussion,

"…several pesticides are chemically related and may exert their effects on humans through a similar mechanism of action, which may explain the wide range
of pesticides that have been related to NHL over time in different countries and with different exposure conditions" (p. 1661). On the other hand, this pattern of positive findings could be a result of bias, including recall bias (or other information bias), selection bias, uncontrolled confounding, or a combination of these and other factors.

Interpretation of Eriksson et al. (2008, ASB2012-11614) in Context of Other Studies. Despite the statement by the authors that, "Recent findings from other groups also associate glyphosate with different B-cell malignancies such as lymphomas and myeloma" (p. 1662), most multi-variate analyses of glyphosate and NHL do not report statistically significant associations (De Roos et al. 2005, ASB2012-11605; De Roos et al. 2003; ASB2012-11606, Hardell and Eriksson 1999, ASB2012-11838; Hardell et al. 2002; ASB2012-11839, Lee et al. 2004; ASB2012-11883, McDuffie et al. 2001; ASB2011-364, Nordström et al. 1998, TOX1999-687) (Tables B.6.5-62 and B.6.5-63). It is notable that Hardell et al. (2002, ASB2012-11839) reported a significant positive association between glyphosate association and NHL, but the multi-variate -adjusted odds ratio was attenuated and not statistically significant. Similar findings were reported by Eriksson et al. (2008, ASB2012-11614). Specifically, the association reported by the authors in the abstract (OR = 2.02; 95% CI: 1.10- 3.71) was adjusted for age, sex and year of diagnosis or enrollment. When other pesticides were added to that model (i.e., agents with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subjects), the adjusted odds ratio was 1.51 (95% CI: 0.77-2.94). Thus, the authors' final statement, "Furthermore, our earlier indication of an association between glyphosate and NHL has been considerably strengthened" is questionable. Their previous findings showed a non-significant association after multi-variate adjustment (OR = 1.85; 95% CI: 0.55-6.20). The 2008 study similarly reported a statistically non-significant association between glyphosate and NHL after multi-variate adjustment (OR = 1.51; 95% CI: 0.77-2.94). The results reported for analyses of duration of exposure and latency of exposure did not adjust for other pesticides, and one would expect that those ORs would also be attenuated.

Summary of Findings: Cohort and Case-Control Studies of Exposure to Glyphosate and Non-Hodgkin Lymphoma

Table B.6.5-62: Cohort Studies

Author Year
Description
No. of Exposed Cases
Type of Relative Risk Estimate
Relative Risk Estimate
95%
Confidence Limits
Variables Included in Statistical Model
De Roos et al.
2005 (ASB2012
-11605)
57-2,678 vs.
1-20
Cumulative Exposure Daysa
17
RR

0.9
0.5-1.6
Age at enrollment, education, pack-years of cigarette smoking, alcohol consumption in the past year, family history of cancer in first-degree
relatives, and state of residence
337.2-18,241
22
RR
0.8
0.5-1.4
Also adjusted for other
vs. 0.1-79.5
pesticides
Intensity-
Weighted
Exposure
Daysb
a Years of use x days per year; categorized by tertiles
b Years of use x days/year x estimated intensity level; categorized by tertiles
Table B.6.5-63: Case Control Studies
Author Year
Exposure Evaluated
Subgroup Description
No. of Expose d Cases
No. of Expose d Control
s
OR
95% CI
Variables Included in Statistical Model
De Roos et al.
2003 (ASB201 2-11606)
Ever exposure to specific pesticide; men only (all 47 pesticides were regressed simultaneously)
Glyphosate (Logistic Regression)
Glyphosate (Hierarchical Regression)
36
36
61
61
2.1
1.6
1.1-4.0
0.9-2.8
Age, study site and other pesticides
Second-level model incorporated what was known about each true effect parameter prior to seeing the study data
Hardell and Eriksson 1999 (ASB201 2-11838)

Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)
Glyphosate (conditional logistic regression; uni-variate analysis)
Glyphosate (conditional logistic regression;
multi-variate analysis)
4
4
3
3
2.3
5.8
0.4-13
0.6-54
Age and country (matching factors)
Multi-variate variables not listed by authors
Hardell et al.
2002 (ASB201 2-11839)
Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)
Glyphosate (conditional logistic regression; uni-variate analysis)
Glyphosate (conditional logistic regression;
multi-variate analysis)
8
8
8
8
3.04
1.85
1.08-8.52
0.55-6.20
Age and county (matching factors); study, study area (county), and vital status
Multi-variate variables not listed by authors
Lee et al. 2004 (ASB201 2-11883)
Exposure to individual pesticides
Glyphosate use, Non- asthmatics
Glyphosate
use, Asthmatics
53
6
91
12
1.4
1.2
0.98-2.1
0.4-3.3
Age, state, vital status

McDuff- ie et al. 2001 (ASB201 1-364)
Exposure to individual active chemicals
Glyphosate (Round-Up)
Glyphosate (Round-Up)
51
NR
133
NR
1.26
1.20
0.87-1.80
0.83-1.74
Strata for age and province of residence
Plus statistically significant
medical variables
Author Year
Exposure Evaluated
Subgroup Description
No. of Expose d Cases
No. of Expose d Control
s
OR
95% CI
Variables Included in Statistical Model
Nordst- röm et al.
1998 (TOX199 9-687)
Exposure to specific herbicides, insecticides, and fungicides
Glyphosate
4
5
3.1
0.8-12
Age and country (matching factors)
Eriksson et al.
2008 (ASB201 2-11614)
Exposure to specific herbicides regardless if they also had been exposed to phenoxyacetic acids or not
Glyphosate
29
29
18
18
2.02
1.51
1.10-3.71
0.77-2.94

Age, sex, and year of diagnosis or enrollment
Age, sex, and year of diagnosis or enrollment and pesticides with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subject
Exposure to herbicide stratified by median number of days among exposed controls
Glyphosate ≤ 10 days
Glyphosate >10 days
12
19
9
9
1.69
2.36
0.70-4.07
1.04-5.37
Age, sex, and year of diagnosis or enrollment
Exposure to Glyphosate:
NR
NR
1.87
0.998-
Age, sex, and specific herbicides
B-Cell
3.51
year of diagnosis according to lymphomas or enrollment different lymphoma entities
Lymphocytic
NR
NR
3.35
1.42-7.89
lymphoma/B-CLL
Follicular
NR
NR
1.89
0.62-5.79

grade I-III
Diffuse large
NR
NR
1.22
0.44-3.35
B-cell
Lymphoma
Other
NR
NR
1.63
0.53-4.96
specified
B-cell
lymphoma
Unspecified
NR
NR
1.47
0.33-6.61
B-cell
Lymphoma
T-cell
NR
NR
2.29
0.51-10.4
lymphomas
Unspecified
NR
NR
5.63
1.44-22.0
NHL

Klimisch evaluation
Reliability of study:
Reliable with restrictions
Comment:
Non-guideline mechanistic study. Scientifically acceptable study with deficiencies (controls with glyphosate alone, and co-formulants were not included)
Relevance of study:
Relevant with restrictions (Glyphosate formulation not
glyphosate alone was tested.)
Klimisch code:

2
Additional comments:
The authors use glyphosate as a synonym for what is really a glyphosate based formulated product. Doses in this study are not representative of human exposures to glyphosate or glyphosate based formulations. Mice in the tumor promoting group VIII received topical applications of concentrated glyphosate formulated product three times per week for over thirty weeks without washing after an initial treatment with the potent tumor initiator DMBA. Glyphosate had been shown to have very low dermal absorption, even in formulated products, and since is non-volatile, would likely accumulate on mouse skin. Surfactants are typically irritating and non-volatile. Given the irritation potential of the unwashed exposed mouse skin over the course of thirty or more weeks, tumor promotion may be a physical response to substantial localized dermal irritation. Epidemiological studies reported above note no association with glyphosate and either skin or lip cancers.
Label directions outline appropriate personal protective equipment such as gloves and long sleeves. Furthermore, any dermal exposure of concentrated product to human skin would prove irritating and prompt handlers to wash off soon after dermal exposure.
Human in vitro dermal absorption studies reported for a range of glyphosate based formulations containing different surfactant systems all demonstrate extremely low dermal absorption of glyphosate active ingredient for concentrated products, of less than 0.2 %. Test material recovery in each of the four reported dermal absorption studies was very good, close to 100 %. Most of the glyphosate was removed during skin surface washing at either eight or twenty four hours of in vitro human skin exposure. This also suggests significant potential for accumulation of glyphosate on the surface of the mice skin in George et al. (2010, ASB2012- 11829).
The up-regulation / down-regulation of protein expression reported after a single dermal dose of a glyphosate formulated product (proteomics experiment, group II), while interesting, does not demonstrate any toxicological endpoint. Rather, perturbations may well represent normal homeostatic fluctuations and be a natural response to insult.
Many studies since 2000 are specifically discussed in a comprehensive glyphosate DART review publication by Williams et al. (2012, ASB2012-12052). Further discussions of significant papers follow.
In addition, glyphosate was included on the US EPA Endocrine Disruptor Screening Program's (EDSP) first list of 67 compounds to Tier 1 Screening. The US EPA published the criteria for inclusion on List 1 was strictly based on exposure potential, not hazard, specifically stating in the Federal Register (2009, ASB2012-12041);
"This list should not be construed as a list of known or likely endocrine disruptors".
A consortium of glyphosate registrants in North America, the Joint Glyphosate Task Force, LLC (JGTF), coordinated the conduct of the glyphosate battery of Tier 1 screening assays under the EDSP and submitted these assays to the US EPA. The US EPA will evaluate the full battery of Tier 1 screening assays together using a weight of evidence approach, for glyphosate's potential to interact with the estrogen, androgen and thyroid endocrine pathways. The following below were submitted by the JGTF to the US EPA in early 2012 and are reviewed. However, the Agency has announced they will not release their Data Evaluation Records (DERs) for individual EDSP studies until a weight of evidence review has been completed for List 1 compounds.
In Vitro EDSP Glyphosate Studies submitted to the US EPA
• Androgen Receptor Binding (Rat Prostate Cytosol); OCSPP 890.1150
• Aromatase (Human Recombinant); OCSPP 890.1200
• Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC); OCSPP 890.1250
• Estrogen Receptor Transcriptional Activation (Human cell Line, HeLa-9903); OCSPP 890.1300; OECD

• Published OECD Validation of the Steroidogenesis Assay (Hecker et al., 2010, ASB2012-11840)

In Vivo EDSP Glyphosate Studies submitted to the US EPA

• Amphibian Metamorphosis (Frog) OCSPP 890.1100; OECD 231
• In Vivo Hershberger Assay (Rat); OCSPP 890.1600; OECD 441
• Female Pubertal Assay; OCSPP 890.1450; OECD None
• Male Pubertal Assay; OCSPP 890.1500
• Uterotrophic Assay (Rat); OCSPP 890.1600; OECD 440
• Fish Short-Term Reproduction Assay; OCSPP 890.1350; OECD 229

The glyphosate Tier 1 screening assay study reports are owned by the JGTF. The European Glyphosate Task Force (GTF) is negotiating to procure access rights to the battery of glyphosate EDSP Tier 1 screening study reports. Results of the Hershberger and Uterotrophic in vivo rat studies, now in the public domain, as are the published results of the OECD validation of the Steroidogenesis assay, in which glyphosate clearly had no impact on steroidogenesis, are discussed below.

In Vitro Glyphosate DART/ED Publications

Many in vitro research publications have characterised pesticide formulations, including glyphosate based formulations, as toxic and endocrine disrupting products. Researchers and editorial boards did in some cases not consider the fact that surfactants (which are often components of formulated pesticide products), by their physico-chemical nature, are not suitable test substances using in vitro cell models. Surfactants compromise the integrity of cellular membranes, including mitochondrial membranes, and thus confound endpoint measurements considered as representative of specific toxicological modes of action or pathways.

A laboratory at the University of Caen, France, has multiple recent publications of in vitro research with glyphosate and glyphosate based formulations (Richard et al., 2005, ASB2009- 9024; Benachour et al., 2007, ASB2009-9018; Benachour and Seralini, 2009, ASB2012- 11561; Gasnier et al., 2009, ASB2012-11629; Gasnier et al., 2010, ASB2012-11628; Gasnier et al., 2011, ASB2012-11630; Clair et al., 2012, ASB2012-11592; Mesnage et al., 2012, ASB2012-11900), with proposed extrapolations to an array of in vivo effects including potent endocrine disruption, aromatase inhibition, estrogen synthesis, placental toxicity, foetotoxicity, embryotoxicity and bioaccumulation. These publications are in some cases replicates of earlier studies, using different cell lines or primary cell cultures and in some cases the same data are reported again in a subsequent publication. Firstly, the in vitro synergism claims are conjecture, because no control groups of surfactant without glyphosate were tested. Secondly, the extrapolations to in vivo effects are unjustifiable based on both the unsuitability of surfactants in such test systems and the supraphysiological cytotoxic concentrations at which in vitro effects are reported. Again often overlooked by in vitro researchers and editorial boards, Levine et al. (2007, ASB2009-9030) presented convincing data demonstrating a lack of in vitro synergism for glyphosate with other formulation ingredients. Regarding Seralini's repeated claims of glyphosate induced aromatase inhibition in mircosomes (Richard et al., 2005; TOX2005-1743, Benachour et al., 2007, ASB2009- 9018; Gasnier et al., 2009, ASB2012-11629), the data are confounded and thus uninterpretable where surfactants are introduced to such in vitro systems. This is noted in the US EPA Aromatase Inhibition Test Guideline, OECD 890.1200, in which notes, "Microsomes can be denatured by detergents [surfactants]. Therefore, it is important to ensure that all glassware and other equipment used for microsome preparations be free of detergent residue."

Another in vitro publication claiming a specific developmental toxicity pathway has gained significant public attention. Paganelli et al. (2010, ASB2012-11986) conducted three in vitro assays, (i) frog embryos exposed to glyphosate formulation; (ii) frog embryos directly injected

without injection blank negative controls; and (iii) fertilised chicken embryos exposed directly to a glyphosate formulation through a hole cut in the egg shell. Key issues surrounding this research include irrelevant routes of exposure as well as excessively high and environmentally unrealistic doses.

In Vivo Glyphosate DART/ED Publications

Relatively few in vivo publications on glyphosate DART and ED exist in comparison with the list of in vitro publications. Some lack appropriate interpretation of basic toxicology; e.g. Daruich et al. (2001, ASB2012-11601).

Both studies are reviewed in Williams et al. (2012, ASB2012-12052).

Dallegrave et al. (2003, ASB2012-11600; 2007, ASB2012-2721) published results of two non-guidelines rat developmental toxicity studies, in which a glyphosate based formulation containing POEA was evaluated. However, reporting deficiencies and inconsistencies pose difficulties in data interpretation. These studies are dicussed in detail in the Appendix on
(please refer to B.6.13).

Romano et al. (2010, ASB2012-12012) evaluated a glyphosate based formulation in a male pubertal-like assay in Wistar rats, reporting decreased preputial separation, reduced seminiferous epithelial height, increased luminal diameter of seminiferous tubules, and increased relative testicular and adrenal weights. Given the gravity of the reported findings in this publication, a review was undertaken by Kelce et al. (2010, ASB2012-11867). Most recently, Romano et al. (2012, ASB2012-12011) reported additional findings in male rats after supposed in utero and post natal exposures which include “behavioral changes and histological and endocrine problems in reproductive parameters and these changes are reflected by a hypersecretion of androgens and increased gonadal activity, sperm production
and libido”. As in their first publication, Romano et al. (2012, ASB2012-12011) base their hypothesis on selectively discussed literature implicating glyphosate as an endocrine disruptor, predominantly with citations to research from the Seralini laboratory.

POEA DART Studies

Polyethoxylated alkylamine (POEA) surfactants are a class of non-ionic surfactant, containing a tertiary amine, an aliphatic group of variable carbon chain length and two separate sets of ethoxy (EO) chains of variable length. A dietary exposure assessment of POEAs was submitted by Bleeke et al. (2010, ASB2010-6123). This exposure assessment report also refers to the US EPA Alky Amine Polyalkoxylates Human Health Risk Assessment, which includes POEAs
(http://www.regulations.gov/search/Regs/home.html#document Detail?R=09000064809b983b).
Williams et al. (2012, ASB2012-12052) recently evaluated and detailed the results of DART studies with two different POEA surfactants.

Epidemiology Glyphosate DART/ED Publications

Several epidemiology studies in which glyphosate exposure was considered have evaluated the following range of reproductive outcomes; miscarriage, fecundity, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, attention-deficit disorder / attention-deficit hyperactive disorder (ADD/ADHD). In most instances, glyphosate and reproductive outcomes lack a statistically significant positive association, as described in a recent review of glyphosate non-cancer endpoint publications (Mink et al., 2011, ASB2012-11904). In evaluating ADD/ADHD, a positive association with glyphosate use was reported by Garry et al. (2002, ASB2012-11626), but cases were reported by parents with no clinical confirmation and the reported incidence rate of approximately 1 % for the study population was well below the general population

incidence rate of approximately 7 %. Regarding in utero exposures, McQueen et al. (2012, ASB2012-11898) report very low measured dietary exposures, from 0.005 % to 2 % of the current glyphosate ADI in Europe. Given the low perfusion rate of glyphosate across the placenta (Mose et al., 2008, ASB2012-11914), human in utero exposures would be very limited.
Walsh et al. (2000, ASB2012-12046) published research claiming that a glyphosate based formulation, but not glyphosate alone, adversely affected the steroidogenesis pathway by inhibiting progesterone production resulting in downstream reduction in mitochondrial levels of StAR protein. Subsequent research by Levine et al. (2007, ASB2009-9030) demonstrated no synergism between glyphosate and the surfactant since the cytotoxic effects were completely independent of glyphosate. Identical dose-response curves were noted for formulated product with and without the glyphosate active ingredient.
Further research addressing the steroidogenesis pathway confirmed glyphosate lacked endocrine disruption potential specific to this pathway. Quassinti et al. (2009, ASB2012- 12007) evaluated effects on gonadal steroidogenesis in frog testis and ovaries on glyphosate and another active substance, noting that glyphosate unequivocally demonstrated no effect. Forgacs et al. (2012, ASB2012-11621) also tested glyphosate alone and demonstrated no effect on testosterone levels in BLTK1 murine leydig cells in vitro. Furthermore, the OECD multi-laboratory validation of the Steroidogenesis Assay used for Tier 1 screening of the US EPA EDSP, evaluated glyphosate and concluded no impact on steroidogenesis (Hecker et al., 2011, ASB2012-11840). Consequently, the US EPA considered reference to the OECD validation report sufficient for meeting the glyphosate Steroidogenesis Assay Test Order in the EDSP Tier 1 screening of glyphosate.
Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia, 2012, ASB2012-12016) for the Hershberger and
Uterotrophic assays. No effects were noted for any potential for the active substance glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.
Klimisch evaluation
Reliability of study:
Reliable with restrictions
Comment:
Non-standard test systems, but publication meets basic scientific principles. However, surfactant blend in
Roundup confounds results.
Relevance of study:
Relevant with restrictions: Different effects of glyphosate alone and glyphosate formulations were observed. No conclusion can be drawn that the
observed effects are result of glyphosate exposure.
Klimisch code:
2
Additional comments:
Glyphosate did not affect steroidogenesis in the test system.
Roundup formulation data was confounded by mitochondrial membrane damage, attributable to the surfactant in the tested formulation.
Roundup results were comprehensively addressed in Levine et al. (2007, ASB2009-9030): Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was

shown to have “indistinguishable” dose response curves for reductions in progesterone production in hCG stimulated MA-10 Leydig cells. Therefore the effect on progesterone levels shown by Walsh (2000, ASB2012-12046) were independent of glyphosate and attributable to the surfactant component of the formulation.
Comparable rates of progesterone inhibition for several different surfactants suggest a common mode of action for surfactants.
Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have almost identical concentration-dependent decreases in MTT activity in MA-10 cells, suggesting the surfactant alone was responsible for the observed cytotoxicity and effect on mitochondrial function.
The JC-1 assay demonstrated the decreased progesterone production in MA-10 Leydig cells was accompanied by loss of mitochondrial membrane potential. These results confirm StAR protein function and steroidogenesis require intact mitochondrial membrane potential.
StAR protein expression were not affected by treatments, indicating that perturbed mitochondrial membrane, not StAR protein inhibition, was responsible for the effects noted by Walsh et al. (2000, ASB2012-12046).
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-guideline study that is not sufficiently described for assessment. Inadequate positive and negative control experiments.
Relevance of study:
Not relevant: Irrelevant routes of exposure and inappropriately high doses. Test system not adequate for human risk assessment.
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Study design is insufficient for risk assessment of real exposure concentrations. Methodological deficiencies (no controls were included). Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems. EPA Test Guideline OCSPP 890.1200 specifically notes that microsomes are denatured by detergents (i.e. surfactants) and that all glassware should be
thoroughly rinsed.
Relevance of study:
Not relevant: Excessive doses exceed typical in vitro
limit doses. In vitro test system is inappropriate with surfactants.
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable

Comment:
Study report has several reporting deficiencies in the methods section (e.g. test conditions for the pH- and temperature dependent assay not reported). There is no information on the suitability of the used HEK 293 cell line for assessment of hormonal activity. Exceedingly high doses above the limit dose for this study type.
Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:
Not relevant: Excessive doses exceed typical in vitro
limit doses. In vitro test system is inappropriate with surfactants.
Klimisch code:
3
Additional comments:
Glyphosate at and above relevant concentrations for this test system was demonstrated to have no effects on aromatase activity.
Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.
Comparable research to Richard et al (2005, TOX2005-1743), but with an additional cell line, HEK 293, derived from aborted human embryo kidneys, transformed by inserting adenovirus DNA.
Excessively high doses tested, not environmentally relevant for human health or environmental risk assessment.
Aromatase production within the steroidogenesis pathway. Therefore, aromatase inhibition would be detected in the steroidogenesis assay. The OECD multi-laboratory validation of the steroidogenesis assay evaluated glyphosate, demonstrating no impact on the steroidogenesis pathway (Hecker et al., 2011, ASB2012-11840).
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption
potential of surfactants are well known for in vitro test systems. No positive controls were included.
Relevance of study:
Not relevant (Excessive doses exceed typical in vitro
limit doses. In vitro test system is inappropriate with
surfactants)
Klimisch code:
3
Additional comments:
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Due to reporting deficiencies (e.g. correlation between concentration used in toxicity tests and concentrations used in comet assay) assessment of results difficult.
Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well

known for in vitro test
systems.
Relevance of study:
Not relevant: Excessive doses exceed typical in vitro
limit doses. In vitro test system is inappropriate with surfactants.
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-guideline in vitro test with methodological (i.e. no
positive controls included) and reporting deficiencies (e.g. dose levels not always specified).
Relevance of study:
Not relevant (Due to reliability. In addition, in vitro data do not reflect real in vivo exposure situations, and therefore not relevant for human risk assessment
purposes.)
Klimisch code:
3
Additional comments:
In vitro test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified). The concentrations used in these experiments are not relevant to human exposures to glyphosate and the experimental system used is not relevant to whole animal outcomes. Importantly, the alleged impacts on endocrine function have not been observed in animal studies of glyphosate or other components of glyphosate formulations at relevant concentrations. Authors state that the lowest concentration of glyphosate tested was 50 ppm, several orders of magnitude higher than an anticipated human intake (based on
pharmacokinetics described in Anadon et al., 2009, ASB2012-11542) following worst case dietary exposure at the ADI.
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Not acceptable in vitro methods for test mixtures containing surfactant. Well documented study publication, but surfactants are inappropriate test
substance in cell lines.
Relevance of study:
Not relevant Temporal altered gene expression is not a biomarker for toxicity, but rather, may be within the range of normal biological responses of homeostasis. In vitro cytotoxicity of surfactants, however, is a significant confounder in data interpretation. Data do
not reflect real in vivo exposure situations, and therefore not relevant for human risk assessment purposes.
Klimisch code:
3
Additional comments:
In vitro cytotoxicity of surfactants is a significant confounder in data interpretation. Relevance of

altered gene expression in a cell line derived from a breast cancer should not be extrapolated to reflect human health endpoints. Altered gene expression should not be confused with adverse health outcomes. Rather altered gene expression may equally be considered a biological response within the range of normal homeostasis.
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-GLP, non-guideline study with major reporting deficiencies. Dose-levels poorly defined as 1/10 and 1/100 LD50. Purity of the test substances, source of animals, environmental conditions, mortality and clinical signs not reported. No testis and epididymis weights were determined or reported and no histopathological examination conducted. In addition, stability and homogeneity assessment of test substance preparations were not done or not reported. Rabbits
have low body weights at study start, suggesting impaired health status.
Relevance of study:
Not relevant (Due to low confidence in study conduct
and the inadequacy of reporting.)
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Basic data given, however, the study is performed with methodological and reporting deficiencies (unknown exposure levels, only cytosolic enzymes measured,
inappropriate controls, lack of consistent dose-response data).
Relevance of study:
Not relevant (Due to reliability. In addition, study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate).
Klimisch code:
3
Additional comments:
The study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate. Test substance administration is poorly described, but rough calculations on approximate surfactant intake show excessively high and unrealistic exposures when compared to DART systemic parental and reproductive/developmental NOAEL values for POEA formulation surfactants.
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Study with methodological and reporting deficiencies or conflicting findings (e.g., increased relative testicular
weights, but decreased testosterone measurements.
Relevance of study:
Relevant study type for investigating male reproductive endpoints, but questionable relevance of this specific study based on low reliability of data and interpretation. Not relevant for glyphosate (test

material was a
formulated product, not glyphosate).
Klimisch code:
3
Additional comments:
Test material was a formulated product, not glyphosate. The authors failed to measure many of the key parameters in the validated pubertal male assay protocol and hence generated data that were internally inconsistent or incomplete.

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-guideline, non-GLP study meeting scientific principles. Unusual and short dosing regiment commencing towards the end of pregnancy (GD18, rather than GD6 as per OECD Test Guidelines 414) through post natal day 5. In vivo study with reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). A
number of atypical endpoints evaluated.
Relevance of study:
Not relevant (due to questionable dosing regimen and
atypical array of endpoints measured).
Klimisch code:
3
Additional comments:
Study with some reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). Dosing was limited to dams, starting on gestation day 18, well after organogenisis, through post natal day 5. No controls for litter effects appear to be reported, confounding interpretation of results. With the very short window of maternal exposure, biological plausibility of any test substance related effects in the mature offspring is questionable. However, the normal variability of some unusual or atypical endpoint measurements, such as “sexual partner preference” along with mRNA and protein expression, is not known. Of particular concern, however, are differences in critical endpoints for control animals reported in Romano et al. (2010, ASB2012-12012) compared to Romano et al. (2012, ASB2012- 12011); these include increased day of preputional sepratarion (PPS) of control male rate (37 days in 2010; 47 days in 2012), body weight at day of PPS (146 grams in 2010; 245 grams in 2012), serum testosterone concentrations (155 ng/dL in 2010; 63 ng/dL in 2012), estradiol concentrations (32 pg/mL in 2010; 1.4 pg/mL in 2012), subular diameter (266 µm in 2010;
479 µm in 2012), epithelial height (86 µm in 2010; 92 µmin 2012) and luminal height (94 µm in 2010; 257µm in 2012). Therefore, results are difficult to interpret, particularly for relevance to human health risk assessment.
system in offspring whose dame were treated with glyphosate”. Romano and Romano (2012, ASB2014-9396) rebutted these comments and conclusions.

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide.
Relevance of study:
Not relevant (Study design is not suitable for assessment
of glyphosate exposure).
Klimisch code:
3
Additional comments:
Pre-conception glyphosate exposure odds ratio for spontaneous abortion is considered of borderline significance (OR = 1.4). Post-conception glyphosate exposure was not associated
with spontantous abortin (OR = 1.1). Authors note multiple limitations of the study relating to exposure, likely misclassification of pesticides and correct assignment of exposure window to pre- or/and post-conception
OFFHS information gathering methodology has high potential recall bias. Blair and Zahm (1993, ASB2012-11567) report 60 % accuracy when comparing self reported pesticide usage with purchasing records.
OFFHS relied exclusively on maternal self-reports of adverse pregnancy outcomes, not all of which were confirmed via medical or other records.
Three highly relevant confounding factors were not considered in the OFFHS questionnaire: history of previous spontaneous abortion(s), maternal age and smoking.
Response summarized from Williams et al. (2012, ASB2012-12052)
395 spontaneous abortions were reported out of 3936 pregnancies; rate of spontaneous aborting in Arbuckle et al. (2001, ASB2012-11545) was 10 %.
The baseline rate of spontaneous abortions in the general populations is much higher, ranging from 12 % to 25 %.
Recall bias is reflected in the recall of spontaneous abortion over the previous 5 years (64 % of all spontaneous abortions reported) being much higher than the recall of those greater than 10 years prior to the survey (34% of all spontaneous abortions reported).
Substantial exposure misclassification may have occurred (pre- versus post-conception) due to likely author extrapolation of exposure data.
Strong confounding variables are not apparent in previous data analyses published by the authors of the OFFHS, and therefore odds ratios are crude.
Published results fail to demonstrate a significant association of glyphosate exposure spontaneous abortion risk and therefore must be considered cautiously.
Klimisch evaluation
Reliability of study:
Not Reliable
Comment:
No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide. Due to study design and evaluation methods, study results are not reliable.
Relevance of study:

Not Relevant (Study design is not suitable for
assessment of glyphosate exposure).
Klimisch code:
3
Additional comments:
Glyphosate is one of many pesticides mentioned in three epidemiological reports that examine possible links between on-farm pesticide use and reproductive outcomes. All three reports - Savitz et al. (1997, ASB2012-12022), Curtis et al. (1999, cited in ASB2012-11545) and Arbuckle et al. (2001, ASB2012-11545) - use data from the Ontario Farm Family Health Study (OFFHS) (Arbuckle 1994, cited in ASB2012-11545). Savitz et al. (1997, ASB2012- 12022) investigated associations between reported pesticide use by males and pregnancy outcomes, specifically: miscarriage, pre-term delivery and small-for-gestational-age birth. Curtis et al. (1999, cited in ASB2012-11545) studied whether reported pesticide use by males or females was associated with delayed pregnancy, while Arbuckle et al. (2001, ASB2012- 11545) looked for associations between reported pesticide use and spontaneous abortion.
In the study by Savitz et al. (1997, ASB2012-12022), a number of specific pesticides had weak statistical associations with miscarriages and pre-term deliveries, but pesticides tended not to be associated with small for gestational age births. There were no statistically significant findings for glyphosate. In the study by Curtis et al.(1999, cited in ASB2012- 11545), for farms on which glyphosate was used, there was no significant association for women being engaged in pesticide activities. For men, glyphosate use was associated with a slight, but statistically significant, decrease in time to pregnancy. The authors dismissed this finding, which was contrary to their hypothesis that pesticide exposure delayed pregnancy, as probably due to uncontrolled factors or chance. Arbuckle et al. (2001, ASB2012-11545) found that reported preconception use of phenoxyacetic acids, triazines, glyphosate, and thiocarbamates were weakly, but statistically significantly, associated with spontaneous abortions. Post conception reported use was not associated with increased risk. The authors characterized the associations between pesticides and spontaneous abortions as "hypothesis generating" pending confirmation from other epidemiologic studies.
These studies are not convincing evidence of a relationship between glyphosate exposure and adverse pregnancy outcomes for a number of reasons:
There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. For example, according to a study by the National Cancer Institute, self-reports of pesticide usage were found to be only 60 percent accurate when compared with purchasing records (Blair & Zahm 1993, ASB2012- 11567). Further increasing the potential for inaccuracy is the fact that study subjects were only asked about pesticide use for the 5 years before the OFFS survey. These responses were
assumed to be applicable to the entire farming careers of study subjects, an assumption inconsistent with changes in agricultural practice. Lastly, basing exposure estimation on questionnaire responses has the potential to be influenced by what epidemiologists call "recall bias." This refers to the likelihood that families that experienced an adverse reproductive outcome are more likely to remember use of certain pesticides than families that had only normal births.
The most widely used pesticides, like atrazine, glyphosate, and 2,4-D, are most easily recalled and most likely to be over-reported.
The OFFHS study relied exclusively on maternal self-reports of adverse pregnancy outcomes with no medical or other validation. Generally, scientists place less confidence in reports of health outcomes

that are not validated with medical records.
A confounding factor is a cause of a disease that is correlated with another exposure being studied. Failure to control confounding factors, especially those that are strong causes of a disease, can create spurious associations between benign exposures and diseases. In the Arbuckle study, there were at least three important potential confounding factors that were not controlled: history of previous spontaneous abortion, maternal age, and smoking. Even a weak correlation between these factors and use (or recall of use) of pesticides would produce spurious associations. In addition, in all three studies, the authors did not control the putative effect of one pesticide for the putative effects of other pesticides. So, for example, since farmers tend to use 4 or more pesticides each year, a disease that is associated with one pesticide will likely be associated with all, since their use patterns are correlated. In the absence of an analysis that controls for multiple pesticides, the best that can be said is that the findings for any individual pesticide might be due to its correlation with another pesticide.
In summary, three publications based on data collected in the OFFHS found associations between several pesticides and various adverse reproductive outcomes. There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. Glyphosate was not significantly associated with adverse reproductive outcomes in two of these studies (Savitz et al. 1997, ASB2012-12022, Curtis et al. 1999, cited in ASB2012-11545). Glyphosate and other pesticides were weakly associated with spontaneous abortion in the study by Arbuckle (2001, ASB2012-11545). However, the author did not control for important personal confounding factors or for multiple exposures and no actual exposure data was used, casting doubt on the validity of the findings in this study.
Biomonitoring data for glyphosate, collected as part of the Farm Family Exposure Study (FFES), provide assurance that human health effects related to glyphosate exposure are very unlikely. In the FFES, researchers from the University of Minnesota collected 5 days of urine samples from 48 farm families before, during, and after a glyphosate application (Mandel et al., 2005, ASB2012-11893, accepted for publication). Only 60% of farmers showed detectable exposure to glyphosate, with a 1 part per billion limit of detection, and the maximum estimated absorbed dose was 0.004 mg/kg (Acquavella et al., 2004, ASB2012- 11528). For farmers who apply glyphosate 10 times per year for 40 years, this maximum dose is more than 30,000-fold less than the EPA reference dose1 of 2 mg/kg/day. For spouses, only 4% showed detectable exposures and the maximum systemic dose was 0.00004 mg/kg/day. Since glyphosate is not a reproductive toxic in high dose animal studies and since actual exposures on farms are so low, it is very unlikely that glyphosate would cause adverse reproductive outcomes for farmers or their spouses.
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Epidemiological study with some methodological / reporting deficiencies (selection of study subjects, no information about exposure duration, exposure concentration, pesticide use frequency).
Relevance of study:
Not relevant because of methodological deficiencies.
Klimisch code:
3
Klimisch evaluation

Reliability of study:
Not reliable
Comment:
Epidemiological study with some methodological / reporting deficiencies (e.g. selection of control subjects/samples, no details of exposure).
Documentation is insufficient for assessment.
Relevance of study:
Not relevant for glyphosate (due to reliability; in addition, no direct assessment of glyphosate exposure
was made).
Klimisch code:
3
Additional comments:
The publication brings little information on endpoints attributable to glyphosate.
Given the subjects were pesticide applicators, little can be drawn from the findings other than perhaps certain endpoints which may be associated with this specific occupation exposed to multiple chemical substances.
Of the 136 participants volunteering blood samples, only one individual (subject D) was noted with one abnormally high thyroid hormone levels associated with glyphosate use; thyroid stimulating hormone (FSH) was about double the normal range in the fall and thyroid stimulating hormone (TSH) higher than normal in the summer.
Another individual (subject E) had abnormally high TSH levels associated with multiple pesticide usage of 12 different active ingredients.
Author(s)
Year
Study title
Bell, E.M.
Hertz-Picciotto, I.

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Epidemoliogical study with methodological deficiencies (e.g. glyphosate was included in the pesticide class of phosphates, thiophosphates, phosphonates; no differentiation between single and multiple exposures).
Relevance of study:
Not relevant (No glyphosate-specific results.)
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Exact levels of PAGMF, glyphosate or AMPA in the diets were not determined. It is not clear if the measured concentrations could have been resulted from other exposure routes.

Relevance of study:
Relevant with restrictions (Provides real life actual exposure concentrations in humans. Data are limited due to the absence of any information on applied
pesticides, application rates, etc.).
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Study design of epidemiological study for developmental toxicity insufficient for assessment, as well as methological and reporting deficiencies (no assessment to which pesticides / active substances the mothers were exposed, use frequency not specified, selection of control group after study period is questionable, no information on exposure situation of mother for this control group assessed, etc.).
Relevance of study:
Not relevant (The exposure to several pesticides was assessed in general, but no pesticide or active substance,
including glyphosate, was specified or assessed).
Klimisch code:
3

B.6.7.1 Published data on neurotoxicity
Several publications over the last decade have evaluated glyphosate with respect to neurotoxicity endpoints. Three papers report a total of two human cases of Parkinson's disease. The first case followed acute exposure to a glyphosate formulation while spraying a garden (Barbosa et al., 2001, ASB2012-11557; da Costa et al, 2003, ASB2012-11598). The second case reported chronic exposures to a factory worker in China, where a variety of pesticides including glyphosate were produced (Wang et al, 2011, ASB2012-12047). Several questions arise in attempting to link glyphosate exposures with each case of Parkinson's disease. Firstly, significant systemic exposures to glyphosate in each instance are questionable, given the poor dermal absorption and low volatility of the compound. Secondly, if glyphosate was a causative agent of this fairly common disease, a significant number of cases associated with either acute and/or chronic exposures would be evident. Glyphosate formulations are sometimes readily accessible for suicide attempts, which are usually unsuccessful, as less than 10% of glyphosate self administered ingestions result in death. No reports of Parkinson's disease in survivors following very acute ingestions of glyphosate products have been documented. Glyphosate has been manufactured and widely used in agriculture and consumer markets for approximately 40 years, so a single case of a pesticide factory worker developing Parkinson's disease, while unfortunate, does not constitute cause and effect; there is no evidence of a higher frequency of Parkinson's disease in glyphosate production workers.
Multiple long term animal studies with glyphosate have failed to demonstrate any evidence of neurotoxicity, and certainly have not shown evidence of Parkinson's-like abnormalities. While some studies have suggested statistical associations with general pesticide exposure or general insecticide or herbicide exposure (Engel et al., 2001, ASB2012-11612), there is no evidence suggesting a specific association between glyphosate and Parkinson's disease. In the largest study to date of US Farmers (Agricultural Health Study), no increased risk of Parkinson's disease was found in association with reported glyphosate use (Kamel et al., 2007, ASB2012-11862). Human non-cancer epidemiologic

outcomes related to glyphosate have recently been reviewed (Mink et al. 2011, ASB2012-11904), and there is no convincing
evidence for an increased incidence of Parkinson's disease or other neurological disorders in individuals reporting glyphosate exposure.
Several publications open with the premise that pesticide exposures are linked with Parkinson's disease, and then proceed to report a priori research linking glyphosate with a measurable endpoint. This endpoint is then extrapolated to link with Parkinson's disease in humans. Despite the lack of compelling human associations between glyphosate exposure and Parkinson's disease, such research continues to be published. Astiz et al., (2009, ASB2012-11549), Negga et al. (2011, ASB2012-11923) and Gui et al. (2012, ASB2012-
11835) all conducted glyphosate research in the above mentioned manner, all in very different test systems. Negga et al. (2011, ASB2012-11923) notes neurodegeneration in Caenorhabditis elegans worms following exposure to glyphosate (trimesium form, which has a different toxicology profile than glyphosate) uses concentrations equal to the LD25, LD50 and LD75, or actual concentrations of glyphosate of 3 to 10 percent, i.e.- the high concentration is approximately 10-fold higher than concentrations applied directly in the field. The relevance of such high-dose exposures to the trimesium salt in this experimental model to human Parkinson's disease is highly questionable and irrelevant to the Annex 1 renewal of glyphosate technical acid. Astiz et al. (2009, ASB2012-11549) and Gui et al. (2012, ASB2012-11835) both affirm their test models (in rats and in PC-12 cells respectively) for evaluating neurodegenerative disorders, then directly link their research results to Parkinson's disease in humans; these two studies are addressed below.
Cole et al. (2004, ASB2012-11594) evaluated 15 different pesticides for neurotoxic end points in C. elegans with analytical grade active ingredients, noting reduced cholinesterase for pesticides with this mode of action, but not glyphosate. Interestingly, the authors report a low pH effect resulting in reduced cholinesterase activity in the high dose of glyphosate and a plant growth promoter. Glyphosate formulations contain salt forms of glyphosate, not the technical acid and thus do not have a low pH. Additionally, human incidents of self induced glyphosate poisonings do not report the common symptoms of acute acetylcholinesterase inhibition; salivation, lacrimation, urination and defecation (SLUD).
Klimisch evaluation
Reliability of study:
Not assignable
Comment:
Medical case report, single incident
Relevance of study:
Relevant with restrictions (Data are limited due to the absence of any information on purity and application concentrations of glyphosate formulation, as well as co- formulations.)
Klimisch code:
4
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Unsuitable test system (i.p exposure route is not relevant for human exposure). No information on purities of test substances used. Small group size (4
males/dose group), reporting deficiencies

Relevance of study:
Not relevant (intraperitoneal injection is a non-relevant
route of exposure for humans)
Klimisch code:
3
Additional comments
This non-guideline study utilized very small group numbers (4 rats/group) and therefore is not sufficiently robust to appropriately identify changes attributable to the test material administration.
The test materials are not well described, without indication of whether a glyphosate salt form or acid was used and purity was not reported.
The publication focuses on the post necropsy data analysis and reporting. Data on animal husbandry, clinical observations, feed and water intake, weekly body weight were not reported, but the authors note there were no adverse observations.
No statistically significant effects were noted for liver endpoints, yet the liver is in close proximity to test material administration via intraperitoneal injection.
Statistically significant effects were noted for brain tissue endpoints in the substantia nigra and cerebral cortex. However, there is a lack of biological plausibility for brain exposures to glyphosate, given the necessity to pass the blood-brain barrier and the known rapid elimination kinetics of this polar molecule via urine.

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Documentation insufficient for assessment (not clearly stated dose levels and duration of exposure, as well as treatment conditions for all tests. In addition, tested doses were much higher than real in vivo concentrations).
Relevance of study:
Not relevant (Due to reliability)
Klimisch code:
3
Additional comments:
In this paper, the authors apply glyphosate to adrenal cancer cells in culture at concentrations sufficient to cause cell death. Two major interacting pathways leading to cell death (autolysis and apoptosis) are evaluated, and the results are hardly surprising - the cells do indeed die via known mechanisms leading to cell death. The authors use these observations, and the fact that Parkinson's disease involves the death of certain nerve cells in the brain, to try and create a link between glyphosate and Parkinson's disease. There are, however, many problems with this extrapolation.
The cells used are not the neurons involved in Parkinson's, but rather a cell line derived from an adrenal gland cancer (pheochromocytoma), and the doses used are very high- the high dose killed nearly 50 % of cells in 72 hours, and the low dose was ¼ this level. The high dose equates to approximately 1/10 the concentration applied directly in the field, and is far higher than any internal glyphosate concentration that could ever occur following glyphosate use. A sufficiently high dose of every substance will kill cells - but this does not mean that every substance causes Parkinson's disease.
Unprotected cells in culture are highly susceptible to changes in pH and other non-specific effects,

and it is not clear that the researchers assessed or accounted for these possible effects. This being said, the concentrations of glyphosate used (40 mM) are known to kill other cell types in culture (Heu et al., 2012, ASB2012-11844) via induction of apoptosis. Thus, no particular specificity or neuronally-specific susceptibility exists for the cell line tested. While 40 mM glyphosate is toxic to cells in culture, the LD50 in rodents is over 5000 mg/kg and C. elegans will have a 25 % survival following exposure to a 10 % solution of glyphosate. In- vitro results do not appear to reflect in vivo events.
Anadon et al. (2009, ASB2012-11542) dosed rates with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved glyphosate peak modeled plasma concentrations of approximately 5 ug/mL (5 ppm). Assuming linear kinetics, the current maximum allowable EU daily intake (0.3 mg/kg/day) would give an approximated blood concentration of 0.17 ppm (170 ppb). This is conservative, as McQueen et al (2012, ASB2012-11898) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4 % of the current European acceptable daily intake.
The lowest glyphosate concentration used in this experiment is 5mM (830 ppm), or 5000 times higher than the estimated blood concentration following the current EU maximum allowable daily exposure. It is also 166 times higher than the concentrations Anadon et al. (2009, ASB2012-11542) achieved using doses of 400 mg/kg glyphosate. In short, the concentrations used in this work are massively higher than any concentration is blood (let alone brain tissue) that can be achieved following normal human exposures.

Klimisch evaluation
Reliability of study:
Not assignable
Comment:
Documentation insufficient for evaluation.
The publication overview provides information on the general application of the sea urchin embryo model for the prediction of “cancerogenicity”. Only a short reference to another study with a glyphosate-containing herbicide is given. Details of the glyphosate product are not provided.
Common surfactants have previously shown the same effects in this model. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using in vitro systems.
Relevance of study:
Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants.)
Klimisch code:
4
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Mechanistic study. Outcome with little additional information compared to the authors’ previously published work. Non-standard, non-guideline.
Commonly used surfactants have previously shown the same effects in this model.

Relevance of study:
Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using in vitro
systems.)
Klimisch code:
3

Klimisch evaluation
Reliability of study:
Not Reliable
Comment:
Non-standard, non-guideline study. Commonly used
surfactants have previously shown the same effects in this model.
Relevance of study:
Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using in vitro
systems.
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-standard, non-guideline study. Commonly used surfactants have previously shown the same effects in this model.
Relevance of study:
Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well
documented using in vitro systems.
Klimisch code:
3
The following two recent publications, by Heu et al. (2012, ASB2012-11843 and ASB2012- 11844) are commented on collectively after the second summary/Klimisch rating, below.

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-guideline in vitro tests with no control for low pH effects. Minor reporting deficiencies (source and purity of glyphosate, replicates per dose level)
Relevance of study:
Not relevant (in vitro data on the effects on an immortalised epidetmal cell-line does consider low exposure potential due to stratum cornea protection. Inappropriate test substance if not adjusted for pH; low pH glyphosate acid is not in formulated glyphosate based

products)
Klimisch code:
3

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-guideline in vitro tests with no control for low pH effects. Minor reporting deficiencies (source and purity of glyphosate, replicates per dose level)
Relevance of study:
Not relevant (in vitro data on the effects on an immortalized epidermal cell-line does consider low exposure potential due to stratum cornea protection. Inappropriate test substance if not adjusted for pH; low pH glyphosate acid is not contained in formulated
glyphosate based products)
Klimisch code:
3
Additional comments:
Glyphosate technical acid evaluated was not reported to be pH adjusted and therefore does not reflect real world exposures to the more neutral pH formulations, which contain glyphosate salts, not glyphosate acid
The pH range of test concentrations (850-1150 mg/L) is very acidic, approximately 1.7-2- 2 pH units. Keeping in mind the pH scale is logarithmic, these values are substantially lower than those of viable skin and in vitro cell cultures.
Exposure potential to live human epidermal skin cells in the field is likely to be considerably lower than the authors have considered. The epidermis is protected by the stratum cornea. Human in vitro dermal absorption studies for a range glyphosate formulated products are presented in the chapter on dermal absorption, showing a very low dermal absorption of glyphosate; nearly all of the glyphosate is washed off the skin surface after 24 hour exposures (88% to >99 % before stratum cornea removal). Therefore, the studies of Heu et al., while
representative of glyphosate spray concentrations, are approximately two or more orders of magnitude higher of those which may result for 8-24 hour dermal exposures.
Klimisch Evaluation
Reliability of study:
Not reliable
Comment:
Incorrect characterisation of glyphosate as an organophosphate pesticide. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactant are well known for in vitro test systems. Exposure route not relevant for human risk assessment. Rationale for chosen test substance concentration not given.
Relevance of study:
Not relevant (in vitro data, do not reflect real in vivo
exposure situations. Pre-exposure to diazinon is not relevant for this submission).
Klimisch code:
3
Klimisch evaluation

Reliability of study:
Not reliable
Comments:
Study report meets basic scientific principles. Study design and documentation is insufficient for assessment.
Relevance of study:
Not relevant because study design not sufficient for assessment of toxicity of the active substance Glyphosate. Toxicity is attributable to high oral dosing of surfactant component. There are several reporting
deficiencies.
Klimisch code:
3
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-guideline, non-GLP in vitro tests meeting scientific principles. Deficiencies: No positive controls were specified, test conditions not described (referenced to a description elsewhere). Exceedingly high doses and an inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:
Relevant with restrictions (Due to reliability. The
assessed combinatory effects are of limited relevance)
Klimisch code:
3
Additional comments:
Direct exposure to cells in culture bypasses normal processes limiting absorption and cellular exposure and avoids normal metabolism, excretion, serum protein binding, and other factors that would protect cells in the intact organism.
Anadon et al. (2009, ASB2012-11542) dosed rates with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved peak modeled plasma concentrations of glyphosate of approximately 5 ug/mL (5mg/L or 5 ppm). Assuming linear kinetics, the maximum allowable US daily intake (2 mg/kg/day) would give an approximated blood concentration of 0.025 ppm (25 ppb). McQueen et al. (2012, ASB2012-11898) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4 % of the acceptable daily intake.
The “Roundup” LC50 concentration used (57.5 ppm) is more than 2000-fold higher than the anticipated concentration (based on Anadon et al., 2009, ASB2012-11542) following maximum allowable intake.
The co-application of Cry protein with the glyphosate-surfactant reduces the apparent degree of cellular injury (as measured by induction of caspase levels). This occurs even at concentrations of Cry1Ab which the authors report to cause cellular injury and membrane disruption. This is worth noting for several reasons:
First, it brings into question the toxicity observations with Cry1Ab, as the argument that membrane disruption and impaired mitochondrial function should be protective seems to be highly untenable, especially in view of the studies (Levine et al, 2007, ASB2009-9030) demonstrating the mitochondrial

membrane activity of surfactants.
Second, it should take off the table any implications of a “synergistic effect” of Cry proteins and glyphosate-surfactant herbicides. (The direction is, if anything, antagonistic, but the entire system is fundamentally irrelevant.)
Third, this probably is demonstrating the artificiality of the system itself. As noted above, this is a protein-free medium. Protein protects cells in culture by multiple mechanisms- binding to toxic materials, binding to potential receptor sites, or other non-specific surface-stabilisation effects. It appears from Mesnage’s own data that simple addition of protein to their system, even at low concentrations (and even if that protein is a Cry protein) protects from toxicity.
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-validated, non-guideline test with methodological and reporting deficiencies (e.g. dose concentrations in media not specified, no positive controls or controls that show the validity of the test system /and concentration range tested). Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:
Not relevant (Due to reliability)
Klimisch code:
3
Additional Comments
Glyphosate at 1 % had no effect on lactobacilli but did impair Geotrichum, which is unsurprising as glyphosate at herbicidal concentrations will impact metabolism of many fungi, which (like plants) use the shikimate pathway for aromatic amino acid production.
Surfactants are known to be bacteriostatic, with (for example) quaternary ammonium compounds typically being active in the 30-150 ppm range.
Clair et al. demonstrate that surfactants are bacteriostatic for 3 microorganisms at concentration ranges well within the range of concentrations generally found to be useful for sanitation purposes. However, surfactant solutions are routinely used to sanitize food processing equipment at concentrations at or above those tested by Clair et al. (2012, ASB2012-11592).
B.6.9 Medical data and information (Annex IIA 5.9)
B.6.9.1 Report on medical surveillance on manufacturing plant personnel
Industrial hygiene air monitoring data for glyphosate with workers at the Monsanto Luling, Louisiana manufacturing facility are available for the years 1981-1998 and have been submitted as part of the GTF dossier (no particular reference available). No such data are available from a Monsanto European manufacturing facility. Based on the measured low exposures to glyphosate in the manufacturing setting (well below the ADI) and low toxicological concern, glyphosate specific medical monitoring was not considered necessary by Monsanto. The following data are air concentration measurements which are conservatively applied as 100 % bioavailable to calculations of mean and maximum daily exposures.
Table B.6.9-1: Particulate exposures from glyphosate technical acid operations involving wetcake, e.g., supersack or container filling operations. Values are time weighted averages
Glyphosate Technical Dust (mg/m3)
Mean Daily

Exposure* (mg/kg/day)
Maximum Daily
Exposure* (mg/kg/day)
Sample Type
Samples
Range
Mean
SD
All
179
0.0003-0.2594
0.00647
0.0218
0.00108
0.04323
Personal
176
0.0003-0.2549
0.00655
0.022
0.00109
0.04248
Area
3
0.0008-0.024
0.00153
0.0008
1
0.00026
0.00400
Operator
158
0.0008-0.2594
0.00727
0.0235
0.00121
0.00393
Maintenance
16
0.0005-0.0053
0.00206
0.0014
4
0.00034
0.00088
Lab

2
0.0003-0.0004
0.00035
N/A
0.00006
0.00007
* based on breathing 10 m3 air/shift and 60 kg worker
Table B.6.9-2: Glyphosate isopropylamine salt liquid formulation bottling, drumming and tote filling operations. Values are time weighted averages
Glyphosate IPA Salt- Liquid Formulations (mg/m3)
Mean Daily Exposure**
(mg/kg/day)
Maximum Daily Exposure**
(mg/kg/day)
Sample Type
Samples
Range
Mean
SD
All
72
0.0001-0.47
0.085
0.105
0.01050
0.05804
Personal
58
0.0001-0.47
0.0251
0.106
0.00310
0.05804
Area
14
0.004-0.28
0.0932
0.105
0.01151
0.03458
Operator
54
0.0001-0.47
0.0966
0.11
0.01193

0.05804
Maintenance
4
0.0041-0.0088
0.00792
0.00187
0.00098
0.00099
** based on breathing 10 m3 air/shift and 60 kg worker and divided by 1.3496 to convert IPA salt to technical acid
Improvements in manufacturing facility containment and ventilation systems over recent years further reduce the likelihood of operator exposures within glyphosate manufacturing facilities.

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests
The summary in this section is based on well over 30 years of experience with numerous formulations of glyphosate in a wide range of situations. The extensive use of glyphosate has encouraged clinical assessment of various interventions and has resulted in reporting of alleged associations of symptoms with exposures to glyphosate products. The clinical toxicology of glyphosate and of glyphosate-surfactant formulations have been the subject of an extensive review (2004, ASB2012-11576), and a review of cases with assessment of clinical prognostic factors was more recently published (. 2008, ASB2012-11879).
Animals do not have the shikimic acid pathway; and no direct target-mediated mode of action in mammalian systems has been clearly identified to date (2004, ASB2012- 11576). Glyphosate does not inhibit the cholinesterases, and has no cholinergic effect. While incidental exposure in glyphosate-surfactant herbicide mixtures is common, review of available case reports (AAPCC 2003-2011) indicates that the vast majority of reported non- suicidal exposures involve skin and/or eye irritation or irritation of the respiratory tract by inhalation of spray mist, and that systemic symptoms are rare following non-suicidal exposures to glyphosate products. Based upon human experience and animal data, even those systemic symptoms reported following incidental exposure appear unlikely to be causally related to exposure (., 2002, ASB2012-11831).
The following clinical effects are divided into those expected following minor and significant exposures for each category based upon expected severity of systemic symptoms. The factors which determine if the exposure was minor or significant include:
• Route of exposure.
Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures for purposes of the symptom descriptions below. Ingestions more than 50 mL (one mouthful if amount unknown) of a product with >10 % glyphosate concentration may be significant.
• Concentration of the product.
Glyphosate concentrations of less than 10 % rarely if ever produce significant toxicity. Most serious illness has historically resulted from ingestion of the 41 % (glyphosate IPA) concentrate. In the absence of extensive clinical experience for the 11-40 % concentration range, any ingestion of greater than 50 mL of a glyphosate preparation having a greater than 10 % concentration of glyphosate salts should be considered potentially significant for purposes of the symptom descriptions below.
• Intent of the exposure.

Accidental ingestion rarely involves large quantities of concentrated formulations. Intentional ingestion cases may not present with a reliable history and may require observation if the amount ingested cannot be reliably determined.
Route and organ system specific syptoms of exposure:
Dermal
Minor exposures:
Contact with skin may produce a dermatitis similar to that of detergents (Bradberry et al., 2004, ASB2012-11576)
It is expected that the severity of injury following skin exposure will be significantly decreased with a less concentrated product and with a reduced duration of contact.
Phototoxic reactions (sunlight or ultraviolet (UV) light induced skin reactions) have been reported. These symptoms are believed to be due to an antimicrobial additive (benzisothiazolone) which is present in selected residential use (i.e. non- agricultural) products containing 10 % glyphosate or less (2004, ASB2012-11576).
Significant absorption through the skin does not occur (see also B.6.12). Significant exposures:
Skin exposures are not expected to cause systemic effects or serious cutaneous effects when the results of animals studies and the low dermal absorption are taken into account. There are no reports that would suggest the contrary. Symptoms as noted in the minor exposure may occur.
Ocular
Minor exposures:
A review of ocular exposures to US glyphosate-surfactant formulations (1513 exposures over a 5-year period), showed no permanent eye injury (1999, TOX2002-699).
Human eye exposures have generally resulted in temporary conjunctival irritation, clearing after irrigation or in 1-2 days and permanent eye damage is said to be “most unlikely” (2004, ASB2012-11576).
It is expected that the severity of injury following eye exposure will be significantly decreased with a less concentrated product or with a reduced contact time.
Significant exposures:
Eye exposures are not expected to cause systemic effects or serious ocular injury (1999; TOX2002-699, 2004, ASB2012-11576).
Systemic exposure – ingestion or inhalation
• Neurologic
Minor exposures:
There is no clinical or experimental evidence that glyphosate or glyphosate-surfactant formulations cause neurological symptoms or injury after exposure by any route.
Significant exposures:
There have been no reports of primary convulsions after ingestion.
One author reports most patients present with a clear sensorium unless another substance, such as alcohol, has been co-ingested or severe hypoxemia has occurred (1989, TOX9552426); however "moderate disorders of consciousness" have been reported within 48 hours of suicidal ingestions of the concentrate (, 1987, Z35531;
, 1988, Z35532). This has occurred in patients with significant systemic illness and is not believed to be the result of reduced organ perfusion (., 2004, ASB2012-11576) or perhaps other factors such as metabolic disturbance but the possibility of a direct toxicological effect cannot be excluded (., 2004, ASB2012-11576).
There are two isolated case report of Parkinson’s disease developing in individuals with a history of

glyphosate product exposure (2001, ASB2012-11557;
2011, ASB2012-12047). These publications are reported in detail with Klimisch rating in chapter B.6.7 (Neurotoxicity) under B.6.7.2 (Published data), because they are discussed there in context with other studies on neurotoxicity and on Parkinson`s disease.
• Gastrointestinal:
Minor exposures:
Minor exposures are likely to be asymptomatic, but the patient may experience an unpleasant taste, tingling, mild self-limited nausea and vomiting.
Self-limited diarrhoea may also occur which is thought to be due to the surfactant.
Significant exposures:
A burning sensation in the mouth and throat, salivation, oral erythema, sore throat, dysphonia, dysphagia, epigastric pain, nausea, spontaneous vomiting, abdominal pain and diarrhoea are common and may last up to a week.
Serum amylase may be elevated; isoenzyme analysis done in a few cases identified a salivary gland origin (1989, TOX9552426).
In severe cases with large ingested doses, hematemesis, GI bleeding, melena and hematochezia may occur. Paralytic ileus has been reported as a rare event.
Endoscopy has noted erosions of the pharynx and larynx, esophagitis and gastritis with mucosal oedema, erosions and haemorrhage. However, transmural injury and perforation have not been noted (1999, ASB2012-11510).
In fatal cases, autopsy notes mucosal or transmural oedema and necrosis throughout the small bowel with erosion and haemorrhage; in the large bowel, mucosal oedema and focal haemorrhage was noted (, 1989, TOX9552426).
Clinical, autopsy and experimental evidence (1987, TOX9552430) indicate a potential for gastrointestinal damage from glyphosate components of glyphosate formulations, but the frequency of severe injury appears to be low.

Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Cardiovascular effects are not expected to result from such minor exposures and no reports are available.
Significant exposures:
Hypotension is common after ingestions of a mouthful or more of the concentrated product (not the diluted forms) and usually responds to IV fluids and pressor amines. Shock as manifested by oliguria, anuria and hypotension which was unresponsive to fluids and pressors, ultimately resulting in death, has been reported. (1989, TOX9552426, 2004, ASB2012-11576). Transient hypertension may be noted.
• Upper respiratory: Minor exposures:
Dermal, eye and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Significant upper respiratory effects are not expected from minor exposures, but minor irritation or discomfort may occur (., 2004, ASB2012-11576).
Significant exposures:
Significant systemic exposures are not anticipated to occur via the inhalational route. However, if occurring, they would most probably also affect the lower respiratory tract (see below).
• Lower respiratory: Minor exposures:
Because of the non-volatile nature of glyphosate and the surfactant, exposures to vapour is not

possible. The spray equipment that is commonly used will produce particles that are non- respirable.
Significant exposures:
Tachypnea, dyspnea, cough and bronchospasm including cyanosis have been seen in severe ingestions (more than a mouthful of concentrated product). These effects are indiactive of systemic toxicity.
Aspiration pneumonia, pulmonary oedema and respiratory failure have been seen although the exact role of aspiration has not been fully investigated.
An isolated case report suggests the development of acute pneumonitis in a worker following his performing maintenance on non-operating spray equipment used to apply a glyphosate- surfactant formulation (1998, ASB2012-11513). However, actual exposure and its extent could not be really substantiated in this case. Accordingly, the occurrence of pneumonitis in this individual is more likely to be coincidental by nature although a (different) occupational origin seems plausible (1999, ASB2012-11511).
There is also a case report from Germany in which a glyphosate-surfactant product (tallowamine or "POEA" based) was applied by knapsack spayer in a 0.5ha forestry application at the registered application rate at 25° C for approximately 3 hours without
respiratory protection (Burger et al., 2009, ASB2013-11831). About 7 hours after application he developed chest pain with rapidly increasing severe respiratory distress and fever up to approximately 38° C. On hospital admission, radiographic changes of lungs could be demonstrated. To further assess possible causes, bronchoscopy and closed lung biopsy was performed. Histopathology revealed "toxic inflammation of the lungs" that was significantly different from bacterial infection. After 7-days of drug treatment, changes in lung reversed but six months after the incident, the patient still experienced moderate respiratory complaints on exertion. In the X-ray examination, there were still detectable lung changes although some improvement had been noted.

Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Renal effects are not expected to result from such minor exposures and no reports are available.
Significant exposures:
Hypotension and hypovolemic shock may result in oliguria and anuria, following severe ingestions (2004, ASB2012-11576). Abrupt rises in BUN and serum creatinine may be seen.
• Metabolic: Minor exposures:
Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Metabolic effects are not expected following minor exposures and no reports are available.
Significant exposures:
Mild fever may be noted even in the absence of infection (2004, ASB2012- 11576)
Metabolic acidosis is often seen in a severely poisoned patient 2004, ASB2012-11576) and this acidosis may fail to respond to bicarbonate therapy. Although the exact nature was not elucidated, a lactic acidosis was suspected.
• Hematologic: Minor exposures:
Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures.
Haematological effects are not expected from minor exposures and no reports are available.
Significant exposures:
Leukocytosis without evidence of bacterial infection has been noted in peripheral blood after

ingestion of the concentrate (, 2004, ASB2012-11576).
Hemoconcentration has been seen as a result of intravascular volume depletion and might indicate severe capillary fluid leakage (1989, TOX9552426).
No primary toxic effects on bone marrow or formed elements have been reported to date.
• Hepatic: Minor exposures:
Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Hepatic effects are not expected from such minor exposures and no reports are available.
Significant exposures:
No direct hepatotoxic effects have been noted; however, minor elevations in transaminases and bilirubin are reported (1989, TOX9552426; 2004, ASB2012-11576).
• Clinical chemistry (electrolytes): Minor exposures:
Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance. However, such signs are not expected following a minor exposure.
Significant exposures:
Electrolytes (Na, K, Cl and Ca) in the absence of renal failure generally remain normal. Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance.
POTASSIUM SALTS: While potentially toxic ingestions of all glyphosate products may result in fluid and electrolyte disturbances, particular attention to potassium may be important following ingestion of the potassium salt products. Close monitoring of serum potassium levels and/or electro-cardiographic monitoring (for peaked T-waves or rhythm disturbances) is recommended following significant ingestion of potassium salt products, particularly for high risk individuals. Individuals with the following may be at elevated risk following acute potassium exposure: known hyperkalemia, renal failure / renal dysfunction, use of potassium sparing diuretics, hypoaldosteronism, co-ingestion of other K+ containing materials, underlying heart disease, use of digoxin, digitoxin, oabain, or exposure to other cardiac glycosides. The quantity of potassium ingested from a glyphosate potassium salt product can be estimated from the weight percent of glyphosate potassium as:
Percent K+ salt x 5.3 = mEq potassium per 100 cc of product
Several case reports indicate that after ingestions of large amounts of glyphosate-potassium salt concentrate solutions, clinically significant hyperkalemia may occur. (2001, ASB2012-11556) reported an intoxication in a 65 year old female who ingested a glyphosate- potassium salt (350 mL Roundup Maxload missing from container, in addition to 250 mL of another glyphosate formulation which was not a potassium salt but amount actually ingested unclear) in a suicidal attempt. On admission, serum potassium level was 9.3mEq/L (typical normal value < 5) with electrocardiographic changes consistent with hyperkalemia. The patient did have a concomitant acidosis (pH 7.272) which may account for some portion of the elevation in potassium (acidosis displaces intracellular potassium). The patient responded to medical management and survived.
(2012, ASB2012-11863) reported the case of a 69 year old female who ingested approximately 500 mL of the same product. On arrival in the hospital, the patient had
hyperkalemia (10.7 mEq/L), pulseless ventricular tachycardia, and a severe metabolic acidosis (pH 7.005, will elevate potassium.) The patient required aggressive cardiopulmonary resuscitation and hemodialysis but did recover.
According to the GTF dossier (no particular reference given), Monsanto is aware of one additional (unpublished) case of a similar ingestion with dramatically elevated potassium level in which the patient was moribund when medical care was instituted. The patient could not be resuscitated. Because serum potassium levels rise rapidly following death (due to redistribution of intracellular

potassium), it is not possible to know how much of the observed hyperkalemia was the result of the ingestion versus profound acidosis and post mortem redistribution (which is partially due to acidosis).
It should be noted that the issue of hyperkalemia is limited to cases involving the suicidal ingestion of glyphosate-potassium concentrates. Potassium is a normal component of the human diet, and potassium intake attributable to occupational glyphosate-surfactant herbicide exposure will be negligible compared to typical dietary intake. While the concentrate formulations may contain up to approximately 250 mEq of potassium per 100 mL, product diluted for use (1 % glyphosate concentration) will contain about 6 mEq potassium per 100 mL. By way of reference, a medium size banana contains about 10 mEq (425 mg) of potassium.
Finally, it should be noted that the apparently very large (>150 mL) ingestions of glyphosate-surfactant concentrates observed in these cases are well within the range isopropylamine salt products reported to produce fatalities, and that elevations in potassium concentrations are reported (probably due to acidosis) following ingestions of glyphosate IPA salt products. While the cases do suggest that potassium salt products likely contribute to the risk of hyperkalemia, it is not clear at this time if the use of postassium salts will increase the overall clinical severity and/or mortality associated with glyphosate concentrate product ingestions.
Specific diagnostic testing and prognostic considerations
Serum or other body fluid measurements of glyphosate will be generally not available in a time frame that would be useful for acute clinical diagnosis. As the management of symptoms associated with glyphosate-surfactant product ingestion is symptom-driven in any event, the lack of rapidly available knowledge on concentrations of glyphosate will generally not impair clinical care. Levels may be more helpful in addressing forensic issues.
Attention should be paid to electrolyte concentrations in individuals with significant ingestion exposures, particularly to glyphosate-potassium concentrate solutions.
Respiratory distress requiring intubation, pulmonary oedema, shock (systolic BP <90 mmHg), altered consciousness, abnormal chest X-ray, ingestion of over 200 cc concentrate (41 %), or renal failure necessitating dialysis have been associated with a higher risk of poor clinical outcomes including mortality (2008, ASB2012-11879). A prognostic index based upon these factors was developed but its use is not expected to contibute significantly to improved medical care. As symptom onset may be delayed, early use of such prognostic indicators may in fact result in under-estimation of clinical severity of a case.
B.6.9.5 First aid measures
The following, quite general measures have been proposed by notifiers but were not evaluated by RMS toxicologists because this is beyond the scope of this RAR :
Skin exposure:
Remove all contaminated clothing and flood the skin surface with water. Wash the exposed skin twice with soap and water.
A close examination of the skin may be required if pain or irritation exist after decontamination.
All clothing that are contaminated should be laundered before they are worn again
Eye exposure:
Remove contact lens from the affected eye(s) if appropriate.
Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes.
Pour the water from a cup or glass held 3 inches from the eye.
A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably with an eye

specialist.
Ingestion exposure:
Dilute preparations (Glyphosate <10 %): An ingestion of a dilute preparation of glyphosate (<10 %) probably does not require treatment other than dilution with milk or water, and symptomatic care. Further gastrointestinal decontamination is not needed, even if spontaneous emesis has not occurred.
Concentrated (> 10 %) preparations: Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 mL/kg in a child or 250 mL in an adult.
Inhalation exposure:
No pulmonary treatment is necessary for occasional, accidental breathing of mist.
Severe, acute pulmonary injury has not been reported following inhalation exposure. Individuals with respiratory distress from any cause should be relocated (if medically stable) to fresh air and receive supplemental oxygen if available.
In the event of respiratory failure or lack of respiration, administer artificial respiration (or if pulse not detectable, cardiopulmonary resuscitation).
B.6.9.6 Therapeutic regimes
The following therapeutic regimes have been proposed by notifiers but were not evaluated by RMS toxicologists because this is beyond the scope of this RAR :
“The registrants believe that the following represent general best practices for medical management of serious ingestions of glyphosate-surfactant products.
Establish respiration and assure adequacy of ventilation. Eye exposure:
Remove contact lens from the affected eye(s) if appropriate.
Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.
A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably by an eye specialist.
Ingestion exposure:
Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 mL/kg in a child or 250 mL in an adult.
patient disposition:
Concentrated preparations (Glyphosate 41 % or greater):
Any person ingesting greater than a large mouthful (50 mL in an adult, 0.5 mL/kg in a child) of a 41 % or greater glyphosate concentrate product should be admitted to a hospital and observed for 24 hours.
Any adult ingesting greater than 100 mL of a 41 % or greater glyphosate concentrate product (>1.4 mL/kg in a child) should be admitted to the intensive care unit.
Any suicide attempt by person ingesting a concentrated product should be evaluated for psychological status and should be admitted if necessary for observation with suicide precautions.
Concentrated preparations (Glyphosate 10 %-40 %):
An ingestion of concentrated glyphosate (10 % - 40 %) will usually result in spontaneous emesis. There is limited experience with glyphosate formulations in this concentration range. In view of this limited information, the registrants currently recommend managing these ingestions in a manner similar to the management of the 41 % concentrate.
Prevention of absorption (This lists various methods for “Prevention of Absorption". These should

NOT be construed as being in order of preference. Consult with Poison Center or medical personnel to determine the need for and preferred method for decontamination. In many instances, no intervention is required.)

Gastric aspiration: If no significant spontaneous vomiting has occurred gastric aspiration may be considered. If performed soon after ingestion, gastric emptying by aspirating liquid gastric content with a lavage or standard NG tube may possibly remove some of the ingested glyphosate. The intent is to remove unabsorbed liquid by aspiration not to use lavage fluid. As absorption of liquids is likely to be relatively rapid, gastric aspiration after 1 to 2 hours is unlikely to be effective.

Emesis: Emesis is controversial at this time. Glyphosate/surfactant products are irritants. The registrants do not recommend the routine use of syrup of ipecac for glyphosate / surfactant ingestions because of the risk of exacerbating the irritant effects on the GI tract.

Activated charcoal: There are no data to support or refute the use of activated charcoal in glyphosate/surfactant product ingestions. Low molecular weight, amphoteric compounds and detergents do not always bind well to activated charcoal. In the event of a mixed ingestion, activated charcoal may be advisable.

Assessment of gastro-intestinal injury

Injury to the upper gastrointestinal tract may occur following ingestion of glyphosate concentrates. A study of upper gastrointestinal endoscopy following glyphosate–surfactant ingestions suggested that Zarger grade 2 lesions (erosions) were associated with longer hospital stay and with a higher incidence of serious complications (Chang 1999, ASB2012- 11510). However, no major esophageal or gastrointestinal injury was observed, and strictures have not been reported following uncomplicated glyphosate-surfactant ingestion.

Because no serious gastrointestinal injury is reported, and because the need for hospitalisation and/or treatment of complications can be determined without endoscopic evaluation, the registrants recommend that endoscopy be reserved for patients with co-ingestions suggesting a need for endoscopy or for patients with signs and symptoms suggestive of more serious injury (serious oral burns, inability to handle secretions, clinical obstruction) regardless of clinical history.

Monitor blood pressure:

Monitor the patient closely for signs of hemodynamic instability. The insertion of a Swan- Ganz catheter may be warranted.

Hypotension:

If the patient is hypotensive, administer IV fluid boluses and place in Trendelenburg position. If the patient is unresponsive to these measures, administer a vasopressor (dopamine, epinephrine, norepinephrine, phenylephrine, isoproterinol, etc.) if needed.

Monitor blood gases and obtain chest radiograph:

Consider the use of repeat blood gases and a peripheral pulse oximeter to monitor hypoxemia. Observe closely for sign of acidosis.

Pulmonary oedema:

Closely monitor arterial blood gases. If PO2 cannot be maintained above 50 mm Hg with inspiration of 60 % oxygen by face mask or mechanical ventilation, then positive end expiratory pressure (PEEP) or continuous positive airway pressure (CPAP) may be needed. Avoid a positive fluid balance by careful administration of crystalloid solutions. Monitor fluid status through a central venous line or Swan Ganz catheter as needed.

Acidosis:

Correction of acidosis should be guided by blood gases, electrolytes and clinical judgment. Attention should be directed to volume status and correction of poor perfusion in mild cases. Sodium

bicarbonate may be used to correct the acidosis in severe cases.
Hyperkalemia (from ingestion of Potassium salt formulations):
For moderate hyperkalemia (K+ of 6.0-7.0 mEq/L), administer sodium polystyrene sulfonate with sorbitol. For more severe hyperkalemia (K+ > 7 mEq/L) or serious complications of hyperkalemia, correct metabolic or respiratory acidosis if present to allow potassium to enter the intracellular space. Additional management may include a glucose/insulin drip, intravenous sodium bicarbonate or calcium, and dialysis to remove excess potassium.
Monitor renal function closely:
Assure adequate urine output. Catheterise severely ill patients. Hemodialysis may be needed in the event of renal failure or electrolyte disturbances.
Enhanced elimination:
Forced diuresis: Glyphosate is excreted very well by the kidneys. Adequate urine flow will ensure the rapid elimination of glyphosate. Although elimination may perhaps be enhanced by forced diuresis, there is no clinical evidence that this is necessary, and fluid overload may precipitate pulmonary oedema.
Hemodialysis: Hemodialysis may be useful to correct fluid, electrolyte and metabolic disturbances in the patient with renal failure. The institution of hemodialysis solely to enhance
the removal of glyphosate or other product components is not of proven benefit. Nevertheless, it is reasonable to consider the initiation of hemodialysis in the significantly ill patient who fails to respond to routine supportive management.
Serious exposure via inhalation is not expected:
Inhalation exposures are not expected due to the aerodynamics of droplet size from sprayers and because the product is not volatile. Monitor the patient for signs of respiratory compromise. Create an artificial airway if necessary. Check adequacy of tidal volume. Monitor the patient for respiratory distress; if a cough or dyspnea develop, evaluate the patient for respiratory irritation, bronchitis and/or pneumonia, but these are not expected.
Serious exposure via skin is not expected:
Significant skin exposures are not expected; however, the patient should be treated empirically if a dermal exposure is suspected. Remove all contaminated clothing and flood the skin surface with water. Wash the exposed skin twice with soap and water. A close examination of the skin may be required if pain or irritation exist after decontamination. All contaminated clothing should be laundered before wearing.
Laboratory:
Monitor electrolytes, especially if the patient is experiencing vomiting and diarrhea.15 Patients ingesting concentrated products based on the potassium salt of glyphosate may ingest large amounts of potassium (see calculations above). Observe serum potassium and/or electrocardiogram carefully. Patients experiencing pulmonary symptoms or having chest radiograph changes should have arterial blood gas monitoring. A peripheral pulse oximeter and a Swan Ganz catheter may be needed."
B.6.9.7 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion
Dermal exposure:
Skin irritation following exposure to glyphosate-only or glyphosate-surfactant materials is generally limited to topical irritation which will resolve within 3 days to 1 week following exposure. If exposure is aggravated by occluded conditions or physical abrasion, more severe skin injury with open skin injury may rarely result and may take longer to fully resolve.
Eye exposure:

Irritant symptoms generally resolve within 3-7 days of exposure. Most irritation is minor, but exposure to concentrate or the occurrence of a foreign body or of abrasions (from rubbing the eye) may result in corneal abrasion requiring topical antimicrobial therapy, often given in conjunction with topical corticosteroids and temporary eye patching to provide symptomatic relief. As noted above, a large study of (U.S.) ocular exposures to glyphosate-surfactant products demonstrated no long term eye injury.

Inhalation exposure:

Glyphosate-surfactant products generally do not contain readily volatile ingredients and thus inhalation exposure is limited to inhalation of agricultural droplets, which will deposit primarily in the upper airway. Resulting irritant symptoms will generally resolve within hours to a few days following exposure.

Ingestion:

Following minor or incidental ingestions, or ingestion of fully diluted formulations, gastrointestinal upset with nausea, vomiting, and diarrhoea may occur. Nausea and vomiting usually resolve within a few hours of ingestion. Diarrhoea may last for several days but is generally not severe. Following a major ingestion, the onset of systemic symptoms may be delayed by several hours. Fatalities due to cardiovascular failure are generally delayed by 12 – 36 hours. For serious but non-fatal cases, primary clinical injury generally is manifest within 72 hours but secondary complications such as infection or respiratory distress syndrome may supervene. The majority of serious but surviving cases will be fully recovered within 7-10 days of ingestion. Individuals with complicated hospital courses may require a more extended and highly variable time to recover.

B.6.9.8 Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment

The outcome of eye, dermal, and inhalational exposures, which are not expected to result in serious injury in any event, will not be significantly altered by delays in medical management. Similarly, minor oral exposures are symptomatically managed and unlikely to result in severe gastrointestinal symptoms. Medical management with intravenous fluids may provide some symptomatic relief in the event of dehydration, but recovery is anticipated in any event.

For serious ingestions having major electrolyte disturbances or life threatening alterations of cardiovascular performance, medical intervention may be life saving. Fortunately, as noted above, the onset of serious symptoms following ingestion is generally delayed by at least several hours, allowing for medical transport in all but the most remote or extreme circumstances.