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EVALUATION OF HERBICIDE FACTSHEET, GLYPHOSATE (ROUNDUP)

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The author is Caroline Cox, editor of JPR.

This piece is an update of two articles that appeared earlier in JPR by the same author, claiming to describe the toxicology, human exposure and ecological effects of glyphosate. Reviews of earlier versions were requested by B. C. Railway and by the City of Santa Cruz, CA.

It is accompanied by a one page editorial responding to criticism of earlier articles on the same subject. It is necessary to respond to this editorial as well as the discussion of glyphosate. My comments are in a few paragraphs at the end of this critique.

There have been some additions from past versions, and withdrawal of a few references that were grossly misrepresented, but overall, this version contains the same flawed approach found throughout the earlier articles.

The tone of the paper is set by generalizations at the beginning suggesting that glyphosate is severely toxic and represents an environmental threat.

No source quoted in the article, or any other evidence supports those contentions.

The ideas that Cox attempts to promote with these statements are contrary to the conclusions of national and international regulatory bodies and their advisors, as well as the extensive scientific literature on glyphosate.

It is unfortunate that so much space must be used in response to this paper, but as is usually the case, review of bad work is much more difficult than evaluating quality.

BACKGROUND OF THE PUBLICATION

JPR is a magazine published by the Northwest Coalition for Alternatives to Pesticides (NCAP), known earlier as the Northwest Coalition Against Pesticides. NCAP is headquartered in Eugene, Oregon. JPR is not a peer-reviewed; articles that purport to be scientific are not sent to independent scientists for review of technical content and validity of interpretation. Neither is JPR supported by the reputation of any established scientific organization. It is best described as a house organ and conduit for the opinions and philosophy of NCAP. Neither NCAP nor its journal can be presumed *a priori* to be a valid source of scientific information.

JPR and its parent organization, NCAP, take a vigorous position against use of pesticides. There is nothing intrinsically improper about that philosophy. However, the author of this article, Caroline Cox, is also Editor of the magazine.

By definition and in practice, Cox holds the same philosophical positions as NCAP, yet represents these articles as independent, objective and accurate. The Editor as author bypasses responsible editorial review. With no external scientific validation there is no assurance of quality, responsibility and accuracy.

That inherent conflict of interest could have been mitigated by a competent external scientific review.

It is self evident, however, that if such a review were heeded the article would not have been published.

There are many nationally recognized toxicologists and environmental chemists close at hand, at Oregon State University, the University of California, Washington State University and the University of Washington, and other institutions. Some or all of these scientists would have been willing to provide advice. On a broader scale, the Society of Toxicology, the Society for Environmental Toxicology and Chemistry or the Society of Toxicology of Canada would probably respond to a request for peer evaluation of these papers.

The only conclusion can be that the rejection of mainstream scientific principles and misrepresentation of scientific literature by this article is deliberate.

GENERAL SCIENTIFIC CONSIDERATIONS

Regulatory officials and their scientific advisors world-wide have concluded that glyphosate formulations have very limited toxicity and that toxicological risks and environmental impacts associated with use of glyphosate are minute for all non-plant species. The basis for these conclusions may be found in the USEPA Reregistration Eligibility Document for glyphosate (ref 4), in the World Health Organization (WHO) Environmental Health Criteria 159, Glyphosate (ref 2) and Agriculture Canada Discussion Document E91-01 for glyphosate (ref 16), as well as numerous other risk evaluations.

Those documents are prepared by panels of expert scientists in and out of government who have reviewed the published research and the data supporting the registration of glyphosate formulations. They state the official positions of the US regulatory system and of WHO. Their conclusions are consistent with international regulatory positions.

However, Ms. Cox lifts words from those official documents out of context in attempts to imply substantial risk with glyphosate use, and ignores the conclusions of minimal risk in the same pages.

Along with misrepresentation, the most consistent lapse in this JPR article and its predecessors is refusal to consider the relationship between dose and effect when discussing the scientific literature. The implication throughout is that if glyphosate (or any other substance) can cause harm at some very high dose, such a response is to be expected at any lower dose.

The concept of the dose-response relationship governs all interactions between chemicals and biological systems. It is the foundation of toxicology and pharmacology. It applies to all substances and all effects, and it applies to every biological system, from cell culture through fish and fowl on up to humans. There are no exceptions.

Given the importance of the dose-response relationship, its essential simplicity is deceptive: As the amount of a chemical taken in increases, so does the effect, and decreased intake is accompanied by decreased effect. This is true of desired effects in therapeutics as well as adverse toxic responses.

Every chemical, natural or synthetic, dietary necessity or hazardous waste, can cause harm at some dose. There is also a level below which systemic effects will not occur. This is a threshold or No-Observed Effect Level (NOEL). Anyone who has consumed several cups of coffee or who has observed a person drinking alcohol has studied the dose-response relationship and the idea of a threshold at first hand.

The same ideas apply to the use of therapeutic medicines, which are intended to exert effects on specific mechanisms in the body. Even these chemicals with their specific physiological and biochemical targets must be given in relatively large doses to cause the desired changes in the body. If the dose, or amount

taken in is too low, the medicine is useless; if too high it may cause unintended side effects, or toxicity.

An evaluation of risk is impossible without examination of effects at several doses or dose rates, both in the laboratory and in the field, and comparison with the amounts that may reach organisms of concern. It must also be remembered that even substances of very low toxicity can cause harm if exposures are high enough, just as sufficiently low exposures to substances of extreme toxicity may cause no adverse effect.

Ms. Cox generally ignores the essential dose-response information in the references she uses as evidence of harmful consequences of glyphosate use. Also cast aside in these references are authors' conclusions that glyphosate use is unlikely to result in harm.

One reason that information exists about toxic effects at high doses is the fundamental requirement that studies supporting registration of any pesticide must include a range of dose rates, the highest of which is expected to cause observable toxicity. The lowest should produce no evidence of toxicity.

In the JPR article, numerous important research studies on glyphosate have been missed or ignored and several clearly incompetent publications are used. There are examples where publications have clearly been misread. These faults further compromise the claim of objectivity and accuracy of these articles.

The author of an article such as this should be aware not only of the principles that govern the science of toxicology, but also the principles that govern responsible reviews of the scientific literature. A reviewer has the same responsibility to evaluate the strengths and weaknesses and implications of a published paper as does the editor who reviews it prior to publication. A reviewer also is obliged to counsel with experts to assure objective and accurate treatment of the material.

This article has met none of the obligations of a scientific review. When the data and the conclusions of authors and regulatory publications reviewed by Cox are compared with her interpretation, it is difficult to avoid a conclusion that the purpose of this paper is political, not informative.

COMMENTS ON SPECIFIC STATEMENTS

P 4, Col 1, Mode of action
The description of the mechanism of action of glyphosate in plants is essentially correct. The statement by EPA that the mode of action is not known is curious. The mode of action is known, in great detail, some of which is described here.

P 4, Col 2. For some reason, under mode of action, is the statement, "In rats, Roundup decreased the activity of two detoxification enzymes in the liver and an intestinal enzyme"
Hietanen et al, (1983, ref. 14) is the quoted authority for this statement. The work has no meaning except in possible suicide attempts. Decreases were modest and probably not sufficient to impair normal function, and were probably a function of general toxicity following the enormous doses.

Roundup formulation was placed in the stomach of rats, by stomach tube five days a week for two weeks. The daily dose was 500 mg/kg/day, reduced to 300 mg/kg/day after four days, probably because of gastrointestinal irritation by the surfactant in the formulation. The dose rate was roughly equivalent to a 150 pound person drinking a quarter cup of Roundup concentrate each day. No effort was made to examine lower doses, to learn the dose response relationship.

If anything, the report illustrates how difficult it is to produce an adverse response with glyphosate.

Page 4, col 3, Acute Toxicity

The statement is made that commercial glyphosate-containing products are more acutely toxic than

glyphosate alone. The references quoted are not pertinent and are misused. (Martinez et al (1990), and Martinez and Brown (1991), ref. 15 and 18)

Martinez and Brown (1991) injected the test substances directly into the lungs of rats in liquid form. The maximum volumes placed in the respiratory tract of the rats were equivalent to about 60 ml of fluid inhaled directly into the trachea of a 50 kg (110 lb) person. 60 ml is about 1/8 of a pint. Anyone who has choked on a sip of water knows how difficult it would be to tolerate any kind of fluid in the respiratory tract, let alone such a large volume.

Cox did not discuss the doses used, but perhaps unwittingly did indicate the rather violent nature of the experiments, mentioning that the products were "forced into the trachea, the tube carrying air into the lungs.---"

The rationale for the research was concern about aspiration of vomit after suicidal ingestion of the herbicide. The objective was reasonable because a surface active material in sufficient amounts may interfere with the delicate natural surfactants of the lung. In fact, aspiration of any ingested and vomited chemical is very dangerous.

The experiments have nothing to do with the real world of exposures due to use of glyphosate; numerous studies have shown that respiratory exposure is a trivial fraction of total exposure of spray applicators.

The experiments of Martinez and Brown do suggest that Roundup causes more damage than the surfactant alone, or a different kind of surfactant. However, the amounts required are very high, and the dose-responses were not entirely clear. The findings are applicable only to the special case of suicide attempts.

The report by Martinez et al (ref 18) is somewhat odd because the products tested are inadequately described; doses are described only by volume with no clear idea of the amount administered per kilogram body weight.

Registration data indicate similar acute toxicity for glyphosate and Roundup, although the formulation produces greater gastrointestinal symptoms. The doses required for significant acute effects are so great that a concerted effort such as a suicide attempt is required to ingest such amounts, making any difference academic. The World Health Organization document (ref 2, pp 71-72) summarizes these data, which would have been more useful to readers of Cox's article.

Page 4, Col 3, middle

Cox claims that "Glyphosate-containing products are more toxic via inhalation than orally." She refers to a discussion document from Agriculture Canada (ref 16), indicating "signs of distress in all test groups".

Readers were not told that the lowest air concentration tested was 1100 mg/cubic metre, which is very high and cannot be achieved outside an exposure chamber. (The active ingredient and surfactant are in practical terms non-volatile, so the only way to produce inhalation exposure is by generating very fine mists of the test mixture.) It is not possible to achieve significant inhalation exposure in the field.

Signs of distress in those animals disappeared shortly after the end of exposure.

The Reregistration Eligibility Document (ref 4) for glyphosate, states that "---adequate inhalation studies were conducted on the end-use product formulations." The data upon which that conclusion was based includes long term studies of inhalation of Roundup aerosols at the maximum concentrations that can be accomplished without the material "raining" out of the atmosphere. Acute median lethal air concentration (LC^{50}) for undiluted concentrate over a four hour period is over 3000 mg/cubic meter.

Inhalation exposure for 30 days to concentrations up to 360 mg/cubic meter of a 1:2 Roundup:water mixture produced nasal irritation but no systemic effects. A three month, 6 hour/day, 5 day/week exposure to 1000 and 5000 mg/cubic meter also produced little effect. These concentrations are orders of magnitude higher than any possible field exposures.

None of these data are discussed by Ms. Cox.

Page 4, col 3, Effects on the circulatory system.

Cox quotes Tai, 1990 (Ref 19) as showing that glyphosate and Roundup can cause a variety of cardiovascular problems. As usual, there is no indication of the amounts required, or how they relate to the real world.

The work by Tai et al was stimulated by a number of suicide attempts in Asia, in which concentrated Roundup was consumed by mouth (see below). The doses were about a million-fold greater than is possible in workplace exposure.

The studies were done in Beagle dogs. To simulate intakes in suicide cases, the dogs were given 8.2 grams of glyphosate intravenously over a one hour period, raising blood glyphosate levels to almost 900 ppm after 30 minutes and about 1200 ppm after one hour of injection. This level was considered by Tai et al to "approximate a moderate case of acute poisoning for a human subject".

The surfactant and full formulation were administered at rates corresponding to their respective concentrations in the formulation. The dogs weighed 10-15 kg, which means that the glyphosate intakes ranged from 550 to 820 mg/kg. Given intravenously over a short period, these are potentially lethal doses. It should be no surprise that any substance loaded to that extent so abruptly would produce dramatic responses.

Failure to discuss the actual dose rates administered, or blood concentrations of glyphosate measured in the experiments makes the comments by Cox meaningless.

Page 5, Inset discussing inert ingredients.

As presented, these statements have no value, and are clearly intended to frighten, not inform.

A number of inert ingredients are identified and qualitative statements are made about aspects of their toxicity. In each case, the exposures through the formulations are very small, and the effects arise from large doses.

A proper description would show: 1, the amounts present in the formulations; 2, the potential exposures resulting from use of the formulation, and 3, the dose-response data from the studies of the materials. No estimate of risk can be made without that information.

The reasons for withholding this information from her readers is known only to Cox.

Page 5, Col 2, Acute toxicity to Humans.

Six publications (refs 66-71) are referenced, all of which describe observations of attempted suicides. Temple and Smith (ref 69) also discuss three dermal exposures, which were not properly discussed in this review. In the first case, concentrated Roundup was involved, and the victim was treated and was at work the following day. In the second case the Roundup was double strength (presumably compared to

the usual dilution); the symptoms disappeared without treatment in 48 hours. The third patient was drenched with the formulation, and was treated with a steroid at "two monthly intervals" according to Temple and Smith, which may or may not represent the duration of symptoms.

Page 6, col 1, Subchronic Toxicity.

The salivary gland lesions were found in the parotid and submandibular glands, and consisted primarily of cellular swelling. The NTP report (ref. 74) suggested that the changes were similar to those caused by high doses of b-adrenergic agonists (epinephrine-like substances). The doses required were very high relative to human exposures. It is of interest that chronic studies at much higher doses have not indicated such changes.

Page 6, Col 1, effects in calves.

This description is somewhat incomplete. Brahma cross heifers were given Roundup by stomach tube daily for seven days, followed by 14-15 days observation. Dose rates were 400, 500, 630 and 790 mg/kg/day, to groups of three animals per dose. At 500 mg/kg/day and above the animals exhibited diarrhea and loss of appetite. One animal at the highest dose died, and was found to have aspirated rumen contents into the lung, which caused a pneumonia. The pneumonia was not caused by Roundup as such.

Since the contention is made that "Glyphosate-containing products are more toxic than glyphosate in subchronic tests", it would seem useful to include data about glyphosate itself, but none is shown.

Page 6, col 2, Carcinogenicity.

After a long description of the findings of cancer studies, and numerous hints that the manufacturer influences EPA decisions, Cox states that "Unfortunately, EPA has not taken that viewpoint (of protecting the public health) in its assessment of glyphosate's cancer-causing potential",

Cox is not competent to pass judgement on the risk assessments of EPA, the World Health Organization, and government regulators throughout the developed world. All have classified glyphosate as non-carcinogenic, for scientifically sound reasons.

Decisions of EPA are made on the basis of several levels of expert review, including external specialists. While it is not philosophically or scientifically possible to prove absolutely that an event such as cancer will not occur, there is a massive body of carcinogenicity, mutagenicity, pharmacokinetic, pathological and toxicological data that supports the position of those expert bodies that glyphosate is not carcinogenic. Cox ignores those tiers of expert advice.

As to the absence of carcinogenicity assays on glyphosate products, enough is known about the constituents of the formulations to support a conclusion of noncarcinogenicity.

Page 7, col 1, Mutagenicity

Cox has used several studies to suggest that glyphosate is mutagenic. It is remarkable that all but one of these reports are demonstrably flawed, and useless for assessment of risk. The one paper that appears to be properly done shows no effect except in plant cells, which is not relevant because of general plant toxicity.

The numerous reports in the refereed scientific literature and in the registration documents, as well as the conclusions of the international community of regulatory authorities, that show the lack of genetic activity of glyphosate are ignored.

Bolognesi et al (ref 81) is said to have shown that "Glyphosate- containing products are more potent mutagens than glyphosate", referring to a stated increase in sister chromatid exchanges. They also are said to have found genetic effects on human lymphocytes at both doses tsted and with glyphosate at all but the lowest dose tested.

For a number of reasons, the paper does not quite provide useful information.

A number of different assays were conducted, and while positive effects are claimed, there are serious questions about the findings.

An assay for sister chromatid exchanges (SCE) was done using lymphocytes from two human donors, which is an insufficient number of sources to assure control of individual variation. There is no data showing the extent of normal variation between the cells of the two donors, or the variation at comparable doses between the donors. These are essential pieces of information.

There is no positive control, in which a known clastogenic agent is tested to be sure the assay is working properly, which is also essential to a valid assay.

There is a scientific question whether SCE is an indicator of genetic toxicity or of cellular damage, which may well have been the basis for the observations. Glyphosate concentrations up to six mg/ml of medium were used, and were maintained in contact with the cells for 48 hours of incubation. It is useful to compare the *in vitro* concentrations to the doses that would be required to achieve such concentrations in an idealized intact animal situation. Some simple arithmetic is informative.

Consider that an animal is 70% water and assume that the test article is distributed uniformly throughout the body water, with no excretion. A concentration of one mg/ml would be equivalent to a dose of 1400 mg/kg body weight. Glyphosate introduced directly into the circulation is lethal at much lower doses than this. The highest concentration tested would compare to 8400 mg/kg; the lowest concentration of 0.33 mg/ml would relate to 462 mg/kg. It is highly likely that the effect is a function of cellular damage.

DNA damage was assessed in mice treated with 300 mg glyphosate or 900 mg Roundup formulation per kg body weight, administered intraperitoneally. No information is given about the condition of the mice after these very high doses. No dose response data were obtained, so we have no idea of effects at doses known to be survivable. Furthermore, DNA damage is not a definitive indicator of mutagenicity, because of the potential for response to non-specific cellular damage.

Oxidative damage assays are also not accepted standard indices for genotoxicity, but are more likely to respond. The animals were treated with very high doses of glyphosate and Roundup, and no dose-response data were obtained. (The same animals may have been used in both assays.)

The absence of effect with Roundup while equivalent doses of glyphosate produced a response is also curious. Typically, a laboratory finding this kind of inconsistency will do additional experiments to clarify the issue. The authors discuss, rather unclearly, the role of oxidative metabolism in producing apparent DNA oxidation. However, they imply that glyphosate undergoes the same kind of metabolic change, with potential for production of reactive intermediates, that characterizes "pollutants and xenobiotics". Presumably they mean such substances as PCBs, polyaromatic hydrocarbons and other fat soluble and persistent substances. Glyphosate is not metabolized in the body but is excreted unchanged, with none of the characteristics they speak of.

In the micronucleus experiments, Bolognesi et al may have seen a small response, but again, the doses of Roundup and glyphosate were very high relative to lethal intraperitoneal doses, and there was no attempt to establish a dose response slope. It is interesting that in this experiment a positive (known active) control

was used, in contrast to other parts of the work.

The report by Kale et al (ref 83) appears meaningless.

One is first struck by a number of errors that have nothing to do with the laboratory, but in themselves cast doubt on the quality of the work and the knowledge of the investigators. They speak of "pesticides and herbicides". Presumably they refer to insecticides and herbicides. Both are pesticides; it is difficult to imagine competent scientists who do not understand terminology. They identify the formulation Crossbow as containing 2,4,5-T. Crossbow contains no 2,4,5-T; it is a mix of triclopyr and 2,4-D. At the same time they comment that 2,4,5-T has been banned, and obviously do not connect the two statements. They state that glyphosate has been classified as a "possible human carcinogen by the EPA". EPA had declared glyphosate to be non-carcinogenic two years before their paper was published. They refer to Vigfusson and Vyse (See ref 39, below) as background for this work, a study that genetic toxicologists reject out of hand. With some nine authors of the Kale et al paper it should be expected that there is enough collective basic background knowledge to prevent such errors. Why the reviewing editor permitted publication of these errors or the poorly conducted research described below is yet another question.

As to the work itself, there is no dose-response information.
The treatment concentrations were apparently based on a determination of the median lethal concentrations of the test materials, but there is no explanation why the animals were seen to be 10,000 fold more sensitive to Roundup than to other, more toxic, chemicals. That in itself is inconsistent with all other data, and inconsistent with the absence of activity of Roundup or glyphosate in other genetic toxicity assays.

Every chemical they assayed was positive, which in itself is quite improbable. Although the pattern was unusual, Kale et al did not check their own work by the obvious step of evaluating responses at a series of concentrations. This approach is particularly important where there is apparent high sensitivity, which can result from some kind of laboratory error. Some of the data is presented without controls, or controls were obtained at a different time, which makes interpretation impossible. It is also not clear how long the test organisms were exposed, and in fact there is some suggestion that exposure periods varied.

I am astonished that reference is still made to the lymphocyte study published by Vigfusson and Vyse (ref. 84). The work was evaluated many years ago by several recognized genetic toxicologists after it was published, and shown to be invalid; it is remarkable that it was accepted for publication. This study was also done on cells from only two donors; a minimum of ten donors are required in such studies just to assure control of inter-individual variation. The data reported in the publication showed no dose-response relationship and the variation between donors was greater than the effect of the compound tested. In other words no effect was shown.

With respect to mutagenicity in Salmonella (Ames tests) the finding of a weak response by Rank (ref 85) was not dose-related and not reproduced in other identical standard assays. Both qualifications are necessary for a conclusion that a substance has a positive effect.

Peluso et al (ref 86) are stated to have shown that "In mice injected with Roundup, the frequency of DNA adducts in the liver and kidney increased at all three doses tested"

This report also is significantly flawed. The dose rates were so high the animals were almost certainly compromised, but the reported adduct frequency was below known normal levels. The nature of the adducts was not known, and the method is not an accepted approach for judging mutagenicity.

In examining the paper, a number of peculiarities are immediately evident that have nothing to do with the experiments, and cannot be ascribed to language problems. The authors do not read critically.

Their description of Roundup is in error; they include all of the chemical forms and formulations of glyphosate under the formulation name of Roundup, including some not manufactured by Monsanto.

They speak of acute toxicity as determined in 13 week feeding tests. Such tests are not indices of acute toxicity.

On page 55, the authors quote WHO (1994) as a source for descriptions of long term studies of Roundup, apparently not understanding that the statements in WHO 1994 related to glyphosate, not Roundup.

It appears that Peluso et al also misread the discussion in WHO of multigeneration studies, "In multigeneration studies, a decrease in body weights and a renal tubular dilatation have been found at 30,000 mg/kg diet in rodents (WHO 1994). As described in WHO, in an early 3-generation assay the only effect noted was an increased incidence of renal tubular dilatation in the second litter of the third generation. A new assay with 2000, 10,000 and 30,000 ppm in the diet, caused only soft stools and decreased body weight in parent animals and slight decreases in litter size and body weight in pups at the high dose rate. There was no histological effect on the kidneys of the pups. (30,000 ppm is 3.0% of the entire diet.)

Peluso et al do not reference the substantial body of evidence that shows no mutagenic response to glyphosate, but do quote the few reports that purport to show positive effects, even though some of these reports are demonstrably inadequate or show no effect. I have discussed certain of these papers already.

Peluso et al used doses that were stated to be lower than the acute oral LD50 of more than 4000 mg/kg/day, apparently with the idea that they were using relatively low doses. However, they administered the compounds by intraperitoneal injection, presumably unaware that the administered doses are in the lethal range. This in itself negates any conclusions from the work.

There is no information in their paper describing the condition of the animals. At the doses said to be used, the animals must be expected to show visible signs of toxicity. As a matter of principle, genetic toxicity assays at a dose level that produces cellular damage are not valid. This is true whether cell preparations or intact animals are used.

These seem like minor matters, but they indicate a level of carelessness that is inconsistent with good science.
These deficiencies are aside from the specific scientific questions about the work.

An expert panel was recently convened to evaluate the method and its capabilities (Nestmann et al, 1996). Among the conclusions of the group, it stated that rigorous scientific criteria must be applied to assure that findings in fact represent true adducts of DNA, and that biological significance of the findings must be critically evaluated. A "problem" with the method is that its sensitivity exceeds ability to define biological significance. The panel also concluded that findings must be examined in light of background incidence of DNA adducts.

There are three fundamental questions that must be asked when considering the utility of this work as an indicator of mutagenicity of glyphosate or its formulations:

1. Is this a standard method for measurement of genetic toxicity and/or mutagenicity? (Genetic toxicity, defined as damage to DNA, does not necessarily result in mutation, defined as transmissible changes in genetic information.

The method is not yet standard. It has value as a research tool in answering questions about biological mechanisms, but as yet it is not applicable to judgement of mutagenicity.

2. Are the low levels of adducts claimed to be found different from controls, and is the observed incidence different from the incidence of DNA adducts that arise as a result of normal metabolic processes and background environmental influences?

There are several types of DNA adducts, and they occur at high frequency naturally. In a review by Gupta and Spencer-Beach (1996), it is noted that an incidence of 70-2100 cyclic adducts/10^9 nucleotides occurs in human liver and on the order of 1400 alkylated bases/10^9 nucleotides occurs in human lung. Natural oxidants add to this total to even a greater extent. (It has been known for at least a decade that the background rate of damage of DNA bases in humans is on the order of 1,000 to 10,000 events per cell per day, a substantial number considering the 12 or 13 trillion cells in the human body.)

The adduct levels reported by Peluso et al ranges from about 8 to 30 per 10^9 bases. This is in the normal range. Inability to find adducts in the control or glyphosate treated animals raises questions about the identity of the presumed adducts, and their application of the method.

3. Are the adducts observed in fact DNA adducts, and if so, what kind of adducts are they?

As the authors point out, the nature of the adducts is not known. Some compounds other than DNA bases, both endogenous biological substances and foreign materials, will label and be visualized in the same way as DNA adducts. Consequently, we have no way of knowing what was seen.

4. Are the doses used relevant to either toxicological or physiological considerations? In other words how do the treatments relate to any events that can occur in the real world, and are the animals intact or systemically damaged to the extent that normal functions are lost?

The treatments were given intraperitoneally, clustered at a narrow and very high range of doses; neither dosage or route of administration is environmentally relevant. The lowest dose used was at least highly toxic but no information about post-treatment health of the animals was provided. The treatments were at levels orders of magnitude higher than can be accomplished in any occupational or public exposure.

At such high dose levels of the surfactant there will be general non-specific cellular injury. It is quite possible that the lipid peroxidation products resulting from such injury may have produced these findings.

Glyphosate is generally accepted throughout the international regulatory and scientific communities as a non-mutagenic substance. The World Health Organization (ref 2) and the EPA (ref 4) state this very clearly, and provide the information to support that position.

Ms. Cox chooses to ignore these authorities, but the papers referenced in her article provide no evidence to support her contention that glyphosate is mutagenic.

Page 7, Col 2, Reproduction

An epidemiological study (Savitz et al, ref 87) is stated to have "found that fathers' use of glyphosate was associated with an increase in miscarriages and premature births in farm families."
Savitz et al are careful not to use such positive language.

This is a study that appears to have been well conducted, and the authors are careful to discuss the limitations. They do not over-interpret their findings, as Cox has done; they correctly indicate that "The overall implication of these results is to add to the interest in a possible role of male pesticide exposure in adverse pregnancy outcome---." They speak to the need for more precise methods.

They have estimated exposures as well as can be expected, but acknowledge that recall of past events, upon which this survey depends, in not entirely dependable. This problem, of remembering materials used

in the past, and estimating exposures from stated practices, has been the downfall of most epidemiological studies relating to pesticides. They state that they cannot be certain that any of the findings are not due to maternal exposure through environmental or household contamination.

Of greatest importance are the data. It is generally accepted that odds ratios with 95% confidence interval (CI) in which the lower limit is less than one are not likely to have meaning. (The CI is the range with a 95% probability of containing the true number.)

None of the findings for glyphosate meet that criterion.

Yousef et al (ref 89) are quoted as showing that glyphosate reduced sperm counts in rabbits at the two highest doses tested. This is yet another paper that provides no usable information; it should not have survived editorial review.

The authors stated that the doses were 1/10 and 1/100 LD_{50}, given orally in gelatin capsules, but they have given no idea what they considered the LD_{50} to be. However, in another paper by the same authors (Yousef et al, J. Envir. Sci Health B31:99-115, we are told that the LD_{50} is "11,829,419 uM" for the rabbit. This also makes no sense. While dosage may rarely be expressed in molar terms, a body weight or other concentration factor must be stated. It cannot represent a concentration in body fluids because such measurement was not made.

How they arrived at such a precise number is a mystery, and we will round it to 12 million micromoles. That amount of glyphosate (molecular weight 169.1, assuming glyphosate acid, weighs more than 2000 grams, a rather substantial dose for a 2,600 gram rabbit. If it is the glyphosate IPA salt, the dose would weigh more than the rabbit.

By this notation, 1/10 and 1/100 LD_{50} would represent 20 and 200 g/2.6 kg, or 7700 and 77000 mg/kg. All of this is clearly absurd, so we have no notion of the doses that were given.

The treatment is stated to have continued over a six week period. We can only assume that treatment was daily, but there is no indication whether the stated dose was a total divided into daily doses over six weeks, or was given each day. It is described as a dose, not a dose rate. If divided, the doses above would have been 183 and 1830 mg/kg/day. The latter dose might be barely tolerable.

Looking at the data, the body weights showed a slight decrement at the "low" dose, and an increase at the "high" dose. Since we have no idea what doses were used, it is not reasonable to judge relative effects on the basis of body weight.

Semen characteristics showed essentially no difference between "high" and "low" doses; in other words there is no dose response, even though there is difference from controls.

The authors' choice of references for discussion on this subject suggests that they may view pesticides as some relatively homogeneous group of chemicals. They discuss a multitude of other pesticides, but have made no reference to the published literature or the available regulatory documents that discuss the reproductive toxicity of glyphosate, particularly the multigeneration studies.

Page 7, col 3, reduced sperm counts in rats, referring to Figure 5. Note that the effective intakes were at 2-1/2 and 5.0 % of the entire diet, which is survivable, but likely to produce general toxicity, with numerous secondary effects.

The study of fetal weights (and other parameters) after glyphosate treatment of rabbits (ref 90) has generated no regulatory response anywhere, including WHO. The data are rather curious, in that over a wide range of doses the responses were the same. In other words a daily dose of 350 mg/kg/day

produced effects very little different from those at 75 mg/kg/day. In addition, the control values appear somewhat higher than historical controls, which may make treated weights appear lower.

Page 8, Col 1, Quality of laboratory testing

There have been two widely publicized cases in which contract laboratories have falsified data intended to support registration of pesticides. A section is devoted to these cases, with the terminal premise that "this fraud casts shadows on the entire registration process."

There is no question that Industrial Biotest Laboratories falsified data on products of a large number of pesticide manufacturers in the seventies, including glyphosate. All of that work was redone acceptably by other laboratories.

The important consideration of that event is that it caused a major overhaul of the registration process. A formal and thorough auditing of tests is carried out by regulatory agencies of the US and Canada in cooperation, and at present a large part of the world regulatory structure is also involved. The institution of "Good Laboratory Practices" (GLP) standards across the entire regulatory structure also resulted, so that assays are now conducted in a uniform and reproducible manner. That rigorous (and burdensome) auditing, coupled with a policy by manufacturers of duplicating part of the registration studies in other laboratories has virtually eliminated fraudulent and incompetent practices.

The Craven Laboratories event is a case in point. Work required to support reregistration of a fungicide was commissioned by a consortium of manufacturers (not including Monsanto). The manufacturers found the work to be inconsistent with their own studies done in parallel, in fact appearing too favorable. After the manufacturers reported suspicions to EPA, the agency investigation uncovered the fraud, which resulted in convictions and jail. Residue studies for Monsanto were being conducted by Craven during the same period. They were found to be properly done, but were repeated by Monsanto, with the same outcomes.

These have been events in which pesticide manufacturers and the public have been defrauded by outside contractors. The fact that these are the only two such cases that have surfaced shows how infrequently cheating occurs.

Page 9, col 1. Human Exposure.

Qualitatively, the routes of exposure listed are correct. The same statement can be made for virtually all chemicals. In the case of glyphosate and most herbicides, workplace exposure on the skin represents the most important potential route. Other incidental exposures are very low.

Residues on food are minor and are discussed in detail in the USEPA Reregistration Eligibility Document for glyphosate (ref 4). (see below)

Page 9, col 1. Contamination of food.
World Health Organization Environmental Health Criteria 159 (ref 1) does in fact state that glyphosate analysis is "in general laborious, complex and costly".

The JPR article further quotes WHO: "For this reason, it is not included in government monitoring of pesticide residues in food."
The WHO document does not make or imply such a statement.

There are dozens of laboratories capable of these analyses, and the literature is replete with development of methods for glyphosate residue analysis. The WHO document quoted discusses many of these methods.

The EPA Reregistration Eligibility Document states, "An adequate enforcement method is available for analysis of residues of glyphosate and its metabolite AMPA in or on plant commodities and in water."

Cox implies that residues in food are not well known and are potentially dangerous, using the USEPA Reregistration Eligibility Document (Ref 4) to support her contention.

However, that document contains a lengthy list of residue tolerances for 50 or more specific food crops, as well as tolerances for processed commodities, meat, poultry, eggs, fish and milk. All of these are based on toxicological analysis and residue data determined in crops after application of the herbicide according to the label. Residues in drinking water and irrigation water and in plants resulting from irrigation have been measured as well. Market basket surveys have not been done, but these are generally conducted for pesticides that for some reason represent a health concern. Glyphosate is not seen by regulatory authorities as a health concern.

Tolerances are the maximum residues of pesticides permitted in foods and forage. They are based in part on toxicological considerations and in part on residues to be expected from appropriate application practices. Usually, appropriate practice governs the residue tolerance because levels that would convey any calculable risk are greater than those resulting from proper use.

Page 9, Col 2, Occupational exposure

The implication that respiratory effects in milling of glyphosate-treated flax were due to glyphosate is highly misleading. The exposure to glyphosate in this study was about 12 million fold less than the lowest no-effect level observed in sustained treatment of experimental animals.

Jamison et al (ref 28) studied respiratory effects resulting from experimental exposure of volunteers to dust from flax that had been retted in the field, compared with dust from flax retted by conventional methods. The processing of flax is a very dusty activity; flax mills are a source of occupational exposure to particulate material and fungi; workers are frequently affected.

Retting is the process of allowing the flax plants to partially decompose, to free the fibers from the woody portion of the plant. It is usually done by soaking or by allowing the plants to lie in the field with normal moisture. Where there is too much moisture for field retting, treating with a herbicide allows the standing dead plants to decompose under somewhat more controlled conditions. When the plants decompose on the ground they are colonized by fungi that produce bronchioactive toxins that are carried in the dust from the flax. Allowing decomposition while standing limits the formation of fungal toxins that might reach the lungs.

The study was done in an exposure chamber designed for suspended dust experiments with humans. It appears from the research as published, that the effects of the dusts differ with different retting methods, but whether the difference is in the physical nature of the particles, the nature of the fungal toxins that are inevitably present, or even from glyphosate could not be stated with certainty.

As the quoted paper points out, specific inhalation studies with glyphosate have not been conducted in humans. However, inhalation by animals of concentrations as high as could be maintained in a respirable atmosphere produced no responses suggestive of the bronchioconstriction associated with flax dust exposure. There has been no clinical evidence of such a response in applicators or other exposed people.

The actual doses of glyphosate inhaled are instructive and should have been shown by Cox. The glyphosate levels in the dusty air were estimated to be a tenth of a microgram (one ten millionth of a gram) per cubic meter, which is a very low concentration. If daily respiratory volume in the workplace is eight cubic metres, the daily dose for a 60 kg person would be 0.013 micrograms glyphosate per kg body

weight. As already remarked, this figure is about 12 million fold less than the lowest no-effect level observed in sustained treatment of experimental animals.

Page 9, Col 2,3, page 10, Col 1, Drift

This section conveys an impression that drift of glyphosate is somehow unique and is a major danger in use of glyphosate.
The evidence in these papers does not support such a contention.

There are two kinds of reports discussed; a few field incidents of drift are mentioned, along with several research studies.

Drift is a concern with any pesticide. Drift is not specific to glyphosate or any other chemical, and is manageable with proper equipment aand attention to conditions and surrounding vegetation communities. It is a fact that incidents of non-target plant damage have been occurring since the advent of modern herbicides 50 years ago. Most have resulted from inappropriate timing or conditions or human error, and are less and less frequent as training, equipment and regulation have advanced.

The incident reports used as examples in this section tell us nothing about specific risks associated with glyphosate.

There have been numerous studies of drift; many use glyphosate because plant effects are quite speciufic and easily observed.

The objective of most of these research studies was to identify both appropriate and inappropriate application methods and practices. Although Cox uses these studies as evidence of inordinate drift, each of them shows that proper methods limit significant drift to the width of reasonable buffer zones and that poor practices result in unacceptable drift.

Cox presents only the findings resulting from poor practices, and states them in such a way that they may be seen as representing all applications. She does not describe the research properly and ignores the stated conclusions of the researchers she quotes.

Atkinson (ref 106) followed the development of perennial plants after exposure to non-lethal drift damage and found, as pointed out by Cox, persistent effects. As did others, he identified methods that minimized drift, adding that careful attention to adjacent sensitive crops and plantings is essential.

Page 9, Col 3, Ground applications

Breeze, et al (ref 107) identified several species of wild plant that are highly sensitive to glyphosate, and modeled ground application drift data in combination with dose-response findings obtained in glasshouse experiments. They concluded that "only glyphosate sprayed at the highest recommended concentration might be unsafe to some of the species examined." In their discussion, they "suggest that a 10 m wide boundary is usually adequate."

There is an allusion on page 9, col 3 to a paper by Freedman (ref 108) relative to the variability of deposition after application. Freedman makes the same point others do, that management of methods and attention to conditions mitigates most unusual drift situations, and that inappropriate methods are responsible for most unintended impact.

Freedman devotes considerable comment to the disparity between public perception of risks associated with herbicide use, and risks as estimated from factual information.

Page 9, col 3

Yates et al (ref 109) is used as evidence that glyphosate drifts enormous distances. The proper question about drift must include both the distance and the levels measured.

The objective of this work, done more than 20 years ago, was to evaluate various application equipment and techniques. All but one of the measurements were made in cross winds ranging from 6.8 to 9.5 miles per hour. In other words, adverse conditions for drift were deliberately chosen. The nozzle and pressure configuration that produced maximum drift did not represent typical applications then, and certainly does not at the present time. Other configurations reduced drift on the order of 150 fold. The same kind of relationship was found in examination of damage to potted grapes and wheat plants in the drift zone.

If one studies the data, it is evident that at 10 meters from the treatment zone, deposition from the best treatment, as measured on Mylar sheets, was about 170,000 fold less than the application rate. As measured on glass fiber air filters the difference was about 210,000, and the application was by helicopter.

The work of Riley et al (ref 113) shows a very sharp early drop in deposition, followed by very low residues extending for some distance. The real issue is not whether some herbicide is present at those distances, but whether those amounts represent detectable adverse impact. One objective of the authors was to "interpret observed deposit data with regard to published toxicity data for sensitive aquatic organisms---". (The authors are employed by the Province of New Brunswick.)

The comment by Riley et al at the end of their abstract is informative: "Given these results there are no plans to change the current buffer zone recommendation." (The current buffer at the time of publication was 65 metres, much wider than most requirements.)

Payne (ref 115) evaluated differences among application methods and meteorological conditions and concluded that appropriate practices and buffers would assure absence of adverse effect.

Page 10, col 1, persistence.

Cox is correct in remarking on the wide variation in degradation rates for glyphosate. Many of the figures quoted by Cox are from studies of conditions that lead to longer persistence, particularly cold climates and soil that is not biologically active. The description of a rapid initial degradation followed by a slower second phase is typical of many chemical reactions in the environment.

Persistence and half-time have quite different meanings. A substance may be said to persist as long as any amount can be detected in the medium under examination. The half-time or half-life is the time required for half of the amount present to dissipate. Two half-times would leave a quarter of the original amount. Half-time is more useful, because it enables prediction of residues without waiting until nothing more can be detected.

the difference is shown nicely by Newton et al (ref 121). The 55 day period mentioned by Cox was the duration of the study, not the persistence; the half-time measured in soil was about the same duration. Feng and Thompson (ref 123) found half-times in British Columbia of 45 to 60 days. Roy et al (ref 124) found half-times in Ontario to be about 24 days. (Cox has located ref 123 in Ontario and ref 124 in British Columbia.) Torstensson et al (ref 125) comment that the very long persistence of small amounts of glyphosate in the north can be attributed to the frozen soil and to slow release from vegetation. Frozen vegetation would retain any herbicide until thawing and restart of microbial activity.

The persistence of detectable levels of any chemical must be considered in the light of its availability to potential target organisms, and the thresholds and low level dose-response for those organisms. The half-times for glyphosate, even in the low temperature zones do not represent ecological or toxicological risks.

Müller (ref 122) expressed this idea well: "It is concluded that glyphosate degraded even at low temperature conditions, and it is not to be expected, that the application of glyphosate will affect directly nitrogen fixation, nitrification or denitrification activity in these soils."

Page 10, col 3, top, soil adsorption

There is a wealth of laboratory and field data showing that glyphosate is in practical terms immobile in soil.

The JPR article refers to a paper by Piccolo et al (ref 128) as evidence that glyphosate is in fact mobile.

A reading of this paper shows that it refutes the very argument Cox claims that it supports. It actually demonstrated the remarkable binding affinity of glyphosate for soils.

This work did not really address the behavior of glyphosate in soil, but rather dealt with its behavior in water containing suspended soil. If it may be related to any environmental context, it is much more a model of very short-term behavior in sediments in ponds and streams than soils as such, and actually shows that a small amount of suspended soil can remove large amounts of glyphosate from water.

The experiments were done with one gram of soil, added to 25 ml of water. In other words the system was 96% water. Soil moisture varies widely, but a typical soil sample will contain on the order of 10% water and capacity to hold water may be above 100%, which means that the mix is half soil and half water.

The water contained 50, 100, 150, 200 and 300 mg glyphosate per liter (ppm) before the soil was added. (For perspective on these concentrations, a typical two kilogram per hectare application, directly to water 30 cm (one foot) deep, will produce a hypothetical concentration in water of about 0.7 ppm. The glyphosate concentrations tested by Piccolo et al ranged from 70 to 400 times higher.

Removal of glyphosate was assessed by filtering the soil out of the suspension and resuspending it in 10 ml of glyphosate-free solution, and measuring the amount of glyphosate that was released from soil into the solution. In other words, this experiment used a single cycle of adsorption (binding) and desorption (unbinding).

In nature, the number of binding-unbinding cycles is so large it is literally uncountable. A molecule of glyphosate (or other soil bound chemical) binds to a soil particle, and in time releases and binds again to another particle. At the same time all the other glyphosate molecules in the soil volume are doing the same thing. Each binding site on soil components binds and releases molecules continuously. The net effect is that glyphosate travels such a short distance that in practical terms it is not mobile. Binding is tenacious enough that plant roots are essentially unable to extract glyphosate from soil.

This exercize can be visuallized in simple numerical terms as well. At the lowest concentration, 1.25 mg glyphosate is present in the solution per gram of soil (1250 ppm). At the highest, the ratio is 7.5 mg/gm (7500 ppm).

These figures may be compared with concentrations in the field measured by Roy et al (ref 124) which were roughly 200 ppm in the organic layer and zero in the upper mineral layers of a cleared forest floor. All of the soils studied by Piccolo et al were of high mineral content.

Page 10, col 3, water contamination

Certainly it is a fact that glyphosate has been found in surface water. Numerous monitoring studies of agricultural and forestry applications have detected low levels of glyphosate and numerous other herbicides following rain events or associated with forestry or agricultural activity.

Statements that a chemical has been found in a medium (refs 131, 132, 133 and 134) have no meaning without discussion of the amounts present, and in the case of glyphosate the extent of binding to soil particles, which will represent most of the water borne material. Frank et al (ref 129) analyzed water from 211 farm ponds and found 132 ponds to be contaminated with a pesticide. Glyphosate was found in just two of those cases. Runoff from a treated field led to a glyphosate concentration of 42 parts per billion (ppb); the other resulted from a spill with a concentration of 112 ppb. While such events should be minimized, the most interesting information is the low concentrations that resulted. In both cases the levels were far below amounts that can harm organisms in or using the water.

Edwards et al (ref 130) followed run-off from a number of fields on which Roundup was used in preparation for no-till cropping. Application rates ranged from 1.1 to 9 kg/ha. The latter is a very high, although legal rate. A sample of run-off after a heavy rain on that site the day after application contained 5.2 ppm, but the maximum for other sites was less than 0.1 ppm.

Cox comments on contaminated surface water in the Netherlands, as shown in World Health Organization Environmental Health Criteria 159 (ref 2). That document includes a list of findings of glyphosate in various environmental media around the world. Why the Netherlands was chosen from this list as an example is difficult to judge. A single sample was reported, at a concentration of one ppb. Some others elsewhere in the world were as much as a thousand times higher.

The Smith et al (ref 135) observations are not overly useful in judging the potential for glyphosate to reach well water. Three wells on substation sites were sampled. Communication with the authors has disclosed that there was no buffering around the well heads, and that the well in which glyphosate was found is a dug well, with a concrete pipe casing. The application rate was 4.63 kg/ha, with hand-gun equipment. It is not a surprise to find trace contamination; there is no assurance that some material was not applied over the top of the casing.

Electrical substations are placed on sand and gravel bases for insulation, and vegetation growth must be totally prevented. The underlying structure may be porous or it may be impervous enough that overland movement may be possible directly to the well head and down the outside of the casing.

Of more importance is the absence of glyphosate detections among the enormous number of samples drawn each year in North America. The detections in wells in Texas and Virginia mentioned by Cox were preliminary findings and were negative on re-analysis.

Page 11, col 1, Ecological effects; Nontarget animals

The report by Hassan et al (ref 138) is only a summary of an international, multilaboratory effort to assess effects of some 21 pesticides on beneficial insects. So much detail is lacking that the work is difficult to understand. Particularly, exposure conditions were not clearly indicated and control (untreated) animal data were not included. Roundup (1%) was apparently applied to a surface occupied by the test species and allowed to dry. There was no indication of the duration of exposures, and it is not certain whether the material was applied on a glass or leaf surface. The organisms were stated to be of a common life-stage, but the stage was not specified, nor was there indication that the stage was of high or low sensitivity.

Of the 21 pesticides, including four herbicides, Roundup appeared to cause the least effect, within the rather broad criteria for effect. The only utility of this report is as a directory to the specific experiments, which can be obtained and judged on their own merits.

Page 11, col 1, bottom.

Brust (ref 139) Brust's study of effects of glyphosate-induced change in habitat on carabid beetles

showed a maximum decrease in population to 72% of control at 14 days. In terms of population biology, this is not a serious decrease.

Brust also conducted direct toxicity studies that raise questions about the toxicity to carabid beetles indicated by Hassan et al (ref 138). Rearing boxes were sprayed at a typical field rate with Roundup, the beetles were dipped in the same tank mix, then maintained for one year. The author's conclusion is that "The results of this study demonstrate no direct acute or chronic toxic effect of the herbicides atrazine, simazine, glyphosate or paraquat to the five common carabid species tested."

In the study reported by Asteraki et al (ref 140) the glyphosate treatment was intended to "kill all flora at the base of the hedge", which was the observation area. A change in population density would certainly be expected.

An important consideration in habitat effects is the area and frequency of use. Temporary decreases in insect population in modest and separated areas recover in a reasonable time, and in fact treatments may enhance insect populations and their predators. Even in forestry, where relatively large areas may be treated, the distance for predators to more hospitable terrain is short for most species.

The 1986 EPA guide for reregistration of glyphosate (ref 142) is cited as indicating that an endangered species of longhorn beetle would be jeopardized by use of glyphosate. As the guidance document points out, this is a habitat question and is to be mitigated by appropriate labeling.

Of more importance, the more recent Reregistration Eligibility Document (ref 4) states: "Based on the toxicity data and the estimated exposure, it is not expected that endangered terrestrial or aquatic organisms will be affected from the use of glyphosate on the registered uses since the EEC's are well below the endangered species criteria (birds= 1/10 LC_{50}, aquatic organisms=1/20 LC_{50})." The document goes on to discuss endangered plants, and the habitat of the Houston Toad.

Given the frequency with which Cox extracts verbiage from this document, it is odd that she didn't see fit to provide this very straightforward statement.

Page 11, col 2, Other arthropods

As stated above, the work reported by Hassan et al (ref 138) is so poorly described that it is not possible to tell what it means.

Mohamed et al (ref 143), in discussing their work, comment that it is likely that any effects in the field are probably due to microhabitat alteration.

The woodlouse species they studied inhabits non-irrigated soil. The exposures were in glass chambers 9.3 cm in diameter. Filter papers saturated with solutions of 25 or 50 ppm glyphosate were placed in the bottom of the dishes and the subjects placed in the chambers with appropriate nourishment. Exposure duration was 7 days. At 25 ppm there was a slight effect, but no lower doses were used. How this kind of exposure relates to a typical soil concentration on the order of a part per million in most cases is difficult to say, since the dose response data was not obtained, but the results indicate that glyphosate at concentrations that may occur in soil (see ref 4) will not harm these insects.

Page 11, col 3, Earthworms.

The report by Springett and Gray (ref 148) tells us nothing. These are poor experiments; there is no information in the paper. I do not understand how it was published, let alone used as evidence.

Single worms were placed in culture vessels 10 cm in diameter and one cm deep, and sprayed every two

weeks for 100 days, with about 0.05, 0.1 and 0.2 times what was stated to be a "commercial" rate of application. However, that commercial rate was stated to be 14.4 grams per hectare, which is about 1/200 of a typical rate. This level is far below any amount known to affect other organisms; it is likely that this is an error in notation. As a result a reader of this paper has no notion of the way the worms were treated. The doses all produced the same response in decreased weight gain; in other words there was no dose response. Furthermore, in parallel experiments, Azinphos-methyl, a potent organophosphorus insecticide, produced less response than glyphosate. Azinphos-methyl has an acute LD_{50} of 10 mg/kg, compared to about 4000 mg/kg for glyphosate.

Page 12, col 1. Fish.

As a general case, the information to the extent it is given is correct, that is, certain concentrations are lethal or cause distress to fish under specified conditions. Missing, however is any information about the concentrations that may be found in a stream under various circumstances, and how those concentrations relate to those that cause harm. Without that connection there is no usable information.

As an example, this lack is illustrated by examination of Morgan et al (ref 153), which shows that effects of glyphosate or its formulations on fish are unlikely. Observing avoidance and behavioral responses, they found thresholds of effect much higher than concentrations that will be found in operational treatments: "Nominal concentrations of the herbicides causing threshold changes in fish behavior were 37.5 ppm Vision-10% surfactant, 13.5 ppm Vision-15% surfactant,---". It is obvious that these responses will not occur in any situation other than near a spill of concentrate.

Those concentrations were maintained over a 96 hour period. They can be compared with 0.7 ppm, the concentration expected after a direct application to water 30 cm deep.

The reference by Neskovik (ref 155), has been misread. The fish were exposed for 14 days, and the concentrations bear only a passing relationship to the acute LC_{50}. There were some adaptive changes in liver enzymes at 2.5 ppm after 14 days and some changes in gill structure at 5.0 ppm. Liver changes were modest and occurred only at 10 ppm. Low as are these concentrations, they are still much higher and longer in duration than will occur in any environmental context.

Page 13, col 2, Nitrogen fixation.

Cox contends that glyphosate interferes with nitrogen fixation and related reactions by microorganisms.

The papers she quotes (ref 167-172) uniformly show that such effects are not likely.

Eberbach and Douglas (ref 167) did find an effect of glyphosate on Rhizobia at 2 ppm in soil, which in is the general range of levels that may be found after application. They did not establish whether the effect was on the bacteria directly or on the plants they depend on for energy. The latter seems more likely, given knowledge from other experiments. They also commented that the only fertilizer used in the soil studied was phosphorus, but there was no data on phosphorus content. Sandy soil, combined with a high phosphorus content will make glyphosate more available to plants. It is interesting that all three concentrations produced the same effect.

Later work of Eberbach and Douglas (ref 168) also showed evidence for effects on nitrogen fixation. They estimated the risk that might be associated with uses of glyphosate. Their comment is useful: "--the predictions in Table 3 suggest that at field application rates, these herbicides would cause little damage to legume nodulation."

Moorman et al (ref 170) are said to have found inhibition of aromatic amino acid synthesis by nitrogen fixing Rhizobia at concentrations "approximately that expected in soybean roots following treatment--".

Cox may have misread the notation mM (millimolar) for parts per million. Moorman et al studied the effect of 0.5, 1.0 and 10 mM glyphosate IPA, or 116, 233 and 2332 ppm, in culture media. These are concentrations vastly greater than would be found in soil, with no microbial degradation or adsorption. Moorman et al do express curiousity whether glyphosate might somehow concentrate in plant roots at concentrations sufficient to have an effect.

Mårtensson (ref 171) did not examine nitrogen fixation as such, but did study components of the system, at concentrations of 10-100 ppm in the medium. At the lowest concentration there seemed to be little effect on growth of Rhizobium species in broth, on growth of small seeded legumes, root hairs of red clover, or dry matter production of clover roots. Nodule formation of red clover roots was inhibited, however.

The author remarks that the concentrations used are not likely in normal application, but, like Moorman et al (ref 170), wonders if unique situations of localized high concentration could occur. These studies were also in culture media, where there is free distribution of added chemical and no degradation by microorganisms. As several authors quoted by JPR point out, such conditions enhance toxicity to cultured organisms. (see below)

Page 13, col 3, Mycorrhizial fungi.

Estok et al (ref 174) evaluated glyphosate toxicity to ectomycorrhizial fungi in agar culture. One of the three species was inhibited nearly 50% at a concentration of one ppm. The other species were not affected at 10 ppm. Estok et al qualify the findings with a caution, that agar medium represents a very different condition from the environment of the forest floor:

1. The medium is designed to enhance growth and increased uptake of herbicide in the hyphae.
2. The organisms used are laboratory stocks grown for many generations on specific enriched media and are highly susceptible to environmental change, unlike wild organisms.
3. Agar is an aqueous medium with no barriers to diffusion of chemicals to sensitive sites, unlike the soil, where a large fraction of the material is immobilized.
4. No degradation of herbicides takes place in the agar medium, nor is there any other mechanism of removal.

The same caveats must be applied to the studies of Chakravarty and Sidhu (ref 175) where one of five species of mycorrhizal fungi was slightly inhibited by 1.0 and 10 ppm Roundup. Other species were unaffected at 10 ppm.

Comments Re: Editorial:

Caroline Cox has written an editorial "Responding to a Chemical Goliath", intended as a response to criticism of earlier articles about glyphosate. Three points are attempted.

1. The Journal of Pesticide Reform is not peer-reviewed. "JPR does not publish original research, and so it is not peer reviewed" Every respectable review journal is peer reviewed. Just a few examples might be Reviews in Environmental Contamination and Toxicology, CRC Critical Reviews in Toxicology, Annual Review of Pharmacology and Toxicology, and many others. A review is even more difficult to prepare than a research publication, and requires great diligence in evaluating research, integrating it with other similar work, finding its weaknesses as well as its strengths. Even the most authoritative scientists expect and welcome external appraisal of their review publications.

The next sentence points out that the "studies we cite in our glyphosate fact sheet are either peer-reviewed research articles, government documents or publications from pesticide manufactureres." The implication here is that if articles quoted are from peer reviewed journals, the conclusions she makes when quoting these papers must be correct. Even the most elegant scientific work can be misrepresented, as is shown again and again in this article. A comparison of the USEPA Reregistration Eligibility Document for glyphosate (ref 4), the World Health Organization (WHO) Environmental Health Criteria 159, Glyphosate (ref 2) and Agriculture Canada Discussion Document E91-01 for glyphosate (ref 16) with Cox's conclusions is evidence enough.

Peer review is not a protection against incompetent reading.

2. "It has been fifty years since pesticide use became widespread. During that time there has been a series of problems caused by pesticides even though these chemicals had been favorably evaluated by government agencies." That statement has nothing to do with glyphosate formulations, of course, but perhaps Cox would find it useful to catalog the long history of re-evaluations and cancellations that have arisen in the registration process.

Then, "People shlould be encouraged to look at the data that are the foundation of these evaluations and independently decide if they agree with the conclusions." This may be the only principle on which I agree with Cox and NCAP. Registration data should be available, and progress is being made in that direction. The idea that NCAP and its misuse of scientific information is a legitimate agent to assist the public in that endeavor strains credulity.

3. In response to a purported claim by the chemical industry that NCAP does bad science, NCAP remarks that pesticide research
is typically conducted or paid for by pesticide manufacturers. I am not sure what that has to do with the argument. The fact that the research and methods are specified by regulatory agencies, and the results interpreted by agency scientists seems to be lost here. As to the bad science, it is self-evident to anyone who reads this "fact sheet".

Lastly, there is a little epilogue to this editorial, speaking about Rachel Carson, and how NCAP is humbly honored to be following in her footsteps. Rachel Carson was a scientist of a high order, somewhat a visionary, but her ideas have done well since 1962. If she were to read that this material is claimed to be in her tradition, she would probably throw up.

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