DOCUMENTS ON GLYPHOSATE DRAFT ASSESSMENT REPORT

Section: Mammalian Toxicology (ECCO 78)

Comments

Date	Supplier	ECCO Ref No.
19 April 1999	Monsanto	6335/ECCO/PSD/99
19 April 1999	United Kingdom	6336/ECCO/PSD/99
19 April 1999	Netherlands	6337/ECCO/PSD/99
19 April 1999	Belgium	6338/ECCO/PSD/99
19 April 1999	France	6339/ECCO/PSD/99
19 April 1999	Greece	6340/ECCO/PSD/99
21 April 1999	Monsanto	6369/ECCO/PSD/99
21 April 1999	Cheminova	6370/ECCO/PSD/99
26 April 1999	ECCO 76	6373/ECCO/PSD/99
27 April 1999	Germany	6376/ECCO/PSD/99
28 August 1998	World Wide Fund for Nature	6006/ECCO/PSD/98

Other documents

NONE

Additional information considered at the meeting but not included in this report

Date	Supplier	Content	ECCO Ref No.
19 April 1999	Germany	Addendum to the Monograph	6341/ECCO/PSD/99

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Registration Department

Date : February 12, 1999

From : Hilde Van Parijs

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This fax contains page(s) (including this one). Please call Sender +32 2 776 4199, if you do not receive all the pages.

P.S.D.
15 FEB 1999
I.D. No. 54854

URGENT MESSAGE:

Re: EC evaluation of Glyphosate (according to regulation EC Nr 3600/92 and concerning inclusion of the active substance Glyphosate in Annex I of Directive 91/414)

Dear Sirs,

Attached is a copy of the Monsanto/Cheminova comments on the second draft of the Monograph on Glyphosate.

We have sent as well full copies (relevant appendixes + separate confidential folder) of the comments
- 4 copies to BBA
- 1 copy to PSD

Yours sincerely,

Hilde Van Parijs
Registration Correspondent

6335/ECCO/PSD/99

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PSD ref.: PRD 3624
Our ref. : RPG/hvp

Brussels, February 11, 1999

URGENT

Dear Sirs,

Re: EC evaluation of Glyphosate (according to regulation EC Nr 3600/92 and concerning inclusion of the active substance Glyphosate in Annex I of Directive 91/414)

Please find enclosed our comments on the second draft of the Monograph on Glyphosate. The major point from our review are:

a) We do not believe that Glyphosate is explosive.
b) An anaerobic metabolism study is not needed as the use on rice is to bare soil, before planting the rice.
c) Beneficial insect laboratory studies are affected by the "sticky nature" of the dried deposit.
d) We have a disagreement on the impact of Glyphosate on aquatic organisms.

We have done the best we can, within the time allocation for review, and look forward to further discussion on the data.

Yours sincerely,

R.P. Garnett
Registration Manager, Glyphosate

cc: ECCO-Team (PSD) - York

- attachments

GENERAL COMMENTS

We suggest that the evaluation should establish a principle for the acceptance of data. Some pivotal studies are utilised where a company's compositional data shows incomplete analysis, i.e. unidentified impurities. The dosing levels for glyphosate studies are so high that slight variations in manufacturers impurity patterns may contribute to the variation in toxicological results which can be seen in the monograph. We would recommend that to be used for any critical endpoint the study concerned should be classified as "Acceptable" and the test material adequately defined, in terms of purity, impurities and description.

There are two main manufacturing routes for glyphosate, one called the Glycine process and the other the IDA process. Although they both produce >95% pure material the impurity profiles are different, and because the toxicological studies have been carried out at extremely high dose levels the significance of the impurities increases.

In a similar manner glyphosate formulations are available with a wide spectrum of Risk and Safety phrases because the major source of toxicological impact comes from the additional components, not glyphosate itself. It follows that critical endpoints for glyphosate which are based on formulation studies, such as ecotoxicological studies, should be considered by the Member States when they assess specific formulations, provided, for the purpose of Annex I listing it can be demonstrated that at least one formulation is within the acceptable limits.

We have given as many comments as possible in the limited time available but may not have covered all the items. We will continue to work on the document.

We are discussing with the rapporteur the most appropriate means of supplying additional data that have become available since the submission was first made - See Appendix I.

Volume 1, Level 1, Statement of Subject Matter and Purpose of Monograph

1.3.7 Manufacturer or manufacturers of the active substance

We believe that a number of the "Manufacturers" quoted in the list are in fact traders and have no manufacturing facility of their own. If they are just traders should they be included in the process? As a minimum we would suggest they have to declare their sources and the process.

We would like to add the following source of Monsanto material to the list of manufactory plant:

MONSANTO SAO JOSE DOS CAMPOS PLANT
AV. CARLOS MARCONDES, 1200
12241-420 SAO JOSE DOS CAMPOS-SP
BRAZIL

TEL : +55 123 327100
FAX : +55 123 327199

1.3.10 Identity of isomers, impurities and additives

In conjunction with FAO we have submitted new specifications to comply with the new requirements for the establishment of FAO specifications. These will be considered at their meeting of 29th June 1999. As a result of the batch analyses carried out on material from our manufacturing plants, there are some slight amendments to our original submission. The new levels are disclosed in CONFIDENTIAL Appendix A.

1.3.11 Analytical profile of batches

As mentioned in 1.3.10 we have carried out recent analyses of our material and the results are attached in CONFIDENTIAL Appendix A - see section "impurity profiles Monsanto glyphosate".

1.5.4-1 Information on the approvals in the EU

Information on the approval in EU for Cheminova's glyphosate products is missing. This is now provided in Appendix J.

The tables for Austria and Belgium were missing from our copy but we assume this is just a copying error as they were included in the original draft.

We submitted the data below for Denmark on a previous occasion and the compounds noted below for France all have 10 year registrations. The product concentrations for Sweden and Greece should be amended as indicated.

Denmark			
Roundup 2000	48-9	SL 400 g/l	1991-1999
Marvel	48-10	SL 120 g/l	1991-1999
Roundup 480	48-15	SL 480 g/l	1991-1998
Roundup Spray	48-19	SL 7.2 g/l	1994-1999

France
Roundup Geoforce, Roundup Bioforce, Roundup360, Hockey Pro, Spasor A, Aristo TS, Durano TX, Honcho TS, Roundup Acti, Nomix Agri 2000, Roundup Alphee, and Ragtime -- all have 10 year registrations in France.

Sweden	
Roundup Bio	SL 360 g/l
Roundup Garden	SL 120 g/l
Roundup Spray	AL 7.2 g/l
Greece	
Roundup Armada	SL 90 g/l

1.5.4-2 Approvals in the EU

The "Details of Intended Uses", Table 1.5.3-1, should be amended with the following uses which are approved in the countries indicated (see also annotated table Appendix B):

Citrus Fruit	Application rate: 0.54-4.32 kg as/ha
Pome and Stone Fruit	Application rate for Southern Europe: 0.54-4.32 kg as/ha
Almonds	Add use in Greece as indicated in Appendix B
Rape Seed	Pre harvest: application rate 1.08-1.44 kg as/ha Harvest Management: application rate 1.08-1.44 kg as/ha Add Germany to countries
Linseed	Pre-harvest: add Germany to countries Harvest Management: application rate 0.36-1.08 kg as/ha Add Germany to countries
Winter wheat, durum wheat, barley, oats	Add use as indicated in Appendix B
Maize	Delete Greece
Sugar Beet	Delete Greece
Annual spring crops	Application: add inter-row equipment Application rate: 0.54-3.6 kg as/ha Max application per season: 3.6 kg as/ha
Stubbles	Spring use: add UK to countries, delete Greece
Stubbles of various crops	Add cotton to list of crops (Greece only)
Conifer sites	Application after finish of shoot elongation: • add SG to first line • second line: application rate 0.54-1.08 kg as/ha, UK only

Home & Garden	- First line (all EU countries): water l/ha 400-700 - Insert second line for watering can application in all EU countries: water l/ha 1600-5000, otherwise as for sprayer Add watering can in same way for SG formulation Ready to Use Italy: 0.72 kg/hl
Railway	Application rate: 0.54-4.32 kg as/ha
Drains	Amend drains to "Drains - only when dry" Add Greece below Italy
Other non-crop land	Southern Europe: application rate 0.54-4.32 kg as/ha
Flower, poplar, shrub, ornamentals	Add all EU countries
Glyphosate tolerant crops:	
Maize	Growth stage 1-10 leaves for N and S Europe
Soya bean	Max/season 2.16 kg as/ha (ie 2 x 1.08 kg as/ha)

2.4.1 Effects relevant to human health

Short Term Studies: Subacute and subchronic toxicity:

Comments concerning the statement "lowest NOEL values between 50 and 100 mg/kg/day, with the first effects occurring in the range of 250 - 300 mg/kg/day." (Volume 1, Glyphosate, Level 2 Section 2.4.1):

We believe that the Conclusion chapter (Volume 1, Level 2) should emphasise more clearly the conclusions reached in Volume 3 that the effects seen in the 24 subchronic toxicity studies, and also in eight chronic rodent studies, were all minor in nature. This position is established clearly in the individual study reviews in Volume 3, and in the conclusions in Section B.5.10.1, but the Conclusion Volume does not completely reflect the reviewers' comments in this regard. A distinction could be drawn more clearly between slight changes in body weights, organ, weights, or clinical chemistry levels, that need not be considered adverse effects, and true signs of toxicity observed at much higher dose levels. Typical NOAELs for many of these subchronic studies were 300 - 500 mg/kg/day. In the overview for Short Term Toxicity (Volume 3, Section B.5.3), which encompasses all the subacute and subchronic findings, the reviewer concludes "The lowest relevant NOAEL was 300 mg/kg bw/day for glyphosate acid as well as for the IPA salt." The purpose of Section 2.4.1 is to identify "Effects having relevance to human and animal health arising from exposure to the active substance..." therefore we consider that the minor effects observed at lower dose levels (below 300 mg/kg/day) should not be considered relevant to protection of human or animal health and the relevant parameter for this section to be the NOAEL.

Based on the above quotation from B.5.3, it appears that the technical reviewers agreed with our position, therefore Section 2.4.1 of the Conclusion Volume would more accurately reflect the data by emphasising that the minor effects seen below the 300 mg/kg bw/day level were not considered adverse, and that the lowest relevant NOAEL in short term studies was that level, as the reviewer stated.

Mutagenicity:

While we agree with the comments on the deficiencies of the micro-nucleus studies we would also highlight the following;

The dose used in this study was 5000 mg/kg once daily for two days. This would result in an exceedingly high cumulative dose of 10,000 mg/kg. Current guidelines recommend a limit dose of 2000 mg/kg. In addition there are two micronucleus studies listed in the review (Kier et al, 1992 and Jensen, 1991) with single doses up to 3,400 mg/kg and 5,000 mg/kg that are clearly negative.

The values for total percentage of erythrocytes with micronuclei in the Suresh study are not consistent with expected responses for negative and positive controls. For example the values for the positive control cyclophosphamide, administered at 100 mg/kg is unusually low at 1.58. In comparison the total percentage in the study by Jensen was 4.8 for cyclophosphamide dosed at 30% of the level used by Suresh.

Chronic toxicity and carcinogenicity:

See relevant comments above under Short Term Studies. We believe the Rapporteur's conclusions should clarify the distinction between minor effects or equivocal study findings that may indicate adaptations to dosing, but which should not be considered adverse. This distinction can be addressed by consideration of the many study NOELs, and selection of an overall NOAEL to represent the chronic findings. We believe the NOAEL of 60 mg/kg bw/day in the chronic rat study of Suresh (1996) is an appropriate reference point for this purpose.

2.4.2 ADI

Without access to the complete studies it is difficult to cover all of the issues which may arise, however we would make the following comments based on the information available in the review.

We feel that the highest NOAEL should be used to establish an ADI. This is distinguished from various study findings that may reflect and adaptation to dosing but which are not adverse. We agree that the alkaline phosphatase increase in females only at the mid-dose level in the Suresh (1996) study is a non-adverse observation (Volume 3, B.5.5.1.2). When discussing the alkaline phosphatase change, the reviewer states "Because concomitant liver pathology was lacking, this particular as well as the other, more equivocal changes, in clinical chemistry parameters were not considered adverse effects". Similarly, the sporadic minor effects on liver, kidney, and salivary gland weights, as well as clinical chemistry signs among the other chronic studies are inconsistent, are not clearly dose-related, and are not accompanied by evidence of degeneration or toxic damage. There is nothing compelling among these which constitutes a sufficient adverse effect. We agree with the Rapporteur's statement that "usually, a chronic study is considered most appropriate to derive the ADI". If this philosophy were followed, a selection of the 60 mg/kg/day dose in the Suresh (1996) rat study would seem the most appropriate chronic reference point.

Concerning the Rapporteur's assessment of the rabbit teratogenicity study reported by Tasker (1980) in Volume 3, Annex B Section B.5.6.2.2.2, the Rapporteur states "Intercurrent deaths were confined to the treated groups with a total number of one, two and ten rabbits in the low, mid and high dose group, respectively. For one mid dose and seven high-dose females, the cause of death could not be elicited and one can not exclude that these deaths were treatment-related. Thus, the low dose of 75 mg/kg bw/day was assumed to represent the NOEL for maternal effects rather than 175 mg/kg bw/day as proposed by the notifier."

Other regulators, including US EPA have reviewed these data and have not considered that these deaths were treatment-related have established the 175 mg/kg bw/day as the NOEL. Indeed, this study represents the basis of the Reference Dose (ADI) in the US, and it has therefore been considered especially carefully.

If the maternal mortality data from the pilot rabbit teratology study (Tasker, 1980, ref. 5.6.2/01 Monsanto Report number IR-79-018) is included with that from the definitive study (Tasker, 1980, ref. 5.6.2/02 Monsanto Report number IR-79-016), we can consider doses that are both higher and lower than the 175 mg/kg/day at which no deaths were observed:

DOSES MG/KG/DAY	0	75	125	175	250	350	500	1250	2500
DEATH FREQUENCY	0%	6%	0%	13%	0%	63%	80%	100%	100%

If the above data are further refined to eliminate the deaths for which a clear non-treatment related cause was identified at necropsy:

DOSES MG/KG/DAY	0	75	125	175	250	350	500	1250	2500
DEATH FREQUENCY	0%	0%	0%	6%	0%	44%	80%	100%	100%

From this latter table, it can be seen that there is no dose response in the frequency of treatment-related deaths below those at 350 mg/kg/day. The single death at 175 mg/kg/day dose should therefore be considered a spurious finding not related to treatment. Therefore the NOEL should be considered to be 175 mg/kg/day. We request that the Rapporteur reconsider this decision in the light of these additional data.

2.4.3 AOEL

We find the process the Rapporteur has used to establish a systemic AOEL interesting and outside our normal experience. The resulting figure (0.22 mg/kg/day systemic) must then always be paired with a figure for the dermal penetration of a given preparation to assess acceptability. The key is to ensure that the concept of systemic exposure is tightly linked to this AOEL figure, and We are concerned that this may not always be scientifically understood and followed by all regulators who may rely on the EU monograph as the basis for their regulatory decisions. For instance, in Section 3.1 (Volume 1, Level 3), the AOEL figure is described by the Rapporteur as 0.2 mg/kg bw/day without any reference to the need for the dermal penetration calculation. Similarly, in Volume 3 (1 of 4) B.5.14.3, when the Rapporteur is comparing existing worker reentry exposure data to the AOEL, the dermal penetration correction was not employed, and it was concluded "The comparison with the systemic AOEL of 0.2 mg/kg bw/day, proposed by the Rapporteur, shows that this value would be exceeded for a worker entering crops after spraying for a full working day." Had the established upper estimate for dermal penetration (3%, Volume 1 Section 2.4.1) been included, the opposite conclusion that reentry does not exceed the AOEL would be reached. This example illustrates how incorrect conclusions can arise from use of the approach proposed by the Rapporteur.

We request that the Rapporteur include a more detailed discussion of the process that should be utilised by Member States and others so as to help guard against possible misinterpretations. The Rapporteur suggests that dermal penetration is 3%, allowing that dermal deposition of a glyphosate formulation during application would be acceptable if it were 7.5 mg/kg bw/day or less. Perhaps the monograph should cite both figures for added clarification.

2.5.3 Methods for Residue Analysis

We agree with the Rapporteur's position that "glyphosate" is the only residue of concern in the great majority of situation involving plants and animal products. Section 2.6.1 properly clarifies that glyphosate-tolerant plants to which an enzyme (GOX) has been intentionally added as a mechanism of providing glyphosate tolerance can also contain significant residues of AMPA, and that MRLs may be required in those situations. The phrase "containing the GOX plant enzyme" could be added to the last sentence on the 3rd paragraph of 2.5.3 to further clarify when the method needs to allow for AMPA residue determinations.

2.6.1 Residue Definition

We agree with the Rapporteur's position that "glyphosate" is the only residue of concern in the great majority of situation involving plants and animal products. In glyphosate-tolerant plants, there may be a need to establish MRLs for AMPA in situations where this is the prominent or exclusive residue, in order to monitor for Good Agricultural Practice. We do not understand reason for the comment that MRLs may be needed for animal products, since AMPA is not of toxicological concern, and there is no GAP to monitor in this case.

2.7.3 Compliance with existing MRLs

The open positions have been closed for olives, beans, peas and mustard seed in 98. We will submit the data again to comply with directive 91/414/EEC - see Appendix H "reference list of submitted reports".

We believe that for soybeans and cotton tolerant to glyphosate, there is sufficient residue of glyphosate "per se" present in treated commodities to conduct safety evaluations and to determine whether GAP has been followed. There is no need to establish separate MRLs for AMPA in these cases. The need for AMPA MRLs should be limited to cases where the GOX enzyme, or a similar glyphosate-degrading enzyme, has been intentionally introduced, thereby converting most or all of the residue into AMPA.

2.10 Classification and Labelling

Glyphosate Acid:
The hazard group for glyphosate acid is recommended to be R50/R53: "very toxic to aquatic organisms" and "may cause long-term aquatic effects".

R50 is based on a 7-day EC_{50} of 0.6 mg/L for the marine algae *Skeletonema costatum* and is acceptable. However, We would like to point out that *S. costatum* represents an outlier, as it is one to several orders of magnitude more sensitive than the other algae or aquatic plant species tested. Toxicity values (EC_{50}) more representative for the majority of algae species are in the 1 - 10 mg/L range, which corresponds to an R51 label ("toxic to aquatic organisms").

R53 is triggered by bioaccumulative potential or persistence of a compound. In the case of glyphosate acid, we believe that the label does not apply:

- glyphosate tech. has a log n-octanol/water partition coefficient (log Pow) < 0, therefore no bioaccumulative potential (Section 2.8.2 and 2.2 of Volume 1, Level 2).
- glyphosate technical is not persistent. According to Commission Directive 93/21/EEC section 5.2.1.3, a compound can be considered readily biodegradable if "*(...) convincing scientific evidence is available to demonstrate that the substance can be degraded (biotically and/or abiotically) in the aquatic environment to a level > 70% within a 28-day period*". In a water/sediment study (Section B.7.6), over 70% degradation was shown in 30 days. Under natural conditions, glyphosate can therefore be considered readily biodegradable.

Based on the above considerations, we recommend changing the label requirements for glyphosate acid to **R50** alone.

List of endpoints

Monitoring data, if available - page 48

The four detections exceeding 0.1 mg/L in the UK have been investigated and are considered false positives - see Appendix I, SSLRC report number CON 82/4132.

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Biologische Bundesanstalt für Land- und Forstwirtschaft
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Attn. : Dr. H.H. Bruno and Dr.-Ing. H. Kohsiek
Messeweg 11/12
D-38104 Braunschweig
Germany

Your ref. : AP-WA1 004282-00

Brussels, 21-04-99

Dear Sirs,

Re: EC evaluation of Glyphosate (according to regulation EC Nr 3600/92 and concerning inclusion of the active substance Glyphosate in Annex I of Directive 91/414); Addendum to the monograph on glyphosate - Evaluation of possible mutagenic effects of formulations.

In response to the addendum to the monograph on glyphosate, please find enclosed Monsanto's and Cheminova's comments.

We decided not to combine our replies into one letter as our comments relate to different formulations.

Yours sincerely,

William Graham
Registration Manager - Glyphosate

cc : Mr. D. Flynn - ECCO-Team (PSD)
K. Lystbaek - Cheminova

Attachments

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Your ref. : AP-WA1 004282-00

Brussels, 21-04-99

Dear Sirs,

Re: EC evaluation of Glyphosate (according to regulation EC Nr 3600/92 and concerning inclusion of the active substance Glyphosate in Annex I of Directive 91/414);
Addendum to the monograph on glyphosate - Evaluation of possible mutagenic effects of formulations.

The Rapporteur has completed an excellent and thorough evaluation of all the data available on the mutagenicity of glyphosate formulations. Monsanto commends the authors for their completeness and scholarly assessment of the information. Monsanto agrees with the Rapporteur's conclusions that neither glyphosate technical nor the tested formulations show evidence of any genotoxic properties which are relevant for human risk assessment. These conclusions are accurately stated in points 1 to 3 in the Abstract.

Monsanto has one major correction, which our partners in this submission, Cheminova, should confirm. We believe that Berol 907, last paragraph of page 2, is a polyoxyethylene tallowamine. This information may affect the overall recommendations of the rapporteur but not the conclusions regarding the lack of genotoxicity.

The following specific correction might improve the understanding of the addendum:

The term "by-products" is used to describe other non-active substances included in the formulation, specifically surfactants. This term most often connotes impurities produced unintentionally during manufacturing. Monsanto recommends the terms "co-formulants" or "non-active formulation ingredients" to avoid misunderstanding.

Page 2, Brief Description of formulations tested: substituted the word "from" for the word "by" in the phrase "According to information obtained by Monsanto..."

Page 3, Table 1. 3rd entry row - there is an extra "t" following "MON 14445"

Page 19, 2nd sentence in Assessment. "MON 0118" is incorrect and should be "MON 0818."

Monsanto believes different wording is appropriate in the following places:

Abstract, Point 4. The last sentence implies that there have been "adverse effects on health and environment" arising from POEA surfactant. In fact, the only adverse effects of significance to the discussion have been observed in abnormal exposure situations, like attempted suicides, or in artificial test systems. Roundup formulations containing POEA surfactant have been used for 25 years very successfully throughout the world without adverse effects from normal usage. It would be preferable to state that "The available data indicate that the surfactant polyoxyethylene tallowamine (POEA) was linked with irritant properties of formulations and with cytotoxicity in certain in vitro laboratory test systems."

Page 13, under Clement. Sentence refers to 108 mg/L as a concentration below recommended application level. Roundup maximum application rate is 12 L/ha, roughly equivalent to 13.2 kg/ha of formulated product. Assuming that tadpoles live in water at least 30 cm deep, the immediate post-application concentration following a 12 L/ha treatment to 30 cm-deep water accompanied by thorough mixing in the water column is 4.4 mg/L. Concentrations of 27 and 108 mg/L are clearly in excess of those encountered in use situations. Monsanto prefers that the doses judged by Clements to cause DNA damage in tadpoles be described as "exaggerated concentrations that are not relevant to those under allowed use patterns".

Monsanto believes that the Rapporteur's Addendum should conclude following the first paragraph under the heading "Assessment".
The subsequent discussion of irritancy, toxicity, and intentional suicide attempts is outside the scope of the stated topic, since it is not relevant to mutagenicity.

The topic of genotoxicity is a very important indicator of characteristics of serious concern. This indicator is generally viewed as a positive / negative characteristic, using the weight of the evidence. The Rapporteur's report clearly addresses this topic, and concludes that the answer for glyphosate and its formulations is "negative". Irritancy, toxicity, and aquatic effects are quite different. They are clearly dose dependent phenomena which are expected for surfactants at high dose levels but which will disappear at more dilute exposure levels. For each formulation that is considered for regulatory authorization, a group of studies is conducted that is specifically designed to evaluate these properties for that particular preparation, in order to judge acceptability and proper labeling. The regulatory decision on individual formulations should be based on these required tests, and not on an a priori judgment that a certain component is too irritating or toxic, regardless of its concentration in the product concerned. There is no need to adopt such a position when the specific data to make a judgment will be provided.. Monsanto would prefer that the Rapporteur restrict the discussion to the topic in the title of the Addendum, and allow the individual Member States to judge the acceptability of irritancy and toxicity properties of individual formulations based on the specific required tests.

If the final paragraphs remain then Monsanto requests that the characterization of effects on humans who have intentionally ingested or aspirated Roundup formulations are called "Human suicide attempts" and not as "human poisonings". The present wording could be considered as inflammatory and is misleading. It is not until several sentences after the discussion begins later that the word "suicide" is used.

Monsanto believes that reference to the work of Martinez and Brown (1991) should be eliminated because of conflicting data (below) or modified to include this information.

It should be noted that in the study of Martinez and Brown (1991), no supporting mathematical analysis or other basis for the conclusion (possible potentiation) was presented. In a similar study, Adam et al (1997) investigated the oral and intratracheal toxicities of POEA, glyphosate, and Roundup herbicide. These authors concluded that there appeared to be no synergism with glyphosate and POEA. A study by Baba et al (1989) demonstrated a lack of synergism. In that study, oral LD_{50}s were determined in rats, and the interactions between glyphosate and POEA were systematically evaluated. The authors concluded that the interaction between glyphosate and POEA was antagonistic rather than synergistic. Heydens and Farmer (1997) used the harmonic mean formula of Finney to compare the "expected" and "observed" LD_{50} and LC_{50} values for rats and aquatic species exposed to several combinations of glyphosate with other herbicides and/or surfactants. Therefore, there is no reliable evidence indicating synergistic interactions between glyphosate and surfactant.

In the final paragraph, it is suggested that the replacement of POEA by other substances may reduce the risk of death or severe health effects. This statement is inappropriate in its present form because accidental ingestions of Roundup herbicide containing POEA surfactant have not resulted in deaths or other serious effects. Furthermore, there is no data to indicate that the intentional ingestion of other surfactants in the large quantities which occur in suicide cases would result in reduced mortality.

Yours sincerely,

William Graham
Registration Manager - Glyphosate
Monsanto Europe S.A., Brussels

cc : Mr. D. Flynn - ECCO-Team (PSD) - York
K. Lystbaek - Cheminova

References

Adam, A., Marzuki, A., Abdul Rahman, H., and Abdul Aziz, M. 1997. The oral and intratracheal toxicities of Roundup® and its components to rats. Vet. Hum. Toxicol. **39** (3) : 147-151.

Baba *et al.* 1989. Japanese Journal of Toxicology Vol. 2 No. 4, 397-400.

Heydens, W.F. and Farmer, D.R. 1997. Combination of toxicology of glyphosate with surfactants and other herbicides. Fundamental and Applied Toxicology, Vol. 36, No. 1, Part 2, page 341.

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Biologische Bundesanstalt für Land- und Forstwirtschaft
Abt. für Pflanzenschutzmittel und Anwendungstechnik
Attn. : Dr. H.H. Bruno and Dr.-Ing. H. Kohsiek
Messeweg 11/12
D-38104 Braunschweig
Germany

Your ref. : AP-WA1 004282-00

Brussels, 21-04-99

Dear Sirs,

Re: EC evaluation of Glyphosate (according to regulation EC Nr 3600/92 and concerning inclusion of the active substance Glyphosate in Annex I of Directive 91/414);
Addendum to the monograph on glyphosate - Evaluation of possible mutagenic effects of formulations.

In response to the addendum to the monograph on glyphosate, please find enclosed Monsanto's and Cheminova's comments.

We decided not to combine our replies into one letter as our comments relate to different formulations.

Yours sincerely,

William Graham
Registration Manager - Glyphosate

cc : Mr. D. Flynn - ECCO-Team (PSD)
K. Lystbaek - Cheminova

Attachments

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Messeweg 11/12
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Your ref. : AP-WA1 004282-00

Lemvig, 21-04-99

Dear Sirs,

Re: EC evaluation of Glyphosate (according to regulation EC Nr 3600/92 and concerning inclusion of the active substance Glyphosate in Annex I of Directive 91/414); Addendum to the monograph on glyphosate - Evaluation of possible mutagenic effects of formulations

Mutagenic potential of glyphosate formulations

We are in agreement with the clear conclusion that neither the active ingredient glyphosate nor glyphosate formulations assessed in the addendum have any mutagenic potential.

We enclose a document addressing the toxicological aspects of the addendum and the findings seen in relation to exposure as result of normal use of the products.

Berol 907

We can confirm that the composition of the Glifos formulation used for the Ames and micronucleus tests conducted in Brazil is identical to the composition of the Glyfos formulation marketed throughout the EU.

With reference to Table 1 on page 3 of the addendum we can furthermore confirm that Berol 907 in fact is a tallowamine surfactant.

With reference to the remark on page 2 of the addendum that acute toxicity studies were not submitted for the EU re-evaluation we can confirm that a full set of acute toxicity studies on Glyfos conducted under GLP are available.

In this connection it can be mentioned that Cheminova Agro for initial registrations throughout the EU developed an extensive Annnex III data package on the standard 360 g/l SI formulation (Glyfos) containing the tallowamine surfactant.

However, Annex III data on Glyfos was not included in the Monsanto/Cheminova dossier since Monsanto products were selected as representative products for the dossier.

Risk reduction recommendations

Referring to the conclusions of the abstract of the addendum, it is for risk reduction purposes recommended Member States to consider replacement of polyoxyethylene tallowamine (POEA) surfactants soonest possible and not to give new authorisations for PPP´s containing this surfactant.

We fully support risk reduction measures to be taken by Member States when unacceptable risks has been demonstated according to existing Member State regulations (for products awaiting Annex 1 listing of the active ingredient(s) or according to Directive 97/57/EC (for products for which active ingredient(s) have been included in Annex 1).

Glyfos is currently authorized for broad spectra of uses in all Member States.

We are very confident that all current uses of Glyfos with the possible exception of a few aquatic uses will be determined to be fully acceptable according to Directive 97/57/EC when Glyfos is being reviewed by the Member States following Annex 1 inclusion of glyphosate.

References

Data protection was mistakenly not claimed for the two Cheminova mutagenicity studies conducted with Glifos (Vargas, A.A.T. (1996) and Zaccaria, C.B. (1996)).

Please accept herewith our data protection claim for these studies.

We hope you find our comments helpful for the further evaluation process.

Yours sincerely,

Kristian Lystbaek
Cheminova Agro A/S, Lemvig

cc : Mr. D. Flynn - ECCO-Team (PSD)
W. Graham - Monsanto

Attachment

Dorrit Søndergaard
April 19, 1999

German proposal to replace POEA in glyphosate products.

The German call to member states not to accept glyphosate products containing POEA (polyoxyethylene tallowamines) is based on the alleged high cytotoxicity of these compounds as well as their contribution to an unacceptable general high toxicity of the products.

- One of the POEAs in question is Berol 907 from Akzo Nobel. The company indicates in the MSDS an acute oral LD_{50} of 1569 mg/kg bw, it is an eye irritant and skin effects are seen after prolonged contact due to defatting of the skin. The EU classification for health effects is indicated to be X_n with the R-phrases 36 (eye irritation) and 22(harmful when swallowed).

The numerous mutagen studies listed in the German evaluation do substantiate the claim that the cytotoxicity of the products containing POEA is higher than that of the active ingredient when the exposure to cells takes place in growth substrates as in the Ames test or the general toxicity is higher when they are injected intraperitoneally as in the Micronucleus test. These properties do to some extend interfere with the interpretation of the above mentioned studies.

Mutagen tests on products are not an EU requirement for approval of pesticides.
OECD states in its guideline for the micronucleus test, that if the results of the study should be used for risk assessment, the application should not be by injection but one relevant for practical conditions i.e oral or dermal.

The claim that the general toxicity is higher for the products is not quite as substantiated. Cheminova does for the time being sell 5 products within the community containing POEA. The acute oral LD_{50} is for all products $>$ 5000 mg/kg bw and the dermal LD_{50} is $>$2000 mg/kg bw. These data are based on limit tests for four of the products and estimation for the remaining one. The inhalation LC_{50}, 4 hours, is or the four of these estimated to be $>$ 4.86 mg/l.
This is to be compared with the acute oral LD_{50} and the acute dermal LD_{50} of glyphosate of $>$5000 mg/kg bw and $>$2000 mg/kg bw respectively also determined by limit tests. The acute inhalation LC_{50} for glyphosate is found to be 5 mg/l for an 4 hour exposure.

The acute intraperitoneal LD_{50} of glyphosate IPA salt when injected is $>$ 2000 mg/kg bw. for males and 1383 mg/kg bw. for females. The products do seem to be more toxic than the a.i. when injected intraperiotoneally.

The products are slightly to moderately eye irritating, not all of them classified as irritants, and not to slightly skin irritating, and none of them classified.
Glyphosate IPA salt, which is the form in which its occurs in CHA products, is neither an eye nor a skin irritant.

It can from this be clearly seen that the toxicological properties of the products containing POEA do not differ from those of the active ingredient, glyphosate, as far as acute toxicity is concerned with the exception of intraperitoneal injection. The oral and dermal studies to determine acute toxicity are related to the conditions of practical exposure and therefore relevant for human risk assessment.
The risk that an exposure into the abdomen should take place must be regarded hypothetical and could only happen in connection with serious accidents.

The fact that the general toxicity of the products is higher than that of glyphosate when injected intraperitoneally and the cytoxicity is higher than that of glyphosate when the exposure takes place in a growth medium has neither relation to practical use and thus nor to human risk assessment. This should therefore not serve as basis for a decision to ban the use the POEA as surfactant in glyphosate products.

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Dr Lundehn,
Biologische Bundesanstalt für Land und
Forstwirtschaft
Messeweg 11-12
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Braunschweig
GERMANY

24 March 1999
Our reference: ASY 43

Dear Dr Lundehn,

EC REVIEW MONOGRAPHS FOR GLYPHOSATE AND GLYPHOSATE TRIMESIUM

RAPPORTEUR - GERMANY

ECCO 78 - MAMMALIAN TOXICOLOGY MEETING

On behalf of the Pesticides Safety Directorate of the Ministry of Agriculture, Fisheries and Food, please find attached our comments on the monographs for glyphosate and glyphosate trimesium regarding mammalian toxicology. We are submitting these comments for your information as rapporteur and for discussion at the ECCO 78 meeting in April 1999. The section of the monograph dealing with exposure is being examined separately - if there are specific comments on operator/bystander exposure these will follow in a separate letter.

With regard to both substances, I have the following general comments from our toxicologist:

- it is considered that an excellent job has been done in summarising the large amount of toxicology data submitted for this review;
- the document generally provides the appropriate detail of key studies and draws conclusions from the wider data base with an explanation of why studies have been given greater weight than others;
- in nearly all areas PSD endorses the conclusions and proposals of the rapporteur - the few exceptions are presented within our attached detailed comments.

The following general technical comments are considered relevant to the submission:

Data applicability - It is noted that that the glyphosate monograph has 10 notifiers with a number of different synthetic procedures. There is variability in the size and quality of the toxicity dossiers submitted by these different notifiers. It is considered that it will be important to ensure that sources are comparable and that the basis for any extrapolations are clearly documented. For a compound such as glyphosate, which is comparatively of low toxicity, the

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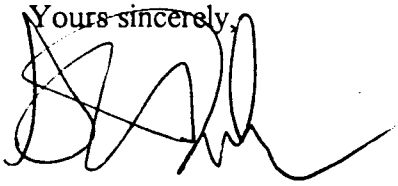
potential for an impurity to influence the toxicity is far greater than for the same impurity in an active substance of high toxicity. Some of these issues relating to the comparability of different sources and specifications will have been examined at the physical & chemical properties meeting, and may also need to be finalised during consideration at the Overview meeting.

Definition of doses/ADIs - In the glyphosate monograph, doses and the ADI appear to be based on the glyphosate ion with no contribution from the isopropylamine constituent. For glyphosate trimesium, it appears that the trimesium constituent has been included in the values given for achieved doses. Could the rapporteur comment on the above and, if correct, give the reasoning behind the apparently different approaches. In the assessments of diquat and paraquat, doses were presented as the *quat ion and there may be merit in adopting a consistent approach for all active substances which contain 2 ionic constituents.

Definition of the residue - As there is not much difference between the repeat dose toxicity of glyphosate and glyphosate trimesium, it would seem appropriate to simplify the residue definition to a single common one and delete reference to the trimesium component.

Our specific points are given in the attached appendices.

Yours sincerely,



S C DOBSON
cc: ECCO Team - PSD

GLYPHOSATE

VOLUME 1 (Report and proposed decision)

LEVEL 2: REASONED STATEMENT OF THE OVERALL CONCLUSION

2.4 Impact on human and animal health

page 7 The summary of short-term toxicity makes no mention of the studies on dogs yet comments on rat, mice and rabbit studies. This implies that one of the requirements of 91/414/EEC has not been met when in fact a range of dog studies exist. This should be clarified.

page 11 The final paragraph of section 2.4.4 regarding by-products/impurities is strongly supported. There could be value in highlighting this issue in any wording associated with Annex I listing..

VOLUME 3 [part 1 of 4] (ANNEX B)

B. 5 TOXICOLOGY AND METABOLISM

Section B 5.1

It is not clear from the text whether the ^{14}C- glyphosate used in the studies was labelled at a specific carbon atom or on all of them. However, as the metabolism of glyphosate in mammals is very limited the positioning of the label would not have a significant effect on the overall results.

The limited (<10%) transfer of radiolabel from an i.v dose to the faeces (B 5.1.1, page 8) indicates a study of biliary excretion of glyphosate would be of no value to the overall risk assessment.

Section B 5.3.2.1.1 (page 43)

The rapporteur has proposed a NOAEL of 300 mg/kg bw/d for this study. However there is clear evidence of effects on the salivary glands at 30 mg/kg bw/d, though whether this relates to an adverse finding is unclear. Such effects were seen in a number of studies and subsequent work confirmed a mechanism for such alterations (section 5.8.2.2; page 123). The rapporteur should thus provide a more extensive argument for setting aside the findings at 30 and 300 mg/kg bw/d - possibly by reference to historic control data or longer term studies.

Section B 5.4.2.1 (page 73) - Suresh 1993

The rapporteur's concerns about the relevance of this study are supported. In addition, it is of note that the incidence of micronuclei in PCE from controls (>0.5%, Table B5.4.2.1-2) is considerably higher than that normally reported in such studies (<0.2%) possibly indicative of some underlying problem within the test animals.

Section B 5.5.1.1 (page 83) - Atkinson et al, 1993

The incidence of parotid salivary gland alteration in low dose females is just statistically significant (p=0.038 by Fisher exact test 1-tailed) and it would be of value to know if any changes in severity had been recorded. Assuming no increase in severity at 10mg/kg bw/d, the rapporteur's proposal to use this as a NOAEL is acceptable.

Section B 5.5.1.2 (page 85) - Suresh 1996

The changes in alkaline phosphatase (AP) levels are all <2 fold. In the absence of histological correlates the variations in AP are not considered adverse.

Section B 5.6.1.1 (page 96) - Reyna 1990

The slight decrease in litter size at 30000 ppm (Table B5.6.1.1-1) may well be a non-specific effect related to reduced body weight in dams. As pup weights at birth in this group are higher than controls, to compensate for fewer pups, the findings up to day 4 are not considered indicative of any specific effect on reproduction.

Section B 5.6.2.1 (page 105) - Brooker et al, 1991

The lack of clear dose response for most of the findings in Table 5.6.2.1.1-1 and unusually low concurrent control values indicates that 1000mg/kg bw/d could be interpreted as a NOAEL. However, given the overall results at this dose, the rapporteur's proposal of 300 mg/kg bw/d as the NOAEL is supported.

Tetratogenicity studies in rabbits

- **Section B 5.6.2.2.1 (page 110) - Suresh 1993**

  The increased incidences of abnormalities shown in Table 5.6.2.2.1-1 are of concern, particularly the heart effects which are also reported in other rabbit studies with glyphosate (page 111, last para and table 5.6.2.2.2-3). The interpretation of this finding must rely on comparison with historical control data. If the typical incidence is approximately 5 fetuses per group then there is no concern. However if this is a very rare finding in control animals and the concurrent controls for this study are typical then there are concerns regarding the potential fetotoxicity of this source of glyphosate.

- **Section B 5.6.2.2.2 (page 111) - Brooker et al, 1991**

  The increased levels of embryonic death/post-implantational loss at all dose levels are of concern, as are the reports of heart defects. PSD considers that a more robust argument should be presented before these findings can be dismissed and a NOAEL set for this study.

- **Section B 5.6.2.2.2 (page 112) - Bhide & Patil, 1989**

  Another study with equivocal evidence of heart defects.

- **Section B 5.6.2.2.2 (page 112) - Anonym 1981**

  Though this study is questioned (bottom of page 103) for showing evidence of fetotoxicity at lower doses than other studies, the study by Brooker (see above) may also indicate fetotoxicity at 50 mg/kg bw/d.

Conclusion - tetratogenicity studies in rabbits
Taken in isolation, none of the findings in these rabbit teratology studies would be clearly of concern. However, overall there is an indication of a pattern. As the toxicity of glyphosate is minimal, the ECCO 78 meeting may wish to focus on the findings of these rabbit studies as they may impact on the ADI and AOEL. Discussions may be aided by comparison with the applicable historical control data for both fetal viability and heart/cardiovascular abnormalities. The rapporteur should ensure that such data are available.

Section B 5.7

The monograph indicates that glyphosate is not an organophosphate (line 5). Technically this is an incorrect statement. Though glyphosate contains an organic component linked to a phosphate group (possibly the reason for some of the neurotoxicity studies) the structure is such that it would produce no or minimal inhibition of acetylcholinesterase activity (the main concern for insecticidal organophosphates). We consider that this should be clarified and recorded at the ECCO meeting.

Section B 5.8.1.1 (page 115)

The most sensitive target organs for glyphosate were the salivary glands (especially the parotid). The rapporteur comments on line 10 of page 115 that AMPA did not cause such effects. It may be useful to give this statement greater prominence by including appropriate text in Volume 1 section 2.4.1 page 8.

Section B 5.9.5

The text on poisoning/suicide cases indicates 'Roundup' may be classifiable as "harmful if swallowed" based on human data. The use of human poisoning data in classification should be considered with great care. Though the reports require no extrapolation between species, in most instance the volume/mass of exposure is not known with any certainty. In the case of 'Roundup' the reports are from outside the EU and formulation details may differ from those approved in Europe. The classification of glyphosate formulations for acute oral toxicity should thus be based on animal studies performed on the formulations to be marketed.

Section B 5.10

Before the **ADI and AOEL** can be agreed, the issues identified in paras 7 and 9 (salivary gland changes) and para 13 (fetotoxicity) should be discussed. If the rapporteur's conclusions on these issues are supported, the proposals for ADI (page 136) and systemic AOEL (page 138) are acceptable.

Section B 5.11 - preparation toxicity

The toxicity of glyphosate preparations seems to be very dependent on co-formulants.

The rapporteur's comments on lines 6-8 of page 140 on the need for member states to address the individual formulations are endorsed.

The rapporteur should check **Table 5.11-7** as the entry for **AGC herbolex** indicates it is not a sensitiser, but other parts of the document (page 140 and table 5.11.3-1) indicate it is a sensitiser.

As some other glyphosate formulations merit R41, it would seem equitable to apply this classification to **Glifogarde (page 163)** where no data on eye irritancy are available. The notifier then has the option to present a case to support a lesser classification (R36) if appropriate.

PSD supports the rapporteur's proposal for **Luxan glyphosate (page 165).** The eye lesions are severe and not reversible at 21 days (study termination?) and meet the criteria for R41.

The description of the eye irritancy for **Glyphosan (page 167**) indicate severe early lesions which had not fully resolved by day 21 (termination?). This appears to meets the criteria for R41 and the rapporteur should justify the proposal of R36.

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Briefnummer 99/1491 JJM/IVH
Behandeld door ir. J.J. Meeussen *doorkiesnummer* 471858
Uw kenmerk
Datum 31 maart 1999

Betreft Comments on the draft-monograph of the active substance **glyfosate**
Ecco-meeting 78
Section: Mammalian toxicology

The Board for the Authorization of Pesticides (CTB) has received the draft monograph of the active substance glyfosate.

Please find enclosed the comments of the CTB with respect to the draft-monograph as prepared by Germany.

These comments also have been sent to the ECCO-team.

Yours sincerely,

Dr. J.S.M. Boleij
(secretary of the Board)

enclosure

cc: ECCO-team (PSD)
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KOPIE

Accoord.

EU Review programme on active substances in Plant Protection Products

GLYPHOSATE

Rapporteur: Germany

Comments of the Netherlands on Toxicology and Metabolism
and Classification and Labelling

Author : C. de Heer and J.J. van Hemmen
Report number : 99-019-F-262
CTB number : 99/0306
Date : 18-03-1999

Volume 1, Level 2 and 4

2.4 Impact on human health

Adjustments should be made according to the comments made on the summaries in Volume 3, Annex B (see below).

In addition, additional studies on the relevance of the histopathological changes in the salivary glands should be requested.

Volume 3, Annex B

B.5 Toxicology and metabolism

General comment: Histopathological changes (described as 'cellular alteration') in the salivary gland were observed in some semichronic toxicity studies in rats and mice, in a chronic study in rats, and in a 2-generation study in rats. Moreover, in rats these cellular alterations were observed in 2 strains. Furthermore, it is noted by the rapporteur that due to the generally limited extent of histopathological investigation of salivary glands, it is questionable whether the indicated effect will be detected in routine toxicological testing. It is not clear to the reviewer whether in studies in which effects on the salivary glands were not found, this endpoint was adequately addressed. When adequately addressed, however, histopathological changes in the salivary gland were consistently observed. Nevertheless, the toxicological significance was judged equivocal and established NOAELs were based on other effects generally occurring at higher dose levels. It is the reviewers' opinion that the rationale for not taking the salivary gland effects into account with setting the NOAELs is insufficiently documented.

In general, the rapporteur establishes NOELs and no NOAELs. It is assumed by the reviewer that these values are comparable for the present studies, i.e. the NOELs are used for risk assessment purposes.

B.5.1 Absorption, distribution, excretion and metabolism

No comments on the summaries and evaluation of oral ADME studies. For comments on the establishment of percutaneous absorption see section B.5B.5.12

B.5.2 Acute toxicology including irritancy and skin sensitization

Acute toxicology
No comments.

Irritation
No comments.

Sensitization
No comments.

B.5.3 Short-term toxicity

See general comments. If the histopathological changes in the salivary glands are judged as toxicologically significant, the lowest relevant oral NOAEL derived from 90-day oral studies in rats is <30 mg/kg bw/d. In contrast, the lowest relevant short-term NOAEL established by the rapporteur is 150 mg/kg bw/d, based on clinical chemistry findings and decreased body weight gain in a 90-day oral study in rats.

B.5.4 Genotoxicity

No comments.

B.5.5. Long-term toxicity and carcinogenicity

See general comments. If the histopathological changes in the salivary glands are judged as toxicologically significant, the lowest relevant oral overall NOAEL derived from chronic oral studies in rats is 10 mg/kg bw/d. In contrast, the relevant long-term NOEL established by the rapporteur is 31 mg/kg bw/d, based on an overall assessment of the chronic studies in rats (liver, stomach and eye effects).

B.5.6 Reproduction toxicity

See general comments. If the histopathological changes in the salivary glands are judged as toxicologically significant, the lowest relevant parental-NOAEL derived from 2-generation studies in rats is 80 mg/kg bw/d. In contrast, the parental NOEL established by the rapporteur is 237 mg/kg bw/d, based on lower body weights and higher food consumption in this 2-generation study in rats.

B.5.7 Neurotoxicity

No comments.

B.5.8 Further toxicological studies

It is noted by the reviewer that effects on salivary glands were also noted in a 2-generation reproduction study in rats (Brooker et al. 1992 in B.5.6.1.2), in addition to the studies referred to by the rapporteur (one long-term study in rats and some subchronic studies in rats and mice). The remark by the rapporteur that due to methodological reasons, histopathological changes in the salivary glands would not be easily detectable in routine testing is considered of great importance. Since studies in which the salivary glands were adequately examined consistently yield lower no-effect levels, it is the reviewers opinion that these effects, although for now of unknown biological significance, should be included in the risk assessment. The rationale for not taking these effects into account is poorly documented (see also general comment).

B.5.9 Medical data

No comments.

B.5.10 Summary of mammalian toxicology and conclusion

B.5.10.1 Summary of mammalian toxicology

Adjustments should be made according to the comments made for the individual summaries. It is noted that although effects on the salivary glands are mentioned as test substance related and apparently toxicologically relevant, they are not included in the establishment of the ADI or AOEL mainly because they were not consistently found. It is however not clear whether in the studies in which histopathological effects on the salivary glands were <u>not</u> found this endpoint was adequately addressed (see general comments).

B.5.10.2 Acceptable daily intake (ADI)

No comments other than indicated in B.5.10.1. If the histopathological changes in the salivary glands are judged as toxicologically relevant, the ADI should be based on the NOAEL of 10 mg/kg bw/d derived from a chronic oral study in rats. Application of a safety factor of 100 results in an ADI of 0.1 mg/kg bw/d.

B.5.10.3 Acceptable operator exposure level (AOEL)

The rapporteur established an internal AOEL of 0.2 mg/kg bw/d for short-term exposure based on a teratogenicity study in rabbits (NOAEL 75 mg/kg bw/d) and using a safety factor of 100 and correction for incomplete (30%) oral absorption.
Given the anticipated exposure scenario (including application in greenhouses), the reviewer considers it more appropriate to use the NOAEL derived from the chronic toxicity study in rats (31 mg/kg bw/d) as a starting point for establishment of the ECCO-AOEL. Use of the indicated safety factor and correction for incomplete absorption results in an AOEL of 0.1 mg/kg bw/d.

The method for the derivation of the AOEL used by the Dutch reviewer is described in a guidance document[1] prepared by TNO. The main differences with the method of the rapporteur are:
- the assessment factors applied;
- the corrections made for incomplete dermal absorption;
- the establishment of an internal and an external (route specific) AOEL;
- the starting point for the AOEL

According to the reviewer, a frequent exposure of contractors throughout the year cannot be excluded for glyphosate and therefore the derivation of an AOEL for long-term exposure in considered justified.

The dossier contains a short-term dermal toxicity study in rats (NOAEL >1000 mg/kg bw/d). The dermal study is considered suitable for the derivation of an AOEL-dermal because (1) the rat is considered to be a sensitive species, and (2) reliable route specific studies are preferred instead of route-to-route extrapolation.
The lowest relevant NOAELs was observed in the 2-year oral toxicity study in rats (31 mg/kg bw/d). This study is used as a starting point for the AOEL-dermal as well as the AOEL-respiratory. The calculations of the dermal and respiratory AOEL's are given in the table below:

NOAEL	oral 2-yr study in rats (31 mg/kg bw/d)	dermal 21-day study in rats (>1000 mg/kg bw/d)
Assessment factors:		
-interspecies[1]	4 x 3	4 x 3
-intraspecies[2]	3	3
-exposure time[3]	1	10 x 1
-dose response	1	1
-confidence of database	1	1
-critical effect	1	1
Correction factors:		
-%oral absorption	30	-
-%dermal absorption[4]	3	-
-%inhalatory absorption[5]	100	-
Results:		
-internal AOEL[6]	0.26 mg/kg bw/day 18 mg/worker/day	- -
-external dermal AOEL[6]	8.6 mg/kg bw/day 603 mg/worker/day	>2.8 mg/kg bw/day >194 mg/worker/day
-external inhalatory AOEL[6]	0.26 mg/kg bw/day 18 mg/worker/day	- -

1 Two factors are used for interspecies differences, one that accounts for the differences in caloric demand (4 and 2.4 in case of rats and rabbits, respectively) and a factor for the remaining differences between species (3).

[1] Rennen, M.A.J., Van de Gevel, I.A., De Heer, C., Hakkert, B.C. (1999) Occupational risk assessment of pesticides; Method used in the Netherlands for the setting of acceptable operator exposure levels for active substances, TNO report no. V99.324, TNO Nutrition and Food Research, The Netherlands, March 1999.

2 A factor 3 is used for the worker population because it is assumed that they are more homogeneous than the general population.
3 The factor for exposure duration is based on comparison of available data on subacute, semichronic and chronic toxicity data. Due to a lack of data a factor 10 is applied for extrapolation from subacute to semichronic exposure, whereas a factor 1 is applied for extrapolation from semichronic to chronic exposure based on repeated-dose toxicity data in rats and mice.
4 See comments B.5.12
5 Default value of 100%, no data available.
6 Assuming a body weight of 70 kg for the average worker.

Since uncertainties have been introduced in both the direct as well as the route-to-route extrapolations, for safety reasons the lowest AOEL is used in the risk assessment process. Therefore, these calculations result in an internal AOEL for long-term exposure of 0.26 mg/kg bw/day, which is equal to 18 mg/worker/day, when assuming a body weight of 70 kg. The external AOELs for long-term dermal and respiratory exposure were found to be 8.6 mg/kg bw/day (equal to 603 mg/worker/day) and 0.26 mg/kg bw/day (equal to 18 mg/worker/day), respectively.

It is noted that if the use of a short-term AOEL can be justified, it is the reviewers' opinion that the AOEL should be based on the NOAEL derived from the teratogenicity study in rabbits. This results in an AOEL for short-term exposure that is approximately 4-fold higher than the AOEL for long-term exposure.

If the effects on the salivary glands are judged toxicologically relevant, the AOEL established for long-term exposure will be 3-fold lower. The AOEL established for short-term exposure should in that case be based on the LOAEL of 30 mg/kg bw/d derived from a 90-day oral study in rats.

In ACCA-TNO report 98-085-C-262 (dd 20-11-1998), the risk assessment was based on a JMPR evaluation which comprised a smaller database. Hence, there were some differences in the toxicological database. The AOELs established were 725 mg/day and 8 mg/day for dermal and inhalatory (long-term) exposure, respectively. These AOELs were based on a LOAEL of 205 mg/kg bw/d for salivary gland lesions derived from an oral semichronic study in rats.

B.5.10.4 Drinking water limit
No drinking water limit was set by the rapporteur. According to the reviewer, the EU drinking water limit for pesticides of 0.1 µg/l drinking water is applicable for glyphosate.

B.5.11 Acute toxicity including irritancy and skin sensitization of preparations
Not evaluated by the reviewer.

B.5.12 Dermal absorption
Based on the data presented in the monograph, the reviewer agrees with the dermal absorption of 3% established in Rhesus monkeys (see monograph section B.5.1.3. Wester et al., 1991). It is noted however that, apparently based on the same study, ACCA-TNO concluded that dermal absorption ranged from 3.7 to 5.5% (based on comparison of urinary excretion following intramuscular (monograph i.v.?!) and dermal application). ACCA-TNO subsequently used a value of 5.5% for dermal absorption. It was noted that it was not clear whether the dermal absorption was studied under occlusion.
It is noted that the quantitative use of *in vitro* dermal absorption data, as proposed by some of the notifiers, is hampered by an adequate validation of different test methods. Therefore, if adequate, these data should only be used semi-quantitatively.

B.5.13 Toxicological data of non-active substances
No comments.

B.5.14 Exposure data

Summary of the exposure analyses
The material presented by industry to the national authority was not available to the reviewers.
- The described formulations of this herbicide are SL and SG.
- For the estimation of operator exposure field studies were presented, as well as some exposure data from the literature.
- For the estimation of exposure of operators, the national authority of Germany uses the German and the UK model.
- For workers and bystanders no exposure estimates have been proposed considering the unlikely or relative low exposures. One of the many notifiers presented exposure some data for workers.
- Experimental data on dermal absorption are obtained from *in vitro* and *in vivo* studies. A dermal absorption value of 3% is used in the risk assessment.

Criticisms on the presented exposure assessment
- The EUROPOEM and Dutch model for mixer/loaders and applicators are not used.
- The data obtained with the German model, using the geometric mean, are not typical for the actual field conditions. By no means do they describe a reasonable worst case of exposure, nor a typical exposure.
- It seems appropriate to use the available European model EUROPOEM for the mixing/loading and the application. For the Dutch approach see the annex.
- The descriptions of the field studies are not very detailed, making it difficult to estimate the value of the exposures for the present risk assessment.

Recommendation
- No specific recommendations. The exposure analysis has a reasonable standard.

[Comparison with the evaluation made for the CTB by TNO in 1998:
- A dermal absorption value of 5.5% was used.
- TNO used a representative field study, for estimating the levels of exposure with liquid formulations for estimation of dermal exposure.]

B.9 Proposals for the classification and labelling

B.9.1 Proposals for the classification and labelling of the active substance

No comments.

Annex

SHORT DESCRIPTION OF THE GENERAL APPROACH TAKEN IN THE NETHERLANDS FOR THE ESTIMATION OF OPERATOR/WORKER/BYSTANDER EXPOSURE TO PESTICIDES FOR EU MONOGRAPHS (COUNCIL DIRECTIVE 91/414/EEC)

Summary of Dutch method

If no adequate field studies are available for estimating exposure, predictive models are used.

- Operators

For mixing/loading and application the databases of the European model EUROPOEM are used only, when a database is of adequate size, i.e. sufficiently large for the choice of the 75th percentile for chronic exposure estimates. If this is not the case, the three available national (European) models are used and the results compared, considering that an estimate is required for potential exposure (no protective measures, i.e. normal work clothing) under reasonable worst case conditions, i.e. about the 90th percentile of the for Europe less accurate national exposure database sets. The relevant data are compared and the median of the three estimates is taken as surrogate for risk assessment, again for chronic exposures.

- Workers

For re-entry activities a model is used based on the scientific literature in which potential dermal exposure is directly related to the amount of dislodgeable foliar residue on the crop, a transfer factor and exposure time.

- Bystanders

For bystanders no suitable model is available. Exposure will be estimated on basis of expert judgement.

Introduction

Generally, operator exposure to pesticides occurs during mixing, loading and application of pesticides. During some activities bystanders might be present and therewith be exposed. After application it may be necessary to handle crops or crop products in such a way that exposure to the workers may occur due to contact with pesticide residues.

For the present purpose the potential exposure will be estimated for an unprotected worker, i.e. wearing normal work clothing, without additional protective measures. The degree of protection required depends on the detailed conditions at work, which may depend on various variables, and which in the context of an EU-monograph cannot be considered in detail. For the bystander even normal work clothing may be an over-estimation of the degree of clothing.

1 Operator exposure

Representative and well-designed field studies with the compound under consideration should form the basis for an adequate exposure assessment (surrogate exposure value(s)). In case such studies are not available the level of occupational exposure must be estimated using appropriate modelling systems. Exposure estimates can be derived using the published models which reflect European conditions. For the present purpose the results of these models will be used for the above-mentioned potential exposure of mixer/loaders and applicators (operators). Presently, the European Predictive Operator Exposure Model (EUROPOEM) is operative, though not optimal for all scenario's. The databases for which the chosen surrogate value in EUROPOEM is based on the 75th percentile, form the best available estimate for chronic exposures in Europe (EUROPOEM, 1997).

When a proper European database is not available, the considered models are the German model (Lundehn et al., 1992), the UK model (PSD, 1992) and the Dutch model (Van Hemmen, 1992). It should be noted that these models have different underlying assumptions, different underlying databases and use different statistics (about the 75th percentile for the UK, indicative 90th percentile for the Netherlands and the geometric mean for Germany) and formats.

The choice of the statistic is especially important, since the variations in actual practice for the level of exposure are large for many reasons, such as work practices, climatic conditions, variations in equipment and especially personal hygiene. For this reason the calculations with the German model will be done with the geometric mean as well as the 90th percentile. The consideration here is that the underlying studies for the UK and German model are not publicly available for consideration, have not been considered according to basic and explicit criteria, as has been done for EUROPOEM, and that the studies have especially local

(national) value, which may not give the required spread for European applications. A more in-depth analysis of the use of different models, not including EUROPOEM, has been published (Van Hemmen, 1993).
For the calculations with all models it will be assumed that a person has a typical weight of 70 kg.
For inhalation exposure the models are applicable for compounds with relatively low volatility (up to 10-100 mPa) at ambient temperature, according to e.g. the Pesticide Manual. When granules have to be considered and an adequate database is not available, it is assumed that the dust content is 10%, unless evidence indicates another percentage. In such cases, exposure to dust is estimated.
For use of the various models it is important to define the reasonable worst case options that are relevant for the calculations. This refers to application rates and volume rates.

Exposure estimates with EUROPOEM (EUROPOEM 1997)
EUROPOEM has not yet considered defaults for application areas and times, nor times for mixing/loading. In the analyses, the same defaults will be used as for the Dutch model, when required (see below).
The format of exposure chosen is mg/kg as probably the best estimate, whenever possible. Only 75th percentiles are used (as far as available from the description of the surrogate values for mixing/loading and application). The model is based on studies that have been considered in detail by the EUROPOEM expert group.

Exposure estimates with the UK model (PSD, 1992)
Some assumptions that are made for the UK model are an application area of 50 ha for downward spraying, 30 ha for upward spraying and 1 ha for manual spraying per day. The format of exposure is volume of spray per unit of time. A typical work day reflects 1 hr of mixing/loading and 6 hours of application. The exposure during mixing/loading is estimated on the basis of package size, type of formulation, and number of operations. The format of exposure is weight or volume of formulation.
The model is largely based on unpublished studies, carried out in England by industry and MAFF.

Exposure estimates with the German model (Lundehn et al., 1992)
Some assumptions that are made for the German model are an application area for downward spraying of 20 ha, for upward spraying of 8 ha and for manual spraying of 1 ha per day. For mixing/loading the nature of the formulation is an important variable. The format of the exposure is mg/kg. 90th Percentiles are calculated from the data in the model.
The model is based on unpublished studies, done by industry and all carried out in Germany.

Exposure estimates with the Dutch model (van Hemmen, 1992; van Golstein Brouwers et al., 1996)
The Dutch model assumes an application area for downward spraying of 10 ha, for upward spraying of 6 ha and for manual spraying of 1 ha. The application time is taken as 6 hr for tractor-driven applications and 3.5 hr for manual applications. The times for mixing and loading are taken as 1 hr for tractor-driven applications and 0.5 hr for manual applications. For greenhouse applications the model considers the full work shift of mixing, loading and application. Indicative 90th percentiles are deduced from the various exposure databases.
The formats of exposure are volume or weight per unit of time, for liquids and solids respectively, expressed for the spray liquid (application) or the formulation (mixing/loading).
The model is based on studies published in the scientific literature and on studies done in The Netherlands.

Discussion of the results
The basic choices are from the truly European model (EUROPOEM), when the databases are considered good enough to calculate the 75th percentiles for surrogate values. This is not done for the national databases which have not been considered according to basic and explicit criteria and which may consist of only local (national) studies, as is the case for the UK and German model. It is evident that the geometric means and 90th percentiles are quite apart from each other. In view of the requirement that reasonable worst case conditions should be estimated, and the considerations given above, the 90th percentile is the best choice for the present purpose.
If relevant surrogate exposures can be estimated by all three exposure models, the median of the assessed exposure values will be used as surrogate for the risk assessment.

2 *Bystander exposure*

The presence of bystanders should be kept at a minimum. This can easily be achieved in greenhouses, where no person should be allowed that is not involved in the spraying process. Outdoors, such measures cannot be taken that easily.

For field crops, the exposure to bystanders during mixing and loading will be insignificant in comparison to the mixer/loader. This is true for the inhalation exposure as well as the dermal exposure which, in many cases, is largely restricted to the hands of the mixer/loader. For downward spraying such conclusions cannot be drawn that easily, although it should be realized that the distance between bystanders and the nozzles will generally be more than a few metres. The highest levels of exposure will be encountered by a bystander when he or she is in the downwind area of the drift. This is unlikely to happen several times for a bystander walking along the edge of the field. Even for people watching the application, the distance between the edge of the field and the closest nozzle on the boom will change with every spraying swath. For high crops the level of exposure to a bystander may get higher than in the case of the field crops. There is, however, presently no explicit means of estimating these levels for a bystander. It is expected that the levels of exposure will be small in comparison to the levels of exposure to the operator. Frequency of exposure will be incidental for bystanders.

3 *Worker exposure*

The exposure to workers in crops after application (re-entry) has been considered by various researchers, but this has, so far, not resulted in a formal data base that can be used for the estimation of the exposure to such crop-workers, especially harvesters. A general approach has been described by Popendorf and Leffingwell (1982) and Popendorf (1985; 1992). A more explicit approach has been described by Van Hemmen et al. (1995) for the harvesting of ornamental flowers grown in greenhouses.

For the present purpose the re-entry activities for workers is mainly considered for tree crops and/or greenhouse crops. For other relevant crops the general approaches are similar, but some general parameters may differ, especially transfer factors.

Exposure estimation for re-entry activities in greenhouse crops (van Golstein Brouwers et al., 1996)

For the estimation of exposure during work with high crops in greenhouses within 1-3 days, i.e. for pesticides with relatively stable dislodgeable foliar residues over that period, an algorithm has been developed for exposure during cutting and sorting/bundling. These activities are considered the most exposure-prone processes for many crops and are considered to be performed each for 3 hr a day. The model is based on studies carried out in The Netherlands on behalf of the Dutch government. The format of exposure is (mg/hr)/(kg/ha).

Exposure estimates for re-entry activities in tree crops and other crops (Van Hemmen et al., 1995)

For the harvesting of fruits from tree crops the dermal exposure level can be estimated in an indirect way assuming no decay of the dislodgeable foliar residue between last application and re-entry activities. Assuming an application rate of AR kg/ha, and a leaf area index of about LAI m^2/m^2, the initial foliar dislodgeable residue is about 0.01 x (AR : LAI) $\mu g/cm^2$ (taking care for the dimensions).

If for the activities in tree crops a typical transfer coefficient is presumed of 10,000 cm^2/hr, the level of exposure per hour can be estimated as about 0.1 x (AR : LAI) mg/hr. For a working day of 6 hr, this would amount to 0.6 x (AR:LAI) mg/day. It must be emphasized that this calculation concerns workers with normal work clothing and bare hands. Furthermore, our knowledge on the various factors that are relevant for the exposure under practical conditions is still far from complete, so these data have to be considered as preliminary estimates.

The inhalation exposure cannot be estimated in a similar way due to lack of data. On the basis of expert judgement it is considered unlikely that the level of inhalation exposure is higher than that of the operators.

Discussion of the results

The exposure data must be considered relevant for the crops with the highest levels of contact with the crop and thus levels of exposure. Exposure levels will be lower when the time between application and re-entry is increased as this is largely dependent on the degree of dissipation of the pesticide residue on the crop. Exposure levels will also be lower for crops with only minor contact between crop and worker during the re-entry activities.

4 Risk management

From the exposure data, it may be concluded that the estimated level of potential dermal exposure to the operators is too high. For that reason a generic assessment of protective clothing is required to estimate the actual exposure for *protected* operators. In view of the fact that the potential exposure is assessed by taking the 75-90th percentile from the relevant exposure models or relevant field data, it is considered appropriate to use values of about a factor 10 for the protection afforded by adequate protective gloves, protective clothing and respiratory protective equipment; this presumes that a reasonable degree of personal hygiene is taken care of by the operators. The value of a factor 10 is appropriate for generic use at the level of the putting on annex I of the active substance under consideration, i.e. for consideration at the Community level. For the registration of plant protection products in Member States, a more elaborate consideration of crops, techniques and work methods may lead to some adjustment of these values.

5 References

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MINISTRY OF SMALL ENTERPRISES, TRADERS AND AGRICULTURE

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TELEFAX

Destination	PSD, Ecco-team	**From**	H. FONTIER
Faxnr.	00.44.1904455722	**Number of pages (1+) :**	4
Attention	Mr. C. Redford	**Date :**	07/04/99

SUBJECT . ECCO 78 meeting

Dear Mr. Redford,

It is my pleasure to send you herewith the Belgian comments on the chapter mammalian toxicology of the monographs on CGA 245704, glyphosate and glyphosate trimesium, to be discussed at the ECCO 78 peer review meeting.

These comments are also sent to the rapporteur member state and the European Commission.

Yours sincerely,

for The Engineer-director, abs.
De Ingenieur,

H. Fontier

Ir. H. FONTIER
Ir. A. VANDERSANDEN

6338/ECCO/PSD/99

MINISTRY OF PUBLIC HEALTH AND ENVIRONMENT

SCIENTIFIC INSTITUTE FOR PUBLIC HEALTH - LOUIS PASTEUR

***

Division - Section - Afdeling

TOXICOLOGY - TOXICOLOGIE

***

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Concerns : active substances under Dir.91/414/EEC
Comments concerning the toxicological assessment in the draft monographs.

Concerns : active substances under Dir.91/414/EEC
Comments concerning the toxicological assessment in the draft monographs.

GLYPHOSATE : monograph prepared by Germany

General comments:
The part of the monograph covering mammalian toxicology and metabolism is clearly reported. All the relevant information required to made the toxicological assessment of the active substance and the formulation was described with sufficient details (protocol, results). The rapporteur also indicate clearly if the studies were acceptable or not and if they comply with EEC methods.
It is noted that the purities of the compounds tested is quite different, ranging from 95-98% for glyphosate, to 90% for the ammonium salt (AM), 80% for the sodium salt (SO) and and only 62-65% for the isopropylamine (IPA) salt. On the other side, identity of the contaminants seems not to have been investigated.
As in the part devoted to glyphosate-trimesium, the reviewer gives seldom NOAEL values; this is sometims disturbing, like in the rat long term-study(SD rats) , were evidence of liver toxicity were observed.
Comments :
Point B.5.1 Toxicokinetics and metabolism:
From table B.5.1.1-1, it appears that glyphosate is absorbed at a low level reaching a maximum of 34%, depending of the studies. Bioavailability was calculated by one of the notifiers and was 12% , confirming that absorption is extremely low. Therefore, we propose to use the value of 12% for the correction of AOEL and not 30% as mentionned in the monograph.
It is not clear why elimination is biphasic : two values of T/2 are mentionned : is the second t/2 (69-337 h) related to a metabolite ? With such a long T/2, is there no posibility for accumulation ?
Point B.5.3. : short term toxicity : NOELs are proposed and it is not clear if these values are also NOAEL.
Point B.5.4: Genotoxicity : from the open literature, data indicate that Roundup is able to induce formation of DNA adducts in the kidney and liver of mice and this effect was attributed to an unknown compound of the mixture . In another publication , it is reported

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that glyphosate can increase structural aberrations, SCEs in bovine lymphocytes (Environ.Mol.Mutagen., 1998, 31, 55-59 and Mutat.Res.,1998, 403, 13-20).

Point B.5.10.2. ADI: the use of 31 mg/kg bw/d from a rat study from which the ADi is derived is acceptable. A lower value of 10 mg/kg bw/d was reported in a more recent rat study (Atkinson, 1993) in which salivary gland lesions were observed at 100 mg/kg bw/d. Unfortunately, no intermediate doses were tested. In our opinion, salivary glands lesions are not occurring frequently and this pathology occurs in two 90 day rat studies , in one reproductive rat study, in one study from open literature and in the long term rat study. Therefore, it seems difficult to accept this effect as an equivocal effect.

Point B.5.10.3 : calculation of AOEL must be performed taking into account that bioavailability is 12%.

AMPA : main plant metabolite of glyphosate.

The monograph summarises the toxicology studies performed with AMPA. From the metabolism study, it appears that absorption of Ampa is low (20%), and excreted mainly in faeces (72%). Urinary excreted compound was unchanged AMPA. From the acute toxicity studies, AMPA is of lower toxicity than the parent compound. Oral short-term toxicity studies suggest no main differences between Ampa and glyphosate. It has no mutagenic activity ans is not teratogenic.

M. Duverger

M.Duverger-van Bogaert
12/3/99

You will find herewith three files relating to lindane, amitraz and glyphosate-glyphosate trimesium. These commentson mammalian toxicology are also sent by e.mail to the RMS.
Comments on thiram and ziram will follow soon.
Best Regards
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EU Review program on active substances in Plant Protection Products

GLYPHOSATE - GLYPHOSATE TRIMESIUM

Rapporteur Member State : Germany

Comments of France on toxicology and metabolism and classification and labelling

Volume I level 2 and 4

2-1. Impact on human health

- p.10 / 2.3.4.
  same comment as vol.3 annex B p.137. **We suggest AOEL 0,8 mg / kg bw / d**

Agreement with -TMDI 16/23 % cf. 2.7.1. p.15
- MRL Table 2.7.31 cf. p. 16 / 17

- **p.26 Luxan / Herbex : PLS to add R 43**

Volume 3 -annex B

General comment

Very comprehensive work.
A lot of tables make this dossier clear.
A huge amount of documentation has been summarised for an easy reading.

B-5.1. Toxicokinetics and metabolism.

a) A table, summarising all toxicokinetic parameters : dose related-species related and salts related, could be useful.

PLS : to add that phosphonic moiety is responsible for bone fixation.

b) If BREWSTER suggests that AMPA is the result of bacterial metabolism, instead of mammalian metabolism, **why didn't MONSANTO perform studies to demonstrate that or to exclude this hypothesis ?**
Germ-free rat or mice studies could clarify this suggestion (B.512, p. 12).

B-5.2 p.14

Is the high acute toxicity by IP route (LD 50 I.P.A. 134 mg / kg bw versus LD50 > 2000 mg / kg bw p.o.) relevant to the fatal ingestion in man ? In rat, peritonitis was noted, perhaps related to causticity of the salt, to be compared to severe intestinal congestion, often fatal, in man.
PLS to ask to the applicant an explanation.

B-5.2.12 - p.18

5.2.12.1. Vehicle " none " to be replaced by water ?
PLS to precise if the data with IP A. are expressed in IP A. as salt or as solution 61.8 /65 %.

B-531. p.42

Minor details.

- A study performed in the United States on 23.03.1981was submitted to GLP requirements (in force from June 1979).
- CD rats means Cesarian -Derived, a " trade mark " of Charles River, usually Sprague Dawly rats.
- At the conclusion of short term toxicity test, can be added (B53 p.40) :
  "In Wistar rats, glyphosate is less toxic by gavage (NOEL 1200 mg / kg bw /d) than by dietary administration (NOEL 150 mg /kg bw/d).
  In beagle dog, the same results: NOEL by capsule is 300 / 500 mg / kg bw/d and by dietary administration 8 mg / kg bw /d. This could suggest an enzymatic target."

- **p.55** : 3 months study : decrease of liver weight **PLS: percentage** ?

Study by G. WILLIAMS at AHF in 1983: GLP was compulsory too.
id. p.95 (B.56.11)
id p.110 (B.52.22.2)
The uncreased heart weight could be related to anemia (parameter nct evaluated in this study).

B-58.11. p.115

AMPA same remark as B512

B-59.5 p.129 (5th line)

The following sentence could be added after... of the formulated product "However the pharmacological profile (adrenergic properties) cannot exclude the responsability of the glyphosate itself, taking into account some symptoms of poisoning.

B-5.10.2 p.136

A.D.I. We agree with.

B-5.10.3

Little inconsistancy between "midterm toxicity" based AOEL, and inclusion of repro studies (2 or 3 generations).

p.137

We disagree with the safety factor 100 (due to the safe profile of the a.i.) 25 seems sufficient, and we suggest AOEL 0,8 mg / kg bw.

Discussion about taking into consideration the low oral absorption rate of about 30 % is agreed. However the dermal absorption is still lower! The most important point is: why the absorption rate of about 30 % have not been considered in A.D.I. calculation ?

Formulations

Table B-5.11.7. p. 145.

The sentence by Luxan has been appreciated!

B-5.11.1. p.146 / 150
B-5.11.2.

Due to the particular toxicity of glyphosate plus surfactant, the comparison by the applicant between MON 52276, MON 44068 and surfactant DODIGEN 4022 is not clear enough. Due to the low toxicity, former formulation, new formulation and surfactant alone have to be evaluated for acute toxicity precisely and not with limit test only.

Tables B-5.11.8.1.
B-5.11.9.1 and 2
B-5.11.10.1

Due to the lack of sensitization test HPQ and both Luxan formulations have to be labelled **R43.**

B-5.14.1

Due to the large range of application rate -from 0,720 kg ai / ha (sommerape)- up to 4.320 kg ai /ha, we agree with the rapporteur 's conclusion, despite the disagreement on AOEL value.

Glyphosate MAMMALIAN TOXICOLOGY - COMMENTS

General: The monograph is written in a clear way which allows the evaluation of the most critical studies from the RMS as well.

Volume 3

Annex B

B.5.3.2.1.1 Subchronic Toxicity Study in Sprague-Dawley Rats

In the conclusion of the study , it is mentioned that a clear NOEL could not be established since the number of animals showing cellular alteration in the parotid salivary glands was increased in all treated groups following a dose related pattern. On the other hand, the rapporteur established a NOAEL of 300 mg/kg b.w./day even though the total incidence of cellular alteration in the parotid salivary gland was highly statistically significant ($p<0.01$) (table B.5.3.2.1.1-2). The reviewer considers that no NOAEL could be established for this study since alterations in the parotid salivary gland is a treatment related effect observed in the majority of the studies.

B.5.10.2 Acceptable Daily Intake (ADI)

The rapporteur proposes an ADI of 0.3 mg/kg b.w./day derived from the Sprague-Dawley, 26 months study in rat (Lankas, 1981) which is considered to provide supplementary information only, according to the rapporteur, with a NOEL of 31 mg/kg b.w./day and an assessment factor of 100.

The reviewer considers that the most appropriate study to establish the ADI is the 2 years Sprague-Dawley rat study (Atkinson et al., 1993). This study is not only the most recent one, but also it is a GLP study. Additionally, in this study the histopathology in the parodid and mandibular salivary glands was investigated. Based on the above study with a NOEL of 10 mg/kg b.w./day and an assessment factor of 100, the ADI proposed by the reviewer is 0,1 mg/kg b.w./day.

B. 5.10.3 Acceptable Operator Exposure Level (AOEL)

The rapporteur proposes an AOEL of 0.2 mg/kg b.w./day derived from the teratogenicity New Zealand White rabbit study (Tasker, 1980) with a NOEL of 75 mg/kg b.w./day, a correction factor of 30% (oral absorption rate) and an assessment factor of 100.

The reviewer agrees with the rapporteur that the most appropriate study to establish the AOEL is a rabbit teratogenicity study but considers that the most relevant study is the rabbit teratogenicity study (Suresh, 1993) with the lowest NOEL of 20 mg/kg b.w./day. This study is the most recent one and also it is the study which the rapporteur have chosen to present in detail in this monograph. By using a correction factor of 30% and an assessment factor of 100 the proposed AOEL (systemic) by the reviewer is 0.06 mg/kg b.w./day.

B.5.14

The calculations of the operator exposure were not presented in the monograph.

B.9.1

The products Glyphosate 360 SL, glycel 41 SL and Ipiglyce 36 SL should have the safety phrase S37 (wear suitable protective gloves) according to the estimations of the operator exposure.

Volume 1

Level 2

We agree with the rapporteur that the genotoxicity issue of some formulations in relation to maximum acceptable levels of toxicologically relevant impurities or by products in Glyphosate technicals needs to be clarified.

2.10 Classification and labelling

The products Glycel 41 SL and Ipiglyce 36 SL should have the safety phrase S37 (wear suitable protective gloves) according to the estimations of the operator exposure.

Addendum to the monograph on glyphosate - Evaluation of possible mutagenic effects of formulations

I.

ORIGINAL STUDIES

A total of eight mutagenicity studies using four different glyphosate formulations was made available to the Rapporteur by the companies Monsanto and Cheminova. For each of these formulations, an Ames test and a mouse bone marrow micronucleus test were submitted. The studies are reliable since they were performed at least to a large extent in compliance with current OECD guidelines (Guideline 471 for bacterial reverse mutation tests and Guideline 474 for mammalian erythrocyte micronucleus tests) under GLP-like conditions. They are all scientifically valid and may be used for risk assessment although the studies with the formulation *Glifos* are considered of limited value for this purpose only. Both test systems are widely accepted for mutagenicity testing of chemicals and respective data for glyphosate active ingredient are available allowing a direct comparison between the active substance and some of its formulations. Unfortunately, these data do not refer to those formulations for which acute toxicity studies have been submitted for purposes of EU re-evaluation of glyphosate.

The studies on *Rodeo®* were submitted as part of the joint dossier of Monsanto and Cheminova since this formulation is considered representative for the glyphosate IPA salt without any further chemicals contained. The six other study reports were kindly provided by Monsanto on request for purposes of this addendum and were not part of the original EU submission. Following a short characterization of the products investigated, test conditions and results are summarized in Tables 1 (*in vitro* testing) and 2 (*in vivo* studies). The individual studies are briefly listed below.

Brief description of formulations tested:

Rodeo® is a formulation containing 54% glyphosate IPA salt and water but no surfactants. According to information obtained by Monsanto, it is especially intended for aquatic use. The studies have been performed and data submitted to facilitate the assessment of genotoxicity of the IPA salt since in most mutagenicity studies the test material was glyphosate acid (see chapter B.5.4 in the monograph).

The *Roundup®* formulation tested by Monsanto *(MON 2139)* is made of 31% glyphosate (acid equivalents), tallowamine (*MON 0818*, i.e., a surfactant), and water.

The third tested Monsanto product *Direct® (MON 14445)* contains 72% glyphosate acid equivalents formulated as ammonium salt with also a tallowamine (Ethomeen T25, C20-C25 tallowamine) surfactant. According to the Rapporteurs database, it is the only glyphosate ammonium salt tested for mutagenicity.

The product called *Glifos* in Brazil (in Europe *Glyphos*) is a formulation of glyphosate manufactured by Cheminova. As indicated by the test facility BioAgri, it contains the IPA salt at a concentration of 360 g/l. According to the German national registration data files, the product is made of the IPA salt, the by-product Berol 907, and water.

Addendum to the monograph on glyphosate - Evaluation of possible mutagenic effects of formulations

Overview on mutagenicity studies:

Table 1: Genotoxicity studies on herbicidal formulations containing glyphosate - *In vitro* testing in bacteria (Ames test)

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Ames test	Rodeo® (containing IPA salt and water only)	S.typhimurium strains TA 98, 100, 1535, 1537	50 - 5000 µg/plate; -/+ S9	Negative; no signs of cytotoxicity	Kier et al., 1992a
Ames test	MON 2139 (Roundup® containing IPA salt, a tallowamine surfactant and water)	S.typhimurium strains TA 98, 100, 1535, 1537	5 - 500 µg/plate (-S9)/ 15 - 1500 µg/plate (+S9)	Negative; cytotoxic at the maximum dose levels, occasionally also at lower concentrations	Kier et al., 1992b
Ames test	MON 14445t (Direct®, containing ammonium salt, a tallowamine surfactant and water)	S.typhimurium strains TA 98, 100, 1535, 1537	5 - 500 µg/plate (-S9)/ 15 - 1500 µg/plate (+S9)	Negative; cytotoxic at the maximum dose levels, occasionally also at lower concentrations	Kier et al., 1992c
Ames test	Glifos formulation (IPA salt, Berol 907 and water)	S.typhimurium strains TA 97a, 98, 100 and 1535	1, 10, 100, 1000, 5000 µg/plate; -/+ S9	Negative; cytotoxic at the two upper concentrations	Vargas, 1996*

* study of limited value for risk assessment only
In all trials, the solvent was distilled water.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992 a): Ames/Salmonella mutagenicity assay of Rodeo®. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91184, Sponsor Project no. ML-91-441. Dates of experimental work: 26 November 1991 - 30 December 1991. GLP: yes (self-certification of the laboratory). A respective statement of the Quality Assurance Unit (QAU) is included. The study is considered acceptable.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992 b): Ames/Salmonella mutagenicity assay of MON 2139 (ROUNDUP® herbicide formulation). Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91183, Project no. ML-91-440, Report no. MSL-11729. Dates of experimental work: 26 November 1991 - 06 January 1992. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992 c): Ames/Salmonella mutagenicity assay of MON 14445 (DIRECT® herbicide formulation). Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91185, Project no. ML-91-442, Report no. MSL-11731. Dates of experimental work: 26 November 1991 - 30 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Vargas, A.A.T. (1996): The Salmonella typhimurium reverse mutation by GLIFOS. BioAgri (Biotecnologia Agricola Ltda.), Piracicaba, Sao Paulo, Brazil on behalf of Cheminova; BioAgri Report G.1.1 - 050/96. Dates of experimental work: 12 October 1996 - 23 December 1996. GLP: No. However, a QAU statement is included. The study is considered of limited value for risk assessment only since a legal statement on GLP compliance is lacking and since there were some minor reporting deficiencies in particular regarding the negative (absolute and solvent) and positive control values.

Table 2: Genotoxicity studies on herbicidal formulations containing glyphosate - *In vivo* experiments (micronucleus test)

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Micronucleus test	Rodeo® formulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single i.p. administration	0-850-1700-3400 mg/kg bw; sampling after 24, 48 and 72 h	Negative for chromosome aberrations; overt toxicity (clinical signs, bw↓, death) at the upper dosages	Kier et al., 1992d
Micronucleus test	Roundup® formulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single i.p. administration	0-140-280-555 mg/kg bw; sampling after 24, 48 and 72 h	Negative(no chromosome aberrations); toxic to mice at 555 mg/kg bw with some deaths occurring, cytotoxic to the bone marrow (PCE/NCE ratio↓ at 48-h sampling) at this top dose level	Kier et al., 1992e
Micronucleus test	Direct® formulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single i.p. administration	0-91-183-365 mg/kg bw; sampling after 24, 48 and 72 h	Negative for chromosome aberrations; signs of general toxicity at the top and, although less pronounced, mid dose level	Kier et al., 1992f
Micronucleus test	Glifos formulation in distilled water	Swiss albino mice (m/f), two i.p. injections with 24-h inverval	0-68-137-206 mg/kg bw; sampling at 24 h after the second dose	Negative. No indications of cytotoxic effects to the bone marrow. No information regarding general toxicity in the main study.	Zaccaria, 1996*

m/f male and female mice used
* study of limited value for risk assessment only

Kier, L.D.; Flowers, L.J. and Huffman, M.B. (1992 d): Mouse micronucleus study of RODEO® herbicide formulation. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study nos. 91201 (toxicity range-finding study, not tabulated above) and 91205 (micronucleus test), Sponsor study nos. ML-91-435/ML-91-438. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Kier, L.D.; Flowers, L.J. and Huffman, M.B. (1992 e): Mouse micronucleus study of ROUNDUP® herbicide formulation. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study nos. 91200 (toxicity range-finding study, not tabulated above) and 91204 (micronucleus test), Sponsor study nos. ML-91-434/ML-91-437. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Kier, L.D.; Flowers, L.J. and Huffman, M.B. (1992 f): Mouse micronucleus study of DIRECT® herbicide formulation. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study nos. 91202 (two toxicity range-finding experiments, not tabulated above) and 91206 (micronucleus test), Sponsor study nos. ML-91-436/ML-91-439. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Zaccaria, C.B. (1996): A micronucleus study in mice for the product GLIFOS. BioAgri (Biotecnologia Agricola Ltda.), Piracicaba, Sao Paulo, Brazil on behalf of Cheminova; BioAgri Report G.1.2 - 060/96. Dates of experimental work: 08 October 1996 - 19 November 1996. Dose levels were chosen on the basis of a preliminary toxicity test (LD50 determination) described in the study report. GLP: No. However, a QAU statement is included. The study is

considered of limited value for risk assessment only since a legal statement on GLP compliance is lacking and since there was no information regarding general health effects of treatment to the animals. Therefore, it is not clear whether the highest possible dose was actually reached.

Assessment:

Four glyphosate formulations were tested for mutagenicity in the reverse mutation assay in bacteria as well as *in vivo* by means of the mouse bone marrow micronucleus test. Unequivocally, all these products proved negative in both test systems. Thus, it can be concluded that the formulations Rodeo®, Roundup® (MON 2139), Direct® and Glifos® containing either the IPA or the ammonium salt of glyphosate, alone or in combination with different surfactants, do not cause point (gene) mutations in various *Salmonella typhimurium* strains and are devoid of a clastogenic potential *in vivo*.

However, when the studies of the same type (Ames test and Micronucleus test) for the active substance and the formulations are compared, it becomes obviously that the highest concentrations or dosages to be tested were generally lower with the formulations except Rodeo®. This is apparently due to a higher degree of cytotoxicity as well as of general mammalian toxicity related to the formulations containing other ingredients than glyphosate salt and water.

To facilitate direct comparison of the Ames tests, the respective table (Table B.5.4.1.1.1-1) from the monograph is reproduced here once more.

Table 3: Glyphosate a.i. - Summary of tests for gene mutations in bacteria

Study type	Test material/ Purity	Test organism	Dose range/ Metabolic activation	Result	Reference	Submitted by (notifier)
Ames test	Glyphosate, 95%	S.typhimurium strains TA 98, 100, 1535, 1537, and 1538	8.0 - 5000 µg/plate; -/+ S9	Negative	Thompson, 1995	Herbex
Ames test	Glyphosate, purity not given	S.typhimurium strains TA 98, 100, 102, 1535 and 1537	50 - 5000 µg/plate; -/+ S9	Negative	Fassio, 1995	I.Pi.Ci.
Ames test	Glyphosate, 96%	S.typhimurium strains TA 98, 100, 1535, 1537, and 1538	1 - 1000 µg/plate; -/+ S9	Negative	Suresh, 1993*	Feinchemie
Ames test	Glyphosate IPA salt, 64%	S.typhimurium strains TA 98 and 100	0.01 - 100 µg/plate	Negative	Wang et al., 1993*	Sinon [Shinung]
Ames test	Glyphosate, 98.6%	S.typhimurium strains TA 98, 100, 1535, 1537	-S9: 160 - 2500 µg/ plate; +S9: 310 - 5000 µg/ plate	Negative	Jensen, 1991	Monsanto/ Cheminova
Ames test	Glyphosate, purity not given	S.typhimurium strains TA 98, 100, 1535, 1537, and 1538	8.0 - 5000 µg/plate; -/+ S9	Negative	Jenkinson, 1990*	Agrichem
Ames test	Glyphosate, purity not given	S.typhimurium strains TA 98, 100, 1535, 1537 and E.coli stain WP-2uvrA	10 - 1000 µg/plate -/+ S9	Negative	Bhide, 1986*	Barclay; Luxan
Ames test	Glyphosate, 98.4%	S.typhimurium strains TA 98, 100, 1535, 1537, 1538 and E.coli WP2 hcr strain	10 - 5000 µg/plate; -/+ S9	Negative	Shirasu et al., 1978; published by Li and Long, 1988	Monsanto/ Cheminova

* The study is considered to provide supplementary information only.

It is clearly to be seen that much higher concentrations of the active substance could be tested without causing significant cytotoxicity. According to the literature (Chan and Mahler, 1992), even concentrations up to 10,000 µg/plate have been reached. With the formulations described above, only Rodeo® which is made of glyphosate IPA salt and water could be succesfully tested at such high concentrations. In contrast, strong cytotoxicity avoided meaningful evaluation of mutagenicity of the three

other formulations at least at the highest of the selected concentrations and was still to be seen at much lower dose levels. Therefore, it can be assumed that cytotoxicity is due to the surfactants contained but not to glyphosate or its salts. The effects appeared more pronounced with Roundup® and Direct® than with Glifos® suggesting a particularly high cytotoxic activity of tallowamine surfactants.

Regarding the micronucleus tests, a similar pattern becomes apparent. A number of micronucleus studies with glyphosate a.i. in mice is available (see section B.5.4.2 in the monograph). However, all these experiments were performed using the oral route. General and cytotoxicity (i.e. bone marrow effects) were confined to very high doses of 4000 or 5000 mg/kg bw corresponding to the known low acute oral toxicity of this compound. The only miconucleus test using the i.p. route (as with the formulations) was performed in rats. The highest dose of 1000 mg glyphosate a.i./kg bw did not cause clastogenicity (Li, 1983; also published by Li and Long, 1988). As shown by Kier et al. (1992d), the IPA salt when dissolved in water (Rodeo® formulation) can be given intraperitoneally to mice at a similar dose level (850 mg/kg bw) without causing neither toxicity nor clastogenic effects. Toxicity was confined to higher dosages (1700 and 3400 mg/kg bw) but genotoxicity was not observed. In contrast, toxicity of the other formulations containing surfactants was much higher although, again, no evidence of a clastogenic effect was obtained (Kier et al., 1992e and f; Zaccaria, 1996).

Addendum to the monograph on glyphosate - Evaluation of possible mutagenic effects of formulations

II.

PUBLISHED LITERATURE

During the past few years, a number of studies was published dealing with possible mutagenic effects of glyphosate formulations in different test systems. Scientific assessment of these data is very difficult for at least two reasons.

- One main deficiency is the lack of precise description of the test material. Usually, source, composition and/or purity neither of the formulations nor, if tested, of the active ingredient are not stated at all or, at least, not sufficiently reported in the publications. It should be also taken into consideration that different formulations may be marketed in different countries under the same trademark, e.g. Roundup®. Further confusion comes from the fact that sometimes by-products in formulations (e.g. surfactants) were replaced by others but the name of the product was not changed. On request, data on the ingredients were submitted by the manufacturer Monsanto but even this information was not sufficient to clarify all uncertainties about the test substances. However, on the basis of the information available so far, it can be be stated that the Roundup products used in the different published studies were not identical. Thus, it is questionable whether results obtained with one product will apply to others containing different non-active ingredients in different concentrations.

- A second point of concern is the frequent use of less validated test systems with no proven relevance of the findings for human health risk assessment even if such systems may be well accepted to predict special environmental hazards. With regard to health effects, there are no current guidelines for these test methods and there is no actual experience how to assess positive findings in such test systems. For other test methods used, OECD guidelines do exist but the experiments were not carried out in compliance with these recommendations.

To facilitate presentation of data, it was decided to start with those experiments for which, in principle, widely agreed guidelines are available. Because of the large background database, the SCE assays were also included here. In the subsequent part of this section, investigations in test systems less frequently used for examination of plant protection products and with no guidelines existing are reported. As a result of this approach, one and the same publication may be referred to repeatedly on different sites.

It should be mentioned that in some publications also experiments are reported which were carried out with glyphosate active substance (i.e., the acid or one of its salts) being the test material. These data were not included in the monograph since the respective publications, for different reasons, were considered unacceptable for evaluation purposes (for justification, see description of experimental conditions below) in particular when the current OECD criteria for assessment of published data were applied. However, the findings are reported in this addendum since a direct comparison between active ingredient and formulation data may be of particular interest.

Although various test systems measuring different endpoints were used, it was tried to summarize the available studies in Table 4 (see next pages) to facilitate general overview before the individual publications were discussed in greater detail below. For practical reasons, in particular to facilitate direct comparison, the studies were divided into sections according to the test systems and methods and the experiments separately tabulated.

Table 4: Overview on published studies on mutagenicity of glyphosate, its salts and formulations

Test method/ test system	**Test material**	**Dose levels/ Dose range**	**Results**	**Remarks**	**Reference**
Ames test in S.typhimurium strains TA98 and TA100 (+/- S9 mix)	Roundup (48% glyphosate IPA; polyoxyethylene tallowamine surfactant)	0 - 1440 µg/plate (calculated as glyphosate IPA salt)	Equivocal. Occasional increase in mutation rate but no clear dose response. Marked cytotoxicity from 360 or 720 µg/plate onwards.	Study not acceptable for evaluation purposes due to serious deficiencies. Reliable assessment avoided by cytotoxicity.	Rank et al., 1993
Micronucleus test in mouse bone marrow; single i.p. administration; sampling after 24 or 48 h	Glyphosate IPA salt (1:1 mixture) and Roundup (48% glyphosate IPA; tallowamine surfactant)	0, 100, 150, 200 mg/kg bw (glyphosate IPA); 0, 133, 200 mg/kg bw (Roundup, calculated as IPA salt)	Negative. Indication of dose-related bone marrow cytotoxicity with the Roundup formulation but not with glyphosate IPA.	Supplementary study confirming previous results.	Rank et al., 1993
Micronucleus test in mouse bone marrow; two i.p. administrations with a 24-h interval between.; sampling after 6 and 24 h after the final dose	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 mg/kg bw (2X150 mg/kg bw/d) for glyphosate; 0, 450 mg/kg bw (2x225 mg/kg bw/d) for Roundup	Weakly positive for glyposate after 24 h and for Roundup at both sampling times. Some evidence of bone marrow cytotoxicity of Roundup.	Supplementary study (methodical deficiencies) revealing an increase in micronucleus frequency, data in contrast to previous results.	Bolognesi et al., 1997
SCE assay in human lymphocytes	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0 - 6 mg/ml for glyphosate; 0, 0.1, 0.33 mg/ml for Roundup	Positive for glyphosate from 1 mg/ml onwards and for Roundup at both concentrations. With Roundup, complete cytotoxicity at concentrations >0.33 mg/ml.	Insufficient data. In addition, a positive result in this assay is of equivocal biological significance against the background of more appropriate mutagenicity studies.	Bolognesi et al., 1997
SCE assay in human lymphocytes	Roundup (not specified)	0, 250, 2500, 25000 µg/ml	Weakly positive at the low and mid dose level (for one of two donors). Cyto-toxic at the high dose.	see comment above	Vigfusson and Vyse, 1980
Alkaline elution assay for DNA single-strand breaks and formation of alkali labile sites in DNA obtained from liver and kidneys of mice following single i.p.administration	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 (glyphosate a.i.), 900 (Roundup) mg/kg bw; sampling after 4 and 24 h	Weakly positive after 4 h in both organs suggesting possible transient DNA damage.	Supplementary study (methodical deficiencies). Biological significance equivocal. Results in contrast to the negative outcome of the UDS assay. Effects might be also due to toxicity.	Bolognesi et al., 1997

a.e. acid equivalents

Table 4: Overview on published studies on mutagenicity of glyphosate, its salts and formulations (continued)

Test method/ test system	**Test material**	**Dose levels/ Dose range**	**Results**	**Remarks**	**Reference**
Investigations for oxida-tive damage in liver and kidney of i.p. treated mice by measuring the number of 8-OHdG (hydroxydesoxyguanosine) adducts	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 (glyphosate a.i.), 900 (Roundup) mg/kg bw (single i.p. administra-tion); sampling after 4 and 24 h	Evidence of stimulation of oxidative metabolism in the liver (only glyphosate) or kidney (only Roundup) after 24 h.	Finding not indicative of mutagenicity but could indicate one possible mechanism of toxicity.	Bolognesi et al., 1997
Measuring of DNA adducts by means of ^{32}P-postla-beling technique in the liver and kidney of mice following single i.p. administration	Glyphosate IPA salt and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 130, 270 mg/kg bw (glyphosate IPA); 0, 400, 500, 600 mg/kg bw (Roundup)	Weak dose-related increase in adducts with Roundup; no adducts seen with the IPA salt alone and in the control group.	Indication of possible DNA damage, however, biolo-gical significance of this finding equivocal. Further characterization of ad-ducts needed. Toxicity not adressed. However, non-mutagenic toxic effects can also cause DNA adducts.	Peluso et al., 1998
Comet assay for single-strand DNA breaks in tadpole erythrocytes	Roundup (41% glyphosate IPA; tallowamine surfactant)	0-1.69-6.75-27-108 mg/l water	Dose-related effect on DNA at 6.75 and 27 mg/l; completely lethal at 108 mg/l.	Impact of this formulation on tadpole DNA under environmental conditions indicated. Effect could be also due to toxicity. No relevance for human health risk evaluation.	Clements et al., 1997
Test for lethal mutations in Drosophila melanogaster after treatment of larvae	Roundup (assumed to contain 41% glyphosate IPA and tallowamine surfactant); Pondmaster (probably made from 41% glyphosate IPA; alkyle sulfate surfactant)	Not specified but indicated to be around LC50 concentration.	Positive.	Not predictive for mutagenicity in mammals. Concentrations used were expected to exhibit high toxicity making evaluation of results very difficult.	Kale et al., 1995
Anaphase-telophase allium test for chromosome aberrations in onion root cells	Glyphosate IPA salt (1:1 mixture) and Roundup (48% glyphosate IPA; tallowamine surfactant)	0-720-1440-2880 µg/l (for Roundup calculated as IPA salt)	Roundup: increase in chro-mosome aberrations at the two upper levels indica-ting rather polyploidy than clastogenicity, no clear dose response Glyphosate IPA: negative	Effects in plant cells not predictive for mutage-nicity in mammals. Testing a herbicide for genotoxic effects in plants generally doubtful since cytotoxicity may be expected.	Rank et al., 1993

a.e. acid equivalents

Studies in test systems for which guidelines exist and/or much experience is available (i.e. Ames test, micronucleus test, SCE assay)

Rank et al. (1993) studied the mutagenic potential of the herbicide Roundup and of glyphosate isopropylamine salt in different test systems *in vitro* as well as *in vivo*. An Ames test (plate incorporation test) was performed with Roundup only in the *Salmonella typhimurium* strains TA 98 and TA 100 with and without S-9 mix for metabolic activation. Evidence of mutagenicity was confined to the strain TA 98 under non-activation conditions as indicated by a slight but significant increase in the mean number of revertants at a concentration level of 360 µg/plate (calculated as IPA salt) which was also confirmed in the repeat experiment. With activation, however, no increase in mutation rate was seen up to this dose level. From the next higher concentration (720 µg/plate) onwards, cytotoxicity became apparent with and without metabolic activation avoiding meaningful evaluation. The study authors also reported a positive result for TA 100 in the presence of S9 mix at a concentation of 720 µg/plate but already the next lower dose of 360 µg had markedly reduced the number of revertants as compared to the control suggesting a cytotoxic effect. Furthermore, a dose response was lacking. Thus, the marked increase in mutation frequency at 720 µg/plate is not reliable. In the second experiment, this dose level was not included. Without activation, concentrations from 720 µg/plate onwards appeared cytotoxic. At lower concentrations, no increase in mutation rate was noted with strain TA 100.
In a micronucleus test in mouse bone marrow erythrocytes following single i.p. administration, Roundup as well as the IPA salt (i.e., a 1:1 mixture of glyphosate technical and isoproplyamine) proved negative up to the highest dose of 200 mg/kg bw. However, with Roundup but not with the glyphosate IPA salt alone, there was evidence of bone marrow cytotoxicity at this top dose level as indicated by a significantly lower percentage of polychromatic erythrocytes.
Comment: According to the publication and to further information submitted by Monsanto, it is assumed that the Roundup formulation used was made of 48% IPA salt, tallowamine surfactant, and water. The study design of the Ames test does not comply with current guideline requirements, e.g. the plate number scored was inconsistent throughout the study. The data obtained are so controversial that a reliable interpretation is not possible. Unfortunately, a complete confirmatory experiment was not performed since repeated testing was confined to the dose of 360 µg/plate and an additional concentration of 180 µg/plate was included. A more extensive study by Kier et al. (1992b, see above in section I) using four S. typhimurium strains including also TA 98 and TA 100 failed to elicit any indications of mutagenicity. This latter trial was conducted in compliance with OECD guideline 471 requirements and is of higher reliability, therefore. Of course, the Roundup formulations tested by Rank and her group and by Kier et al. were not identical but similar since both contained only the active substance formulated as IPA salt, tallowamine surfactant, and water. The cytotoxicity of Roundup was described by both groups but the respective concentrations were different.

The design of the micronucleus test was also not in compliance with guideline requirements. A direct comparison between results obtained with the IPA salt and Roundup is not feasible since not exactly the same dose levels were used and since there was a difference in sampling time (24 and 48 h post dosing for the IPA experiment versus only at 24 h after administration of Roundup). The negative outcome of previous micronucleus studies with the IPA salt (Rodeo® formulation, Kier et al., 1992d) and with a similar Roundup formulation in mice (Kier et al., 1992e) was confirmed. The reported weak bone marrow cytotoxicity occurring already after single i.p. administration of 200 mg Roundup/kg bw (amount calculated as the IPA salt to facilitate comparison) may be considered a possible formulation-related effect when the observations in other miconucleus studies (see section I) are taken into consideration.

In contrast, **Bolognesi et al. (1997)** reported positive results from a micronucleus test in mouse bone marrow erythrocytes. Either glyphosate a.i. (declared as 99.9% pure) or a Roundup formulation were administered to Swiss mice once daily by the i.p. route on two consecutive days. Cell samples were harvested at 6 and 24 hours following the final dose. A weak positive effect was observed at total dose levels of 300 mg/kg bw (2 x 150 mg/kg bw/day) after 24 hours for glyphosate and of 450 mg/kg bw (2 x 225 mg/kg bw/day) at both sampling times for Roundup.
Further data in this publication indicated for high purity glyphosate a significant and dose-dependent increase in SCE frequency in human lymphocyte cultures obtained from two female donors from a concentration of 1000 µg/ml onwards. For Roundup, this effect became apparent even at lower concentrations of 100 and 330 µg/ml.
Comment: The outcome of the micronucleus test with glyphosate a.i. is at least surprising since much higher doses of this compound had been tested before and did not reveal indications of clastogenicity (see section B.5.4.2.1 in the monograph). A direct comparison is not possible since the only available test using the i.p. route in which the highest dose of 1000 mg/kg bw proved negative (Li, 1983) was performed in rats. The respective study by Rank et al. (1993, see above) was conducted in mice but the test material was glyphosate IPA salt and the dose administered was probably too low for meaningful evaluation. However, a number of well-performed micronucleus tests with oral administration to mice is available. Even when the low oral absorption rate of glyphosate (about 30%) is taken into account, the dose levels (up to 5000 mg/kg bw nominal) are much higher than those given by Bolognesi and her co-workers but no convincing evidence of a potential to cause chromosome aberrations in vivo was obtained. It should be emphasized that the increase in the incidence of micronucleated polychromatic erythrocytes as reported in this publication was rather weak only. The test was not performed according to the current OECD guideline. In particular, the number of animals used (three male mice per dose group) was too low since a group size of at least five is recommended. A dose response cannot be assessed since only one dose level was included. The basis for statistical comparison is questionable since it is not clear when the six control animals were sacrificed because only one group mean value was indicated. Due to these deficiencies, this isolated positive finding is not considered to provide sufficient evidence to contravene the previously obtained negative results regarding the active substance.
The same methodical shortcomings apply to the experiment with the Roundup fomulation. The formulation tested is reported to contain 30.4% glyphosate acid equivalents. The a.i. is formulated as the IPA salt. Alkyl sulfate surfactant (MON 8080) is also contained (source of information: Monsanto). The weak positive response is in contrast to the beforementioned GLP-like study by Kier et al. (1992e) in which Roundup® proved negative. However, these two Roundup formulations were not identical since the glyphosate concentrations were nearly the same but the surfactants contained were different making a direct comparison of the study results difficult. Little is known on mutagenicity of alkyle sulfate itself, however, MON 8080 proved negative in the Ames test but was clearly cytotoxic at relatively low concentrations (see section III of this addendum). Some evidence of bone marrow cytotoxicity was obtained with both Roundup products as indicated by a decrease in the ratio between polychromatic and normochromatic erythrocytes. Cytotoxicity could have also an impact on chromosome aberration frequency. An overall, unequivocal conclusion from the experiment of Bolognesi and her group cannot be drawn, however an actual clastogenic response is not very likely. Even if a positive result could be confirmed, it would not be applicable to products containing other surfactants.
A higher SCE frequency is not considered to provide evidence of mutagenicity against the large number of studies in which glyphosate proved clearly negative. The two other studies of this type which have been submitted for purposes of toxicological evaluation of glyphosate (Jenkinson, 1990 and Wang, 1993, the latter using the IPA salt) did not reveal an increase in sister chromatid exchange frequency but,

unfortunately, did not include the high concentrations as tested by the group of Bolognesi (see section B.5.4.1.3 of the monograph). Apart from general doubts about biological significance of a positive result in an SCE assay, some methodical deficiencies became obviously in this publication. For statistical reasons, the number of only two subjects to be included in the study appears too low for meaningful evaluation. Furthermore, the data from two experiments were pooled for the two donors and individual values were not given. Therefore, a possible influence of interindividual variation could not be sufficiently assessed by the reviewer. As shown below, this variation may well reach a considerable level. Again, the positive result obtained with Roundup at least might be also due to cytotoxicity of the formulation avoiding further testing at dose levels exceeding 330 µg/ml since no mitotic cells were present any more.

Vigfusson and Vyse (1980) also reported a weak but statistically significant increase in SCE frequency in human lymphocytes obtained from two donors when the cultures were exposed to Roundup (not specified) at concentrations of 250 and 2500 µg/ml. At the next higher concentration of 25000 µg/ml, the test substance was absolutely cytotoxic.
Comment: The reported increase is doubtful since a dose response was seen in the cultures from one of the two donors only. Furthermore, this increase in SCE frequency over the control was weak only and the statistically increased values in the cultures provided from donor 1 were below the control value from donor 2. Furthermore, possible cytotoxicity was not adressed in this paper. Generally, the SCE assay is not accepted to provide convincing evidence of mutagenicity but is rather a screening test. For clarification, the study authors themselves recommended further mutagenicity tests to be conducted.

Other test systems (Comet assay in tadpole erythrocytes, tests for DNA adducts in rats and mice, *Drosophila melanogaster*, plant cells)

Clements et al. (1997) investigated the genotoxicity of selected herbicides in *Rana catesbeiana* (bullfrog) tadpoles using the single-cell gel DNA electrophoresis test (`Comet´ assay). After a previous study had shown a higher amount of DNA damage in bullfrog tadpoles inhabiting small bodies of water in agricultural areas as compared to non-agricultural regions, the impact of Roundup and some other commonly used herbicides on the DNA of tadpole erythrocytes was investigated in this test system under alkaline conditions. This modification allows the detection of single-stranded DNA breaks which are indicated by an increase in length:width ratio of the DNA mass following electrophoresis. DNA was obtained from tadpole erythrocytes (nucleated cells in amphibians) after the animals had been exposed to different concentrations of Roundup in the surrounding water for 24 hours. Whereas the low dose of 1.69 mg/l did not cause evidence of DNA damage, a clear and dose-dependent effect became apparent at the following concentrations of 6.75 mg/l and 27 mg/l. At 27 mg/l, the effect level caused by the positive control substance methylmethanesulphonate (MMS) was already approached. The intended top dose level of 108 mg/l could not be evaluated since all tadpoles died during the exposure period. According to the study authors, the concentrations tested were well below the recommended application levels suggesting an environmental mutagenic hazard in particular for organisms living in small adjacent bodies of water that are usually the first to be affected by pesticide runoff.
Comment: Generally, information on genotoxic effects of pesticides under natural conditions is scarce and, thus, this test system may provide important information regarding environmental effects.
In this special case, however, it appears equivocal whether the observed impact on the DNA was indicative of a true mutagenic effect or rather caused by cytotoxicity. It is known that a positive response in the Comet assay may be not only the result of direct interaction with cellular DNA but can be also mediated by toxic and other effects causing apoptosis or necrosis. Cytotoxicity is not addressed in the publication because it is not directly measured in this test system. A certain degree of general

toxicity can be assumed since the highest dose was completely lethal to the tadpoles. This effect could be well in line with the toxicity of certain glyphosate formulations to aquatic organisms as reported in the monograph. The Roundup product tested by Clements et al. was made of 41% glyphosate IPA salt and MON 0818, i.e. the tallowamine surfactant which is already known to cause toxic effects in different test systems in vitro as well as in vivo. Of course, although there is some evidence of a cytotoxic mechanism behind the positive result in the Comet assay, a direct impact of the test compound on the DNA cannot be completely excluded.
At this time, it is not clear whether a positive result of this test obtained in tadpole erythrocytes, even if it was actually due to mutagenicity, would be of any relevance to human beings exposed. In particular, this is doubtful when the strong body of evidence that neither glyphosate nor its formulations are mutagenic as coming from many studies in various test systems is taken into consideration. Thus, the outcome of the Comet assay should be rather used for environmental hazard evaluation only. Again, the application of results obtained with one formulation to others must be critically regarded.

A possible impact on the DNA was also investigated by **Bolognesi et al. (1997)** in further experiments. A transient but significant effect towards DNA damage in liver and kidney was noted in the alkaline elution assay after glyphosate (300 mg/kg bw) or Roundup (900 mg/kg bw) had been administered once by the i.p. route to mice. This assay may indicate the induction of DNA single-strand breaks and alkali labile sites. A test for DNA oxidative damage suggested glyphosate and the formulation Roundup to stimulate oxidative metabolism in the liver (glyphosate) or in the kidney (Roundup) at 24 hours after application.
In a subsequent study from the same institute **(Peluso et al., 1998)**, a low incidence of DNA adducts was found by means of the very sensitive ^{32}P-postlabeling technique in the liver and kidney of mice following single intraperitoneal administration of Roundup. All tested concentrations (400, 500 and 600 mg Roundup/kg bw, corresponding to 122, 152, and 182 mg glyphosate salt/kg bw) caused DNA adducts in both organs. A dose response was to be seen. In contrast, treatment with the vehicle (i.e., a DMSO/olive oil mixture) and with doses of 130 and 270 mg glyphosate IPA salt/kg bw did not result in DNA adduct formation.
Comment: The data from the tests for DNA damage and stimulation of oxidative metabolism (Bolognesi et al., 1997) are hardly to interpret since the results are given in summary figures only which are based on pooled individual data. There are reporting inconsistencies, e.g. it is not clear how many animals were actually used for testing. A positive control substance was not included. Taking into account that glyphosate proved negative in the UDS assay which is generally accepted to indicate a more frequent occurrence of DNA damage and repair (see section B.5.4.1.3 in the monograph), the published findings are not considered to provide convincing evidence of an interaction with the DNA. Positive results in the alkaline elution assay may also occur as a result of toxic but not-mutagenic effects. Stimulation of oxidative metabolism is not a sign of mutagenicity but may elucidate a possible mechanism behind toxic effects.
The results of Peluso and his group suggest a direct effect on the DNA. It has been shown that the observed effects were related to administration of the formulation only but not to glyphosate IPA salt. Biological significance of the results is equivocal. Generally, it is questionable whether findings after i.p. administration can be applied to more realistic exposure conditions. Of course, the occurrence of such effects also after oral intake would be much more relevant for human health evaluation. Furthermore, some deficiencies of this study make a definitive assessment difficult. It is rather equivocal what a low incidence of DNA adducts per animal as compared to no adducts in the control group actually means since a positive control substance was not included. The degree of variation between the animals is not known because only mean values for the groups comprizing of 3 to 6 mice were reported and individual values are not given but would be helpful for interpretation of the results. Another point of concern is the lacking information on toxicity. At least with Roundup, one

could expect marked general toxicity when the observations reported from the micronucleus tests (see section I of this addendum) and from the acute intraperitonal toxicity studies (see section B.5.2.4 in the monograph) were taken into account. It is known that DNA adducts may be formed not only as a result of direct interaction of cellular DNA with chemicals but also occur naturally or can be even related to a treatment-dependent increase in endogenous metabolites. Thus, further characterization of these adducts and clarification of their nature would be desirable.

Kale et al. (1995) examined nine agricultural chemicals in the sex-linked recessive lethal test in *Drosphila melanogaster* for their ability to cause genotoxic damage to the germ cells leading to lethal mutations in the subsequent generations. The group of test compounds included two insecticides and seven herbicides among those were the glyphosate formulations Roundup and Pondmaster. Unlike the generally used method of feeding the test substance to adult males only, larvae were treated in this experiment. This modification was expected by the study scientists to improve the sensitivity of the test system. All products tested proved positive.

Comment: *This test system is not considered predictive for mutagenicity in mammals. Generally, tests in Drosophila are considered helpful for screening purposes. For glyphosate, however, a large database on the basis of much more reliable test systems does exist. Furthermore, since lethal changes in spermatogonia and spermatocytes were the relevant endpoint, it appears difficult to distinguish between mutagenicity and general toxicity. The dose level tested was not specified but it is stated in the publication that concentrations around the LC50 were used. At such a high dosage, some toxicity must be expected.*

An anaphase-telophase allium test in onion root cells was conducted by **Rank et al. (1993)** to detect a possible induction of chromosome aberrations. The exposure period was 24 hours. In this plant system, a significant increase in the occurrence of chromosome aberrations was noted at the two upper dose levels when Roundup was tested. However, there was no dose response, since the total incidence of aberrations at 1440 µg/l was twice that seen at 2880 µg/l. The authors attributed this lack of a clear dose response to cytotoxicity, however, mitotic index was not dramatically reduced (24.2 in the mean at 2880 µg/l versus 28.2 at 1440 µg/l). According to the investigators, the type and pattern of aberrations suggest rather spindle disturbances than clastogenicity in particular when compared to the effects caused by the positive control substance MMS. In contrast, the glyphosate IPA salt did not increase the frequency of chromosome aberrations in this experiment.

Comment: *The Roundup product tested was made of the IPA salt, tallowamine surfactant, and water (for details see description of the Ames test and the micronucleus test portions of this study above). The more pronounced effect of the formulation as compared to the IPA salt could be explained by an improved uptake by the onion root cells as mediated by the surfactant. However, genotoxic or aneugenic effects in a plant system are generally not accepted to be indicative of mutagenicity in mammals. For glyphosate and its formulations, a number of well-performed studies in mammals is available for this purpose. Generally, it appears questionable whether a herbicide should be tested for mutagenicity in a plant cell system since at least a certain degree of cytotoxicity must be expected.*

Assessment

In the whole, the published data are not sufficient to provide convincing evidence of mutagenic effects caused by glyphosate or its formulations. Of cours, the effects observed in different test systems cannot be totally ignored. Looking for an explanation, the data obtained in the mutagenicity studies with formulations (see section I) must be also considered. Taking all the findings together, the effects reported in the literature appear rather due to cytotoxic properties of the formulations than to a genotoxic mode of action. The same conclusion was also reached by the Danish EPA in

an assessment **(Rasmussen, 1997)** which was made available to the Rapporteur. It has been already known before, that cytotoxicity is much more pronounced with glyphosate formulations than with the active substance and, therefore, is probably due to by-products or impurities. In particular, surfactants are the agents to be suspected for causing such effects.
There are even data suggesting the possiblity of a direct interaction of glyphosate formulations with cellular DNA in some test systems. This is evidenced by a higher frequency of DNA adducts in mouse liver and kidneys following i.p. administration **(Peluso et al., 1998)** as well as from the Comet assay in tadpole erythrocytes **(Clements et al., 1997).** Since glyphosate active ingredient is apparently devoid of a DNA damaging potential (see monograph), these effects, if occurring, can be certainly assumed to be related to co-formulants. Damage to the DNA is not essentially indicative of mutagenicity but could also result from cytotoxicity. Irrespective of the origin of these effects on DNA level, they appear to be confined to very special exposure situations only and not to represent a health hazard to human beings.

Addendum to the monograph on glyphosate - Evaluation of possible mutagenic effects of formulations

III.

MUTAGENICITY TESTING OF SURFACTANTS

On the basis of the extensive mutagenicity database for glyphosate a.i. and formulations, the conclusion can be drawn that neither the active substance nor the formulations so far investigated are genotoxic. A certain potential of some formulations to cause damage to the DNA, however, cannot be excluded and might be related to the marked cytotoxic acitivity of these products. Data suggest that cytotoxicity is rather due to certain by-products used as surfactants than to the active ingredient. Thus, it is of particular interest to look at mutagenicity tests performed with such chemicals which are contained in glyphosate formulations mostly with the intention to improve the uptake of the herbicide glyphosate by the target plants. For three different surfactants, studies on mutagenicity of surfactants have been submitted. The studies are summarized in Table 5 and briefly listed below.

Table 5: Mutagenicity studies with surfactants contained in glyphosate formulations

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Ames test	MON 8080 dissolved in distilled water	S. typhimurium strains TA 98, 100, 1535, and 1537; plate incor-poration and spot test# performed	0.003 - 3.0 µl/plate (+/- S9) in the plate incorporation test	Negative up to 0.9 µl/ plate with and without activation; cyto-toxicity occurring at this dose, complete toxicity at 3 µl/plate avoiding counting of revertants	Flowers, 1981
Ames test	MON 0818 dissolved in DMSO	S. typhimurium strains TA 98, 100, 1535, 1537; plate incorpora-tion test with/ without metabolic activation	Lowest concentra-tions: 0.3 or 1 µg/plate, diffe-rent maximum amounts per plate reached for the strains, i.e. TA98:300µg (-S9) 1000µg (+S9); TA100 and TA1535: 100µg (+/-S9); TA1537:100µg(-S9) 300µg (+S9)	Negative. Cytotoxic effects occurring at the maximum dose levels avoiding evaluation and occasionally also at lower concen-trations. (Mutagenicity data for TA 1535 (+S9) not given probably due to excessive toxicity.)	Stegeman and Li, 1990
Ames test	Dodigen 4022 dissolved in distilled water	S. typhimurium strains TA 98, 100, 1535, 1537, 138; E.coli strain WP2uvrA; plate incorporation test	4 µg/plate - 10000 µg/plate (+/-S9)	Negative for mutagenicity. No cytotoxic effects observed.	Stammberger, 1992a
Cytogeneti c study for chromosome aberration s in vitro	Dodigen 4022 dissolved in cell culture medium	Chinese hamster V79 cells	0-600-3000-6000 µg/ml; (+/-S9); 4 h exposure, sampling at 7, 18 and 28 h after start of treatment	Negative for clasto-genicity and poly-ploidy. Reversible inhibition of cell cycle (mitotic in-dex↓) after 7 h at the highest dose (+/- S9). Cell survival rate↓ at 3000 µg/ml and above (only without activation).	Stammberger, 1992b
Micronu-cleus test	MON 0818 dissolved in corn oil	CD 1-mice (m/f), bone marrow erythrocytes	0 and 100 mg/kg bw; single i.p. injection; evaluation at 24 and 48 h after dosing	Negative. Also, no indications neither of general toxicity nor of bone marrow cytotoxicity to be observed.	Stegeman and Kier, 1998*

The spot test did not provide indications of a mutagenic response, however, does not allow quantitative assessment. This variation of the Ames test is no longer in use in routine genetic toxicology. Therefore, the data are not shown here.
* supplementary study

Flowers, L.J. (1981): Ames/Salmonella mutagenicity assay of MON 8080. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; Project no. ML-80-294/800281; Report no. MSL 1538. Dates of experimental work: 31 Oktober 1980 - 28 November 1980. GLP: No. When the study was performed, GLP was not compulsory. However, a Quality Assurance Audit statement is included. The study is considered acceptable.

Stegeman, S.D. and Li, A.P. (1990): Ames/Salmonella mutagenicity assay of MON 0818. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; Project no. EHL 89178/ML-89-461; Report no. MSL-10625. Dates of experimental work: 28 November 1989 - 29 January 1990. GLP: Yes (self-certification of the laboratory). A respective Quality Assurance Audit statement is included. The study is considered acceptable.

Stammberger, I. (1992 a): Dodigen 4022: Study of the mutagenic potential in strains of Salmonella typhimurium (Ames test) and Escherichia coli. Pharma Development Central Toxicology, Hoechst AG, Frankfurt/Main, Germany; Study no. 92.0336, Report no. 92.0467. Dates of experimental work: 03 June 1992 - 19 June 1992. GLP: Yes. The study is considered acceptable.

Stammberger, I. (1992 b): Dodigen 4022: Chromosome aberrations in vitro in V79 chinese hamster cells. Pharma Development Central Toxicology, Hoechst AG, Frankfurt/Main, Germany; Study no. 92.0337, Report no. 92.1024. Dates of experimental work: 22 July 1992 - 03 November 1992. GLP: Yes. The study is considered acceptable.

Stegeman, S.D. and Kier, L.D. (1998): Mouse micronucleus screening assay of MON-0818. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; Project no. EHL 89182/ML-89-463. Dates of experimental work: 06 November 1980 - 05 February 1990. GLP: Not stated in the report. The study is considered supplementary only since it was not in compliance with OECD recommendations for tests of this type. In particular, the only dose level used was too low for definitive assessment.

Furthermore, the notifier Monsanto submitted to the Rapporteur published data suggesting that also the sorbitol ester surfactants Tween 20 and Tween 80 proved negative in either the mouse lymphoma test or in the Ames test and the mouse micronucleus test, respectively. However, since these co-formulants were not contained in the glyphosate formulations for which mutagenic effects had been reported, the respective data were not reviewed in detail.

Assessment

The available studies clearly show a lack of mutagenicity of the tested surfactants in the limited number of test systems used confirming the negative outcome of respective studies with glyphosate formulations. In contrast, marked cytotoxicity was caused in the Ames test by the tallowamine surfactant MON 0118 as well as by the alkyle sulfate surfactant MON 8080 suggesting that cytotoxicity observed in mutagenicity testing of formulations (see sections I and II of this addendum) are mainly due these surfactants. This assumption is supported by the result of an Ames test using the surfactant-free Rodeo® formulation (Kier et al, 1992a) with no signs of cytotoxicity occurring. The more recently introduced surfactant Dodigen 4022 proved non-cytotoxic in the Ames test and caused cytotoxic effects in V79 cells at very high concentrations only.

It is widely accepted that cytotoxicity of a compound can result in positive results in mutagenicity assays and it is often difficult clearly to distinguish between true substance-related genotoxic effects and "mutagenicity" mediated by excessive cytotoxicity. A close relation between cytotoxicity and mutagenicity became apparent also in the chromosome aberration test with Dodigen 4022 (Stammberger, 1992b). The markedly reduced mitiotc index at the first sampling time indicating an adverse

effect at least of high doses (only the top dose concentration of 6000 µg/ml and the solvent control were assessed after 7 hours) was accompanied by a slight increase in chromosome aberration frequencies including and excluding gaps in the absence as well as in the presence of the metabolically activating S9 mix. However, at the later sampling times (18 and 28 h following substance application), the mitotic index had normalised again and there was no increase in the incidence of chromosome aberrations any more. Therefore, the test substance was considered negative in this test system. This example provides further evidence that suspected mutagenic effects of formulations as reported in section II might be readily due to cytotoxicity.

These results are in line with data suggesting a higher toxicity and irritancy of certain surfactants as compared to the active substance and to formulations as reported in chapter B.5.11 in the monograph. It can be also assumed that specific adverse effects of surfactants might have significantly contributed to the rather unexpected mammalian toxicity of some glyphosate formulations. Despite the low general toxicity of glyphosate technical, a number of poisoning incidents in humans sometimes resulting in death was reported in particular from asian countries (see chapter B.5.9 in the monograph).
Severe intoxication was mainly characterized by a decrease in blood pressure and further cardiovascular symptoms followed by pulmonary dysfunction and renal failure and by signs of irritation in the gastointestinal tract. Pathophyiology of poisoning is assumed to include irritation or corrosion of the intestinal mucosa resulting in electrolyte imbalances, hypovolemic shock and disturbances in the cardiovascular system. The respiratory signs, as well as renal symptoms, are considered secondary to this mechanism beeing caused either by pulmonary edema related to disturbed circulation or by aspiration pneumonia following emesis (**Sawada and Nagai, 1987**; see also monograph, chapter B.5.9). There is evidence that the first step, i.e. damage to gut mucosa, might be primarily caused by tallowamine surfactants due to their irritating properties.
Of course, the clinical reports on human posionings with glyphosate formulations are often difficult to interpret since most of the severe intoxications were attempts of suicide. In such cases, also the frequent concomitant intake of drugs and alcohol should be considered.
However, the hypothesis of surfactant effects being involved is further supported by mechanistic and pharmacological studies (see section B.5.8.2.3 in the monograph) suggesting that the acutely toxic effects may be caused by the tallowamine surfactant alone, too, and that toxicity may be even enhanced when complete Roundup formulations were tested.

Furthermore, according to the information available to the Rapporteur, the cases of severe or even fatal intoxication were related to the ingestion of glyphosate products containing tallowamine surfactant. **Sawada and Nagai (1987)** reported two cases of human poisonings with surfactants causing clinical signs resembling very much those observed after ingestion of large amounts of Roundup.

A possible potentiation of toxicity of glyphosate IPA salt and POEA in animals was reported by **Martinez and Brown (1991)** who tested the acute oral toxicity of Roundup formulations in rats. Using the intratracheal route of administration being of clinical relevance in cases of aspiration, the same authors observed a marked toxic effect of Roundup and of POEA alone to the lungs but this was much less pronounced with Polysorbate-80, i.e. another non-ionic surfactant.
Mucosal irritation in the respiratory tract caused by tallowamine surfactant may be also behind the much lower threshold level for adverse effects of a Roundup formulation as compared to glyphosate a.i. upon subacute inhalative exposure (see section B.5.3.3.2 in the monograph, also reported by WHO/IPCS in 1994).

A statement of the notifier Monsanto was submitted to the Rapporteur in October, 1998. In this paper, it is suggested that the toxic and cytotoxic effects of polyoxyethylenamine (POEA) were responsible for the observed

adverse effects on health and environment. Since it is an important objective to use environmentally safe and less toxic products, the polyoxyethylen tallowamine surfactants were replaced at least in some Monsanto products by others. The company stated that this decision was mainly based on the eye irritation potential and the aquatic toxicity related to the formerly used substances. Accordingly, in the formulations for which toxicological data have been submitted as part of the joint dossier of Monsanto and Cheminova, surfactants of this type are not contained any more. Indeed, cytotoxicity of other surfactants, e.g. Dodigen 4022, and their potential to cause acutely toxic or irritating effects are much lower as compared to POEA.

Thus, it can be expected that the replacement of toxic and irritating surfactants like POEA by other and less critical substances may reduce the risk of death or severe health effects following intentional or accidental ingestion of glyphosate products as well as the severity of eye or respiratory tract irritation.

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Author(s)	Annex point/ reference number	Year	Titel source (where different from company) report no. published or not BBA registration number	GLP or GEP status (where relevant)	Data protection claimed Y/N	Owner
Sawada, Y. and Nagai, Y.	AIIA-5.9	1987	Roundup® poisoning - its clinical observation. Possible involvement of surfactant. J. Clin. Exp. Med., 143, 25-27. Published	N		
Stammberger, I.	AIIA-5.4.1	1992	Dodigen 4022: Study of the mutagenic potential in strains of Salmonella typhimurium (Ames test) and Escherichia coli. Report no. 92.0467. Unpublished TOX 1999-324	Y		MOD
Stammberger, I.	AIIA-5.4.1	1992	Dodigen 4022: Chromosome aberrations in vitro in V79 Chinese hamster cells. Report no. 92.1024. Unpublished TOX97-50446 / Tox 1999-325	Y		MOD
Stegeman, S.D. and Kier, L.D.	AIIA-5.4.2	1998	Mouse micronucleus screening assay of MON 0818. Project no. EHL 89182/ML-89-463. Unpublished TOX 1999-240	N	Y	MOD
Stegeman, S.D. and Li, A.P.	AIIA-5.4.1	1990	Ames/salmonella mutagenicity assay of MON 0818. Report no. MSL-10625. Unpublished TOX 1999-241	N	Y	MOD
Vargas, A.A.T.	AIIA-5.4.1	1996	The Salmonella typhimurium reverse mutation by GLIFOS. BioAgri (Biotecnologia Agricola Ltda.), Piracicaba, Sao Paulo, Brazil on behalf of Cheminova; BioAgri Report G.1.1 - 050/96. Unpublished TOX 1999-321	N		MOD
Vigfusson, N.V. and Vyse, E.R.	AIIA-5.4.1; AIIA-5.8.2	1980	The effect of the pesticides, Dexon, Captan and Roundup, on sister-chromatid exchanges in human lymphocytes in vitro. Mutation Res., 79, 53-57. Published TOX97-00576	N	N	
Zaccaria, C.B.	AIIA-5.4.2	1996	A micronucleus study in mice for the product glifos. BioAgri Report G.1.2 - 060/96. Unpublished TOX 1999-253	N	N	MOD

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Your ref. : AP-WA1 004282-00

Brussels, 21-04-99

Dear Sirs,

Re: EC evaluation of Glyphosate (according to regulation EC Nr 3600/92 and concerning inclusion of the active substance Glyphosate in Annex I of Directive 91/414);
Addendum to the monograph on glyphosate - Evaluation of possible mutagenic effects of formulations.

The Rapporteur has completed an excellent and thorough evaluation of all the data available on the mutagenicity of glyphosate formulations. Monsanto commends the authors for their completeness and scholarly assessment of the information. Monsanto agrees with the Rapporteur's conclusions that neither glyphosate technical nor the tested formulations show evidence of any genotoxic properties which are relevant for human risk assessment. These conclusions are accurately stated in points 1 to 3 in the Abstract.

Monsanto has one major correction, which our partners in this submission, Cheminova, should confirm. We believe that Berol 907, last paragraph of page 2, is a polyoxyethylene tallowamine. This information may affect the overall recommendations of the rapporteur but not the conclusions regarding the lack of genotoxicity.

The following specific correction might improve the understanding of the addendum:

The term "by-products" is used to describe other non-active substances included in the formulation, specifically surfactants. This term most often connotes impurities produced unintentionally during manufacturing. Monsanto recommends the terms "co-formulants" or "non-active formulation ingredients" to avoid misunderstanding.

Page 2, Brief Description of formulations tested: substituted the word "from" for the word "by" in the phrase "According to information obtained **by** Monsanto..."

Page 3, Table 1. 3rd entry row - there is an extra "t" following "MON 14445"

Page 19, 2nd sentence in Assessment. "MON 0118" is incorrect and should be "MON 0818."

Monsanto believes different wording is appropriate in the following places:

Abstract, Point 4. The last sentence implies that there have been "adverse effects on health and environment" arising from POEA surfactant. In fact, the only adverse effects of significance to the discussion have been observed in abnormal exposure situations, like attempted suicides, or in artificial test systems. Roundup formulations containing POEA surfactant have been used for 25 years very successfully throughout the world without adverse effects from normal usage. It would be preferable to state that "The available data indicate that the surfactant polyoxyethylene tallowamine (POEA) was linked with irritant properties of formulations and with cytotoxicity in certain in vitro laboratory test systems."

Page 13, under Clement. Sentence refers to 108 mg/L as a concentration below recommended application level. Roundup maximum application rate is 12 L/ha, roughly equivalent to 13.2 kg/ha of formulated product. Assuming that tadpoles live in water at least 30 cm deep, the immediate post-application concentration following a 12 L/ha treatment to 30 cm-deep water accompanied by thorough mixing in the water column is 4.4 mg/L. Concentrations of 27 and 108 mg/L are clearly in excess of those encountered in use situations. Monsanto prefers that the doses judged by Clements to cause DNA damage in tadpoles be described as "exaggerated concentrations that are not relevant to those under allowed use patterns".

Monsanto believes that the Rapporteur's Addendum should conclude following the first paragraph under the heading "Assessment".
The subsequent discussion of irritancy, toxicity, and intentional suicide attempts is outside the scope of the stated topic, since it is not relevant to mutagenicity.

The topic of genotoxicity is a very important indicator of characteristics of serious concern. This indicator is generally viewed as a positive / negative characteristic, using the weight of the evidence. The Rapporteur's report clearly addresses this topic, and concludes that the answer for glyphosate and its formulations is "negative". Irritancy, toxicity, and aquatic effects are quite different. They are clearly dose dependent phenomena which are expected for surfactants at high dose levels but which will disappear at more dilute exposure levels. For each formulation that is considered for regulatory authorization, a group of studies is conducted that is specifically designed to evaluate these properties for that particular preparation, in order to judge acceptability and proper labeling. The regulatory decision on individual formulations should be based on these required tests, and not on an a priori judgment that a certain component is too irritating or toxic, regardless of its concentration in the product concerned. There is no need to adopt such a position when the specific data to make a judgment will be provided. Monsanto would prefer that the Rapporteur restrict the discussion to the topic in the title of the Addendum, and allow the individual Member States to judge the acceptability of irritancy and toxicity properties of individual formulations based on the specific required tests.

If the final paragraphs remain then Monsanto requests that the characterization of effects on humans who have intentionally ingested or aspirated Roundup formulations are called "Human suicide attempts" and not as "human poisonings". The present wording could be considered as inflammatory and is misleading. It is not until several sentences after the discussion begins later that the word "suicide" is used.

Monsanto believes that reference to the work of Martinez and Brown (1991) should be eliminated because of conflicting data (below) or modified to include this information

It should be noted that in the study of Martinez and Brown (1991), no supporting mathematical analysis or other basis for the conclusion (possible potentiation) was presented. In a similar study, Adam et al (1997) investigated the oral and intratracheal toxicities of POEA, glyphosate, and Roundup herbicide. These authors concluded that there appeared to be no synergism with glyphosate and POEA. A study by Baba et al (1989) demonstrated a lack of synergism. In that study, oral LD_{50}s were determined in rats, and the interactions between glyphosate and POEA were systematically evaluated. The authors concluded that the interaction between glyphosate and POEA was antagonistic rather than synergistic. Heydens and Farmer (1997) used the harmonic mean formula of Finney to compare the "expected" and "observed" LD_{50} and LC_{50} values for rats and aquatic species exposed to several combinations of glyphosate with other herbicides and/or surfactants. Therefore, there is no reliable evidence indicating synergistic interactions between glyphosate and surfactant.

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In the final paragraph, it is suggested that the replacement of POEA by other substances may reduce the risk of death or severe health effects. This statement is inappropriate in its present form because accidental ingestions of Roundup herbicide containing POEA surfactant have not resulted in deaths or other serious effects. Furthermore, there is no data to indicate that the intentional ingestion of other surfactants in the large quantities which occur in suicide cases would result in reduced mortality.

Yours sincerely,

William Graham
Registration Manager - Glyphosate
Monsanto Europe S.A., Brussels

cc : Mr. D. Flynn - ECCO-Team
(PSD) - York
K. Lystbaek - Cheminova

References

Adam, A., Marzuki, A., Abdul Rahman, H., and Abdul Aziz, M. 1997. The oral and intratracheal toxicities of Roundup® and its components to rats. Vet. Hum. Toxicol. **39** (3) : 147-151.

Baba *et al.* 1989 Japanese Journal of Toxicology Vol. 2 No. 4, 397-400.

Heydens, W.F. and Farmer, D.R. 1997. Combination of toxicology of glyphosate with surfactants and other herbicides. Fundamental and Applied Toxicology, Vol. 36, No. 1, Part 2, page 341.

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Lemvig, 21-04-99

Dear Sirs,

Re: EC evaluation of Glyphosate (according to regulation EC Nr 3600/92 and concerning inclusion of the active substance Glyphosate in Annex I of Directive 91/414);
Addendum to the monograph on glyphosate - Evaluation of possible mutagenic effects of formulations

Mutagenic potential of glyphosate formulations

We are in agreement with the clear conclusion that neither the active ingredient glyphosate nor glyphosate formulations assessed in the addendum have any mutagenic potential.

We enclose a document addressing the toxicological aspects of the addendum and the findings seen in relation to exposure as result of normal use of the products.

Berol 907

We can confirm that the composition of the Glifos formulation used for the Ames and micronucleus tests conducted in Brazil is identical to the composition of the Glyfos formulation marketed throughout the EU.

With reference to Table 1 on page 3 of the addendum we can furthermore confirm that Berol 907 in fact is a tallowamine surfactant.

With reference to the remark on page 2 of the addendum that acute toxicity studies were not submitted for the EU re-evaluation we can confirm that a full set of acute toxicity studies on Glyfos conducted under GLP are available.

In this connection it can be mentioned that Cheminova Agro for initial registrations throughout the EU developed an extensive Annnex III data package on the standard 360 g/l SI formulation (Glyfos) containing the tallowamine surfactant.

However, Annex III data on Glyfos was not included in the Monsanto/Cheminova dossier since Monsanto products were selected as representative products for the dossier.

Risk reduction recommendations

Referring to the conclusions of the abstract of the addendum, it is for risk reduction purposes recommended Member States to consider replacement of polyoxyethylene tallowamine (POEA) surfactants soonest possible and not to give new authorisations for PPP's containing this surfactant.

We fully support risk reduction measures to be taken by Member States when unacceptable risks has been demonstated according to existing Member State regulations (for products awaiting Annex 1 listing of the active ingredient(s) or according to Directive 97/57/EC (for products for which active ingredient(s) have been included in Annex 1).

Glyfos is currently authorized for broad spectra of uses in all Member States.

We are very confident that all current uses of Glyfos with the possible exception of a few aquatic uses will be determined to be fully acceptable according to Directive 97/57/EC when Glyfos is being reviewed by the Member States following Annex 1 inclusion of glyphosate.

References

Data protection was mistakenly not claimed for the two Cheminova mutagenicity studies conducted with Glifos (Vargas, A.A.T. (1996) and Zaccaria, C.B. (1996))

Please accept herewith our data protection claim for these studies.

We hope you find our comments helpful for the further evaluation process.

Yours sincerely,

pp Kristian Lystbaek
Cheminova Agro A/S, Lemvig

cc : Mr. D. Flynn - ECCO-Team (PSD)
W. Graham - Monsanto

Attachment

Dorrit Sondergaard
April 19, 1999

German proposal to replace POEA in glyphosate products.

The German call to member states not to accept glyphosate products containing POEA (polyoxyethylene tallowamines) is based on the alleged high cytotoxicity of these compounds as well as their contribution to an unacceptable general high toxicity of the products.

- One of the POEAs in question is Berol 907 from Akzo Nobel. The company indicates in the MSDS an acute oral LD_{50} of 1569 mg/kg bw, it is an eye irritant and skin effects are seen after prolonged contact due to defatting of the skin. The EU classification for health effects is indicated to be X_n with the R-phrases 36 (eye irritation) and 22(harmful when swallowed).

The numerous mutagen studies listed in the German evaluation do substantiate the claim that the cytotoxicity of the products containing POEA is higher than that of the active ingredient when the exposure to cells takes place in growth substrates as in the Ames test or the general toxicity is higher when they are injected intraperitoneally as in the Micronucleus test. These properties do to some extend interfere with the interpretation of the above mentioned studies.

Mutagen tests on products are not an EU requirement for approval of pesticides.
OECD states in its guideline for the micronucleus test, that if the results of the study should be used for risk assessment, the application should not be by injection but one relevant for practical conditions i.e oral or dermal.

The claim that the general toxicity is higher for the products is not quite as substantiated. Cheminova does for the time being sell 5 products within the community containing POEA. The acute oral LD_{50} is for all products > 5000 mg/kg bw and the dermal LD_{50} is >2000 mg/kg bw. These data are based on limit tests for four of the products and estimation for the remaining one. The inhalation LC_{50}, 4 hours, is or the four of these estimated to be > 4.86 mg/l.
This is to be compared with the acute oral LD_{50} and the acute dermal LD_{50} of glyphosate of >5000 mg/kg bw and >2000 mg/kg bw respectively also determined by limit tests. The acute inhalation LC_{50} for glyphosate is found to be 5 mg/l for an 4 hour exposure.

The acute intraperitoneal LD_{50} of glyphosate IPA salt when injected is > 2000 mg/kg bw. for males and 1383 mg/kg bw. for females. The products do seem to be more toxic than the a.i. when injected intraperiotoneally.

The products are slightly to moderately eye irritating, not all of them classified as irritants, and not to slightly skin irritating, and none of them classified.
Glyphosate IPA salt, which is the form in which its occurs in CHA products, is neither an eye nor a skin irritant.

It can from this be clearly seen that the toxicological properties of the products containing POEA do not differ from those of the active ingredient, glyphosate, as far as acute toxicity is concerned with the exception of intraperitoneal injection. The oral and dermal studies to determine acute toxicity are related to the conditions of practical exposure and therefore relevant for human risk assessment.
The risk that an exposure into the abdomen should take place must be regarded hypothetical and could only happen in connection with serious accidents.

The fact that the general toxicity of the products is higher than that of glyphosate when injected intraperitoneally and the cytoxicity is higher than that of glyphosate when the exposure takes place in a growth medium has neither relation to practical use and thus nor to human risk assessment. This should therefore not serve as basis for a decision to ban the use the POEA as surfactant in glyphosate products.

GLYPHOSATE - INFORMATION/QUESTIONS FOR THE MAMMALIAN TOXICOLOGY ECCO MEETING FROM THE PHYS CHEM ECCO MEETING

	INFORMATION	QUESTIONS
1.	**Sources – general information** 18 different sources of glyphosate. Two main pathways to manufacture glyphosate : the glycine process and the IDA process. Glyphosate is produced in 3 forms – the isopropyl salt, the sodium salt and the ammonium salt.	
2.	**IDA process** The Monsanto source was considered the definitive profile as it had the most comprehensive analytical suite, impurity profile and data package for the IDA process. For the IDA process, the other sources were compared to the Monsanto source. **Glycine process** For the glycine process, all sources were compared to the Agrichem source as it was the first source listed in the summary table produced by the glycine route for which data were provided.	
3.	**Sources that don't meet the FAO spec of 950g/kg (in some cases this may be because they have not presented tech spec on dry weight basis)** Sinon Industrias Afrasa Calliope (IDA process) Nufarm	
4.	**Sources for which a decision on comparability of sources is not possible until further data submitted** Feinchemie Herbex Sundat Pinus Alkaloida	
5.	**Potential differences between sources, need advice as to the significance of the impurities listed in summary table attached.** <u>IDA process – compared to Monsanto source</u> Agrichem Aragonesas Barclay Portman Sanachem <u>Glycine process – compared to Agrichem source</u> Calliope	Open point – ECCO Mammalian Toxicology meeting to provide advice to the overview meeting about the impurities in the different sources of glyphosate – are they of toxicological significance? (please see attached summary table)
6.	**Sources with FAO compliance and acceptable 5 batch analysis data and which are considered comparable.** Monsanto Cheminova Luxan IPC	

6373/ECCO/ASD/99

Glyphosate – impurity profile from the different manuf. ..rers (key attached)

	Content (g/kg)																	
	1	2	3	4	5	6 Tulip Task Force						7	8	9	10		11	12
Substance	**MOD**	**CHE**	**FSG**	**MAR**	**HPQ**	**AGC**	**ARA**	**INA**	**SDT**	**CAL**	**PIN**	**LUX**	**IPC**	**NUF**	**BCL**	**POR**	**ALK**	**SAC**
glyphosate	950	960	980	900	980	960[a] 950[b]	990	940	950	950[a] 900[b]	970	960	960	940	970	970	960[a] 960[b]	950
# Mod No 1	28	<0.65	-	2	-	<7 <6	<6	<6	-	<6 <20	<6	<6	28	1	2	-	- 20	1.6
# Mod No 2	17	10	7	3	-	3 2	3	-	-	<1 <7	-	<8	17	1	15	3.9	- 5	-
Mod No 3	9	-	-	-	-	-	-	-	-	-	-	-	5	1	-	-	-	-
# Mod No 4	10	7	-	1.5	-	<4 <4	<6	-	-	- <5	<30	<8	10	10	1	9.2	- 8	0.4
Mod No 5	9	7	2	-	-	-	-	-	-	-	-	-	-	10	6	0.54	-	-
MOD No 6	9	9	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-
# MOD No 7	12	1	-	<0.4	-	-	-	-	-	-	-	-	-	0.1	-	-	- <0.001	-
SO_4^{2-}/H_2SO_4 MOD No 8	4	-	-	-	-	-	-	-	-	-	-	<5	4	4	-	5	-	-
MOD No 9	5	-	-	-	-	- <1	<1	<1	-	<1 <1	<1	-	-	5	2	-	-	-
MOD No 10	8	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0.03	-	-
MOD No 11	7	0.43	-	0.3	-	- <2	<4	-	-	<1 <4	<10	<7	-	8	8	1.61	-	-
MOD No 12	3	0.43	-	-	-	<24 <5	<5	<40	-	<60 <1	<5	-	-	3	1	-	10	14.9

	Content (g/kg)																	
	1	2	3	4	5	6 Tulip Task Force						7	8	9	10		11	12
Substance	**MOD**	**CHE**	**FSG**	**MAR**	**HPQ**	**AGC**	**ARA**	**INA**	**SDT**	**CAL**	**PIN**	**LUX**	**IPC**	**NUF**	**BCL**	**POR**	**ALK**	**SAC**
formaldehyde MOD No 13	3	1.5	-	-	-	- -	-	-	-	-	-	-	-	3	-	-	<5 ppm	-
  MOD No 14	2.5 ppm	1 ppm	-	-	-	nq* nq*	nq*	nq*	-	nq* nq*	nq*	-	-	2.5 ppm	2.5pp m	-	2 ppm <0.001 ppb	-
water CHE No 1	-	0	16	164	17.5	<1 60	<1	<3	-	<2 <100	1	<5	8		10		2	
Cl⁻/HCl CHE No 2	-	1.5	-	-	1.5	<1	1	4	-	3 2	1	-	-	-	-	-	7	-
formic acid AGC No 3	-	-	-	-	-	<0.05 -	-	<0.05	-	<0.05 -	-	-	-	2	-	-	-	-
  AGC No 4						<0.05 -	-	<0.05	-	<0.05 -								
  AGC No 5	-	-	-	-	-	<11 <1	2	<11	-	<15 <1	<1	-	-	-	2	1.4	5	-
  AGC No 9	-	-	-	-	-	<9 <9	<5	<5	-	<5 4	<5	-	-	-	-	-	-	-
  AGC No 11	-	-	-	-	-	<4 <4	<5	-	-	-	<5	-	-	-	1	-	-	-
  AGC No 12	-	-	-	-	-	<4 <11	-	-	-	-	-	-	-	-	-	-	-	-
  AGC No 13	-	-	-	-	-	- <1	-	-	-	-	-	-	-	-	-	-	-	-
  NUF No 12	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	--
  NUF No 13	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-

	Content (g/kg)																	
	1	2	3	4	5	6 Tulip Task Force						7	8	9	10		11	12
Substance	**MOD**	**CHE**	**FSG**	**MAR**	**HPQ**	**AGC**	**ARA**	**INA**	**SDT**	**CAL**	**PIN**	**LUX**	**IPC**	**NUF**	**BCL**	**POR**	**ALK**	**SAC**
NUF No 14	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
NUF No 19														4				
POR No 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	11	-
ALK No 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-
ALK No 7	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	<0.01	-
ALK No 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	-
ALK No 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- <0.015	-
ALK No 12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5 ppm	-
SAC No 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.7
SAC No 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.4
HPQ No 1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
unknown	-	-	>1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7

# = impurities listed in FAO spec
a= glycine process
b= IDA process
*nq= not quantified

KEY

MOD	Monsanto
CHE	Cheminova
FSG	Feinchemie Schwebda
MAR	Sinon
HPQ	Herbex
AGC	Agrichem
ARA	Aragonesas
INA	Industrias Afrasa
SDT	Sundat
CAL	Calliope
PIN	Pinus
LUX	Luxan
IPC	IPC
NUF	Nufarm
BCL	Barclay
POR	Portman
ALK	Alkaloida
SAC	Sanachem

Comments on Reports of Lioi et al. (1998a and 1998b) on Studies of Genotoxicity and Oxidative Stress for Glyphosate and Other Compounds in Culture Human and Bovine Lymphocytes

Recent Lioi et al. published two papers examining genotoxic and toxic effects of glyphosate and other pesticides on *in vitro* cultured human (Lioi et al., 1998a) and bovine (Lioi et al., 1998b) lymphocytes. These papers claim that glyphosate induced chromosome aberrations (CA's), sister chromatid exchanges (SCE's) and glucose-6-phosphate dehydrogenase activity (G6PD) in cultured lymphocytes. The authors suggest that induction of G6PD is evidence for induction of oxidative stress. Close examination of the data in these papers and comparison with other experimental results reveals some very disturbing features of the data. There are some clear protocol deficiencies and contradictions between experiments and with published literature that may indicate inexperience in scoring, interpreting and reporting results for these types of assays. The effects for glyphosate are reported at dose levels that are hundreds or thousands of fold lower than dose levels required to elicit biological effects in a number of other studies using cultured mammalian cells. These include an *in vitro* human lymphocyte cytogenetic assay of glyphosate (van de Waart, 1995) that did not find any induction of clastogenic effects at upper dose levels that were10-30 times higher than the highest levels used by Lioi et al.;. This study directly contradicts the findings of Lioi et al. Given the negative responses for glyphosate in a number of *in vitro* and *in vivo* genotoxicity assays and the very unusual aspects of the Lioi et al. data, the Lioi et al. reports should not be accepted as valid indications of *in vitro* glyphosate genotoxicity without confirmation and explanation of the unusual features of the data.

The protocols used for the Lioi et al. experiments used an exposure of the lymphocytes for 72 hr which is not an unusual practice for this assay. As acknowledged by the authors in their discussion (Lioi et al., 1998a), this is not commonly considered the best exposure time for detection of CA's because CA's are observed at the first mitosis after induction of damage. Examples of positive compounds requiring even 48 hr of incubation for detection are very rare (see e.g. Henderson et al., 1996). The authors have not presented any data on other time points and, particularly for the human lymphocyte experiments, the mitotic index data do not suggest that there is any reason to suspect cell cycle delay induced by the test compounds.

Several features of the Lioi et al. CA and SCE scoring observations are unusual and some are in conflict between the author's own experiments or with literature data. The reported frequency of gaps is very low and the observation that CA's, but not gaps, increase with treatment is strange. Usually, increases in gaps occur in parallel with increases in chromatid breaks. The data on chromosome aberrations are inconsistent between the human and bovine lymphocyte studies. In the human lymphocyte studies large increases in "chromosome type" breaks were reported, but the authors reported "no fragments or chromosomal rearrangements" in the bovine lymphocyte study. This discrepancy is not discussed at all by the authors. It is possible that there may be some

confusion between isochromatid breaks and chromosome breaks, but if so this reflects inexperience of the authors in characterizing and reporting aberrations. The SCE per cell control values reported for human lymphocytes by Liao et al. (1.9-2.2) are considerably lower than common literature values (generally ranging >5-10 per cell, see table on control values). This discrepancy may also reflect some inexperience in scoring by the authors.

Yet another unusual feature of the data in these papers is the remarkably similar dose responses for different pesticides. In the human lymphocyte studies very similar dose response patterns for percent aberrant cells, SCE's per cell, induction of G6PD and cell killing were observed for glyphosate, vinclozolin and atrazine. These data are so similar that they appear to be replicate experiments using a single compound rather than experiments with three different compounds. The fact that this concordance extends to the putative mechanistic endpoint, G6PD induction, requires a rather extraordinary postulate that the same mechanism is operating at the same dose levels for compounds with totally different modes of action for their pesticidal actions and quite dissimilar chemical structures. It is quite difficult to understand why all of these compounds would induce oxidative stress equally at the same dose levels. It is also not clear that induction of oxidative stress, per se, would be expected to result in the CA and SCE effects observed in these studies. In the bovine lymphocyte studies there are the same parallels between results for glyphosate and vinclozolin (atrazine was not tested) and similar comments apply.

A surprising feature of these data relating specifically to glyphosate are the indications of biological effects of glyphosate on mammalian cells at dose levels ranging from 8.5-170 μM. Glyphosate is an inhibitor of an enzyme involved in aromatic amino acid synthesis in plants and is quite non-toxic to mammals. This observation extends to experiments with cultured mammalian cells. In the CHO/HGPRT system toxicity of glyphosate was only achieved at >100 mM levels for a 3 hr exposure and no toxicity was observed at levels up to 0.74 mM in primary hepatocytes exposed for 18 hr (Li and Long, 1988; see attached table). Vigfusson and Vyse (reported toxicity in human lymphocytes exposed for 72 hr to 65 mM glyphosate, although there is a discrepancy in their reporting of dose levels on a molar or mg/ml basis. These levels are in the range of several hundred to a thousand fold higher than levels reported by Lioi et al. to induce chromosome aberrations, SCE's , G6PD and toxicity. These differences cannot be simply dismissed as a difference in endpoints (e.g., toxicity vs. chromosome effects) because it would be highly unusual for genetic damage to be observed at dose levels hundreds or thousands of times lower than toxic dose levels. Furthermore, there are reported indications of glyphosate toxicity (e.g. lower MI) in bovine lymphocytes at 17 uM and higher reported by Lioi. The length of exposure is longer in the Liao studies of CA and SCE than the CHO or hepatocyte studies but they reported G6PD induction and increases in cell killing at very low dose levels after only 6 hr of exposure.

A study performed by Notox laboratory (van de Waart, 1995) clearly contradicts the results of Lioi et al. This study, conducted according to OECD Good Principles of Good Laboratory Practice, found that glyphosate did not induce CA's in cultured human lymphocytes. The dose levels tested in this study were up to 1.4 mM in the absence of S9 for 24 and 48 hr exposures and up to 3 mM in the presence of S9 with a 3 hr incubation. Results were replicated in independent experiments. Thus, this study produced no evidence of induction of chromosome aberrations by glyphosate at upper dose levels ranging from 10-30 times those used by Lioi et al.

Evaluation of the data reported by Lioi et al. clearly reveal some unusual features, suboptimal study design and internal contradictions that raise questions about the reliability of these studies. Comparison with results from other cultured mammalian cell studies indicates that the Lioi observed effects at dose levels of glyphosate that are much lower than dose levels giving biological effects. A comparable human lymphocyte study conducted at dose levels 10-30 times higher than those employed by Lioi et al. did not indicate any CA aberration induction by glyphosate in independently repeated experiments. A number of well validated in vitro mammalian and in vivo mammalian studies have not found genotoxic effects, including several studies measuring chromosome aberration or micronucleus induction in vivo. It is clear that the results reported by Lioi et al. are very aberrant and should not be given weight in evaluating the genotoxicity of glyphosate unless they can be replicated and the curious features of the data satisfactorily explained.

Even though the glyphosate used was claimed to have high purity (≥98%) the form of the glyphosate (e.g. salt or free acid) used by Lioi et al. is not clear and the possibility of toxic impurities must be considered given the surprising toxicity of glyphosate reported in their experiments.

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Lioi MB, Scarfi, MR, Santoro A, Barbieri, R, Zeni O, Salvemini F, Di Berardino D and Ursini MV (1998a). Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed in vitro to gliphosate, vinclozolin, atrazine and DPX-E9636. *Env. and Molec. Mutagenesis 32*, 39-46.

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van de Waart IEJ (1995). Evaluation of the ability of glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat). Notox Project 141918

Vigfusson NV and Vyse ER (1980). The effect of the pesticides, dexon, captan and roundup, on sister-chromatid exchanges in human lymphocytes in vitro. *Mutation Res. 79*, 53-57.

Comparison of Reported Biological Effects of Glyphosate in Cultured Mammalian Cells

Cell Type	Exposure Time	Concentration	Effects	Ref.
CHO	3 hr	29-133 mM	reduced r.s. at >103 mM	Li & Long (1988)
Primary rat hepatocyte	18-20 hr	up to .0.74 mM	no toxicity	Li & Long (1988)
Human lymphocytes	72 hr	.065-65 mM	toxic at 65 mM SCE at 0.65 and 6.5 mM	Vigfusson & Vyse (1980)
Human lymphocytes	72 hr	0.0085-0.051 mM	CA and SCE induction	Liao et al. (1998a)
	6 hr	0.0085-0.051 mM	G6PD induction	Liao et al. (1998a)
	6 hr	0.0085-0.051 mM	% of cell killing	Liao et al. (1998a)
Bovine lymphocytes	72 hr	0.017-0.170 mM	CA and decreased MI	Liao et al. (1998b)
	72 hr	0.017-0.170 mM	SCE	Liao et al. (1998b)
	6 hr	0.0017-0.170 mM	G6PD induction	Liao et al. (1998b)
Human lymphocytes	24 hr (-S9)	0.19-1.97 mM	decreased MI	van de Waart, 1995
	48 hr (-S9)	0.33-1.97 mM	decreased MI	van de Waart, 1995
	3 hr (+S9)	0.19-3.00 mM	decreased MI	van de Waart, 1995

Abbreviations:

r.s.--relative survival
CA--chromosome aberrations
SCE--sister chromatid exchange
G6PD--glucose-6-phosphate dehydrogenase
MI--mitotic index

References:

Lioi MB, Scarfi, MR, Santoro A, Barbieri, R, Zeni O, Salvemini F, Di Berardino D and Ursini MV (1998a). Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed in vitro to gliphosate, vinclozolin, atrazine and DPX-E9636. *Env. and Molec. Mutagenesis 32*, 39-46.

Lioi MB, Scarfi MR, Santoro A, Barbieri R, Zeni O, Di Berardino D and Ursini MV (1998b). Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. *Mutation Res. 403*, 13-20.

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In Vitro Human Lymphocyte Chromosome Aberration and SCE Control Data

Chromosome aberrations

Category	Unit	Mean	Ref.
gaps	per 100 metaphases	0.50	Anderson et al. (1991)
chromatid deletions	per 100 metaphases	0.27	Anderson et al. (1991)
chromatic exchanges	per 100 metaphases	0.02	Anderson et al. (1991)
chromosome deletions	per 100 metaphases	0.33	Anderson et al. (1991)
chromosome exchanges	per 100 metaphases	0.04	Anderson et al. (1991)
aberrant metaphases	percent	0.68	Anderson et al. (1991)
aberrant metaphases	percent	1-2 (est.)	Nordic Study Group (1990)
achromatic lesions	per 100 cells	4.147	Bender et al. (1989)
chromatid deletions	per 100 cells	0.813	Bender et al. (1989)
isochromatid deletions	per 100 cells	0.039	Bender et al. (1989)
chromatid exchanges	per 100 cells	0.045	Bender et al. (1989)
chromosome deletions	per 100 cells	0.388	Bender et al. (1989)
crhomosome rings	per 100 cells	0.02	Bender et al. (1989)
dicentrics	per 100 cells	0.160	Bender et al. (1989)
translocations	per 100 cells	0.044	Bender et al. (1989)

Sister Chromatid Exchange

sister chromatid exchanges	per cell	6.57	Anderson et al. (1991)
sister chromatid exchanges	per cell	5-14 (est.)	Nordic Study Group (1990)
sister chromatid exchanges	per cell	8.057	Bender et al. (1989)

References:

Anderson D, Francis AJ, Godbert P, Jenkinson PC and Butterworth KR (1991). Chromosome aberrations (CA), sister-chromatid exchanges (SCE) and mitogen-induced blastogenesis in cultured peripheral lymphocytes from 48 control individuals sampled 8 times over 2 years. Mutation Res. 250, 467-476

Bender MA, Preston RJ, Leonard RC, Pyatt, BE and Gooch PC (1989). Chromosomal aberration and sister-chromatid exchange frequencies in peripheral blood lymphocytes of a large human population sample. Mutation Res. 212, 149-154.

Nordic Study Group on the Health Risk of Chromosomal Damage (1990). A Nordic data base on somatic chromosome damage in humans. Mutation Res. 241, 325-337.

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EUROPEAN COMMISSION
DIRECTORATE-GENERAL VI
AGRICULTURE
Public, animal and plant health
VI.B.II.1 Legislation relating to crop products and animal nutrition

Brussels,
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P.S.D.
28 AUG 1998
I.D. No. 51955

Dear Dr Lundehn,

I am enclosing a document which the Commission has received from the World Wide Fund for Nature. This document contains evaluation information from third parties which ave been submitted within the framework of Article 6 (4) of Commission Regulation 3600/92/EC concerning the rules for the implementation of the first stage of the programme of work for the re-evaluation of plant protection products.

May I ask you to table this document at the relevant peer review meetings for due consideration and discussion. Can I also ask that the results of discussions be noted in the meeting reports together with reference to the document.

I am sending a copy of this letter to Mr Darren Flynn as the designated co-ordinator of the peer review meetings being organised in the PSD, York, UK.

Yours sincerely

G.del Bino
Head of Division

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57 ECCO/PSD/98

Enclosures: World Wide Fund for Nature Report, Agriculture and Environment "*Evaluation of pesticides which disrupt the endocrine and the reproductive system - summary*" (ref:A/120412)

c.c.: Mr Darren Flynn, ,MAAF, PSD, York,UK.

Mr Redford.

Please arrange for this document to be tabled for consideration at ECCO 64, ECCO 66 and ECCO 72.

cc Mrs Harris
Mr Warren
Mr Cook

DF 1/9/98

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WWF®

A World Wide Fund for Nature Report

Agriculture and Environment

A1/120412 4/6/98

Evaluation of Pesticides which disrupt the Endocrine and the Reproductive System

-Summary-

EVALUATION OF PESTICIDES LICENSED IN GERMANY WHICH DISRUPT THE HORMONE- AND REPRODUCTIVE SYSTEM

A WWF-Germany Report - Summary

by Dr. Andrea Dankwardt*

A number of chemicals such as pesticides are known to interfere with the endocrine system and thereby impair fertility and the development of animals and possibly of humans. Different attempts to formulate a definition of endocrine disrupting or modulating substances are existing. WWF-Germany prefers the definition of the US EPA document from 1997 *Special Report on Environmental Endocrine Disruption: An Effects Assessment and Analysis:*

> *"An environmental endocrine or hormone disruptor may be defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour."*

nicals may bind to sex hormone receptors, activate them and thus lead to responses similar to endogeneous estrogens and androgens. They may also bind to hormone receptors without activating them. However, by this they block binding of endogeneous hormones which therefore cannot be active. Beside such receptor-mediated direct actions a number of indirect (anti)estrogenic and (anti)androgenic reactions are possible. Those include changes in the concentration of hormone receptors in the target organs, interference with the biosynthesis of hormones in the endocrine organs, or effects on the biotransformation in the liver. Furthermore, binding of hormones to proteins in the blood plasma as well as the endocrine activity of pituitary and hypothalamus may be influenced.

In this study the test methods usually applied for the investigation of endocrine effects are listed. These are in vivo and in vitro assay systems. The following pesticides licensed in Germany showed estrogenic activity in different assays systems: Amitraz, lindane (not licensed in 1998), parathion-methyl, permethrin, triadimefon and s-triazines (simazine, atrazine (not licensed in 1998). No anti-estrogenic or androgenic properties were observed with li- d pesticides. Atrazine, lindane, linuron, procymidon, pyrethroides, vinclozolin and its metabolites showed anti-androgenic activity.

Some pesticides may also disrupt steroid metabolism. Atrazine, for example, induces the aromatase, an enzyme that transforms androgens to estrogenes. Conazole fungicides also interfere with the steroid hormone biosynthesis by inhibiting necessary enzymes. This can lead to a reduction in steroid hormone level. Carbofuran and lindane also influence steroid metabolism.

Amitrole, metribuzin, some dithiocarbamtes such as maneb, mancozeb and zineb as well as some pyrethroids can disturb thyroid function. Usually the synthesis of thyroid hormones is inhibited. This leads to an enlargement of the thyroid.

An influence on the gonadotrophic hormones is assumed by some pesticides such as amitraz, some organophosphorous pesticides and dithiocarbamates. For example, interferences with

the release of luteinizing hormone (LH) was observed. The disturbances of the hormone balance may then lead to changes in the gonads, e.g. to a lower weight of the testis or the ovaries.

Many pesticides also influence spermatogenesis and the number and quality of sperms, for example organophosphorous compounds, dithiocarbamates, copper fungicides and some pyrethroids. Some pesticides have been reported to impair female reproduction and the development of offspring, respectively. Among those pesticides are 2,4-D and some dithiocarbamates. Organophosphorous pesticides probably lead to reproductive effects by interfering with hormones of the pituitary-gonad-axis. Furthermore, pesticides can influence the nervous and immune system as well as the behaviour.

In the following table pesticides are listed that are endocrine disruptors or toxic to reproduction. For an estimation of the ecological relevance market volumes of pesticides, physicochemical properties such as the octanol-water partition coefficient and bioconcentration factor are given. The effects of the pesticides on the endocrine system and reproduction is listed.

Different in vivo and in vitro assay systems are currently used by regulatory agencies for the evaluation of reproductive and developmental toxicity. However, these assays may not be competent in detecting endocrine disrupting activity. Some of the assays may be improved in that regard by adding new endpoints such as determination of sex hormone concentrations, induction of vitellogenin, gonad histology, vaginal cytology, etc. For some applications and organisms, however, new assays have to be developed and validated, respectively. General monitoring programmes for the determination of wild populations should be extended and contain parameters for the evaluation of potential endocrine disruption, e.g. vitellogenin measurements in fish.

Already licensed pesticides should be reassessed for their potential endocrine activity. There is especially the need to investigate the endocrine effects of pesticides in the same group as those substances already identified as endocrine disruptors, for example other triazines or diuron. For the registration of new compounds assays to evaluate endocrine activity should be included in the approval requirements of the EC Directive 91/414. The process to select and validate assays systems to be used by regulatory agencies has to be speeded up.

For a better risk evaluation more data on tissue concentrations of the target organs are needed. The bioavailability of endocrine active chemicals should be investigated and more emphasis has to be laid on the investigation of the effect of mixtures. It should also be analysed whether important metabolites of pesticides that themselves show no hormonal activity may have an endocrine disrupting effect. There is a need to reassess adjuvants used in pesticide formulations for their endocrine activity. Compounds showing effects on the endocrine system or reproduction, such as alkylphenolic surfactants, should be phased out.

Bremen/München, 5.5.1998

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TABLE: EVALUATION OF PESTICIDES LICENSED IN GERMANY THAT DISRUPT HORMONES AND REPRODUCTION

(Explanations see legend at end of table)

Pesticides	Group	Market volume (t/a)	WHO-Cl / Cancerog.	Physico-chem. parameters log P	log BCF	Probability of exposition	Direct effects SH, SR	Indirect effects Thyroid	Indirect effects GtH, Gon	Sperm quality	Repro-ductive toxicity
Abamectin	A	low	?	9.9×10^3	n.D.					+	
Amitraz	A Amidin	low	III	5.5	n.D.		+		+		+
Amitrol*	H Triazole	> 100 (IVA '92)	-	-0.85	n.D.	-		+		+	+
Benomyl	F Benzimidazole	low	-/Cq (EPA)	23.4	2.46	?			+	+	+
Bromoxynil	H Hydroxy-benzonitril	> 100 (IVA '95)	II	2.8	2.11	+			+		
Carbendazim	F Benzimidazole	< 100 (IVA '95)	-	1.6	n.D.				+	+	+
Carbofuran	I Carbamate	32 (H, 8)	Ib	1.42	2.07	?	+		+	+	+
Chlorfenvinphos	I OP	low	Ia	2.99	2.67	?			+		
Chlorpyriphos	I OP	low	II	4.7	2.88	?				+	+
Conazole:	F Conazole			n.D.	n.D.		+				
Bitertanol		> 100	-			?					+
Cyproconazol		low	III								+
Epiconazol		> 100				?					+
Ketoconazol		low									+
Propiconazol		> 100	II	3.65	n.D.	+					
Tebuconazol		> 200	-								
Triadimefon		low	III								+
Triadimenol		> 100	III								+
2,4-D	H Phenoxyacetic acid	> 200 (IVA '95)	II/2B (IARC, EPA)	2.81	1.85	+				+	+

Pesticides	Group	Market volume (t)	WHO:G1/ Cancerog.	Physico-chem. parameters		Propability of exposition	Direct effects SH, SR	Indirect effects Thy	Indirect effects GtH, Gon	Sperm quality	Reprod. toxicity
				log P	log BCF						
Demeton-S-methyl (Oxydemeton-S-methyl)	I OP	low > 50 (IVA'95)	Ib	-1.52	n.D.				+	+	+
Dichlorvos	I OP	< 100 (OSPAR)	Ib/ 2B (IARC), Cq (EPA)	1.9	1.9	?			+	+	
Dimethoat	I OP	> 50 (IVA'95)	II	1.9	1.6	?			+	+	+
Diuron	H Urea derivative	low	?	n.D.	n.D.						
Fentinacetat	F Organotin	95 (H, 14)	II	3.4	n.D.	?			+		+
Fluazifop-butyl	H Phenoxyalkanic acid	low	-	4.5	n.D.	?			+		
Glufosinat	H OP	low	III	n.D.	n.D.						+
Glyphosat	H Aminophosphoric acid	> 1000 (IVA'95)	-	n.D.	-0.5						
Ioxynil	H Hydroxy-benzonitrile	low	?	0.9	0.48			+			
Kupferoxychlor	F Copper compounds	low	?	n.D.	n.D.				+	+	
Kupfersulfat	F Copper compounds	low	?	n.D.	4.48				+	+	
Lindan*	I Halogenated hydrocarbons	< 100 (OSPAR)	II/2b (d-B) (IARC) B2/c (EPA)	4.0	5.0	+	+			+	+

Pesticides	Group	Mar... volu... (t/a)	WHO-Cl/... Cancerog...	Physico-chem. parameters		Propabi... of exposition	Direct effects SH/SR	Indirect effects Thyroid	Indirect effects GH/Gon	Sperm... quality	Reproductive toxicity
				log P	log BCF						
Linuron (Diuron?)	H Urea derivative	low	-/ C (EPA)	3.0	2.2		+			+	
Mancozeb	F Dithiocarbamate	> 1000 (IVA´95)	-/ B2 (EPA)	n.D.	n.D.	-		+		+	
Maneb	F Dithiocarbamate	> 200 (IVA´95)	-/ (IARC) B2 (EPA)	n.D.	1	-		+			
Metam-Na	B Dithiocarbamate	> 200 (IVA´93)	II	-2.04	-0.5	-			+		
Metiram	F Dithiocarbamate	< 500 (IVA´95)	-	n.D.	n.D.	?			+		
Methylbromid	B Halogenated hydrocarbons	low	?	1.19	0.67				+		
Metribuzin	H Triazinon	low	-	1.6	1			+			
Paraquat	H Bipyridinium	low		n.D.	0.08					+	
Parathion	I OP	> 50 (IVA´95)	Ia/ C (EPA)	3.8	2.9	?			+		+
Parathion-methyl	I OP	low	Ia	1.9	1.65		+		+	+	+
Phosphamidon	H OP	low	?	0.8	0.04				+	+	
Procymidon	F Dichloranilid	low	-	2.98	n.D.		+		+		

Pesticides	Group	Market volume (tons)	WHO/IARC Cancerog.	Physico-chem. parameters		Probability of exposition	Direct effects SH, SR	Indirect effects Thyroid	Indirect effects GTH, Gon	Sperm quality	Reprod. derived toxicity
				log P	log BCF						
Pyrethroide	I			n.D.	n.D.		+		+	+	+
Bioallethrin	Pyrethroide	low					+				
Cypermethrin		low	II							+	+
Deltamehrin		2 (H, 19)	II							+	+
Esfenvalerat		low	II								+
Fenvalerat		low	II								+
Permethrin		6 (H, 17)	II/3 (IARC)		3.3		+			+	
Pyrethrine		low	Cq (EPA)				+				
Quizalofop-ethyl	H Phenoxyalkanic acid	low	III	3.72	n.D.				+		
Thiram	F Dithiocarbamate	335 (H, 17)	III	3.4	3	+			+	+	
Triazine	H	?	?								
Atrazin*	s-Triazine			3.1	3.4	?	+		+		
Simazin				3	3.2	?	+				
Trifluralin	H Toluidin	> 100 (IVA'95)	-/ (IARC) Cq (EPA)	5.07	3.7	+		+			
Vinclozolin	F	low	-	3.03	n.D.		+		+		
Zineb	F Dithiocarbamate	low	-/ (IARC)	?	2.2		+	+			
Carbaryl*	I	-	?	2.36	1.9		+		+	+	
DDT*	I	-	?	6.1	6.1	+	+				+
Endosulfan*	I	-	?	4.65	4.1	+	+		+		+

*Pesticides not licensed in Germany

Legend

Group:
H: Herbicide I: Insekticide F: Fungicide A: Akaricide B: Compound for soil fumigation OP: Organophosphorous compounds

Market volume (in tons per year):
IVA'92 - IVA'95: Important pesticides in Germany according to market volume, IVA (Industrieverband Agrar), 1993-1996, Frankfurt a. Main.
H: Results of the "Hille-Erhebung" (1988), calculated for Germany (1 = most important pesticide). These values are only given, if no new data were available.
low: Market volume not known, but relatively low (because no IVA-Data).

WHO-Class/Cancerogenicity:
Ia: Extremely hazardous
Ib: Highly hazardous
II: Moderately hazardous
III: Slightly hazardous
-: Unlikely to present acute hazard
?: No data available
References: WHO (1997) The WHO recommended classification of pesticides by hazard and guidelines to classification 1996-1997, ICPS, WHO/PCS/96.3.
Plygers, E., Sadowska, A. (1994) Pesticides et Cancer Humain, Revue, AVES Societe d'Etudes Ornithologiques.

Cancerogenicity
IARC 2a: Probable human cancerogen.
2b: Possible human cancerogen
3: Not classifiable as to human carcinogenicity
4: Probably not cancerogen for humans

EPA A: Human carcinogen: sufficient evidence of cancer causality from human epidemiologic studies
B1: Probable human carcinogen: limited evidence of carcogenicity from human epidemiologic studies
B2: Probable human carcinogen: sufficient evidence of carcogenicity from animal studies
C: Possible human carcinogen: limited evidence of carcogenicity from animal studies
Cq: A risk evaluation can be performed by extrapolation from animal experiments according to the "low dose" model
D: Not classifiable as to human carcinogenicity
E: Evidence of non-carcinogenicity for humans

Physico-chemical parameters:
log P: Octanol-water-partition coeffizient
log BCF: Bio concentration factor
n.D.: No data available
References: IVA (Industrieverband Agrar), Karlstraße 21, Frankfurt a. Main.
Koch, R. (1989) Umweltchemikalien. VCH Verlagsgesellschaft, Weinheim.

Probability of exposition:
Estimation due to market volumes and log P, log BCF
-: No exposition probable
?: Possible exposition due to high market volumes.
+: Probable exposition due to high market volumes and middle to great log P and log BCF
no symbol: Estimation not possible

Effects:
Direct effects: SH, SR: Oestrogenic, anti-androgenic effects and direct effects on steroid hormones, receptors
Indirect effects: Thyroid: Indirect effects by interference with thyroid hormones, metabolism
Indirect effects: GtH, Gon: Indirect effects by disturbance of hypothalamus and pituitary and gonadotrophic hormones, effects on gonads
Sperm quality: Effects on spermatogenesis and sperm quality (number, morphology, motility)
Reproductive toxicity: Effects on fertilization, implantation, embryo, offspring