Summary of criticism and questions on “Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize” collated from published expert commentary and from correspondence with Sense About Science.

21st September 2012

The introduction

• Most of the supporting literature quoted in the introduction is from the researchers’ own group.

• Most of the effects seem to be dependent on the herbicide. Since herbicides have to go through vigorous testing - why was this effect not discovered years ago?

Animals, their diet and photographs

Use of Sprague-Dawley rats was discussed in relation to its disposition towards the pathologies reported:

• Female Sprague-Dawleys frequently develop mammary tumours in well-fed controls. This is comprehensively discussed in The Effects of Diet, Overfeeding and Moderate Dietary Restriction on Sprague-Dawley Rat Survival, Disease and Toxicology (J Nutr. 127: 851S–856S, 1997). It appears the researchers allowed the rats to eat an unrestricted diet but there is no discussion about how many of the tumours were as a result of this.

• No food intake data is provided or growth data.

• It is unclear whether diets were iso-caloric between groups which might make a big difference.

• There is a lack of information on the composition of the diet. One concern is whether there were mycotoxins in the maize meal because of improper storage. Zearalanone is a well know phytoestrogen produced by filamentous fungi that grow on maize.

Experts criticised the apparent lack of blinding among researchers with regards to both sacrificing the rats and for measuring tumours:
• The researchers did not provide details of what steps they took to ensure investigators were unaware which rat belonged to which group until after the data had been gathered.

The photographs of the animals were criticised:

• As well as not really adding anything quantifiable, the researchers only included photos of treated rats and failed to include pictures of control group rats as a comparison. Sprague-Dawley rats frequently develop mammary tumours in well-fed controls. Are we to conclude from this that no controls developed tumours?

• The labelling of the photographs in the paper is inadequate. It is not clear which lesions are tumours and which are hyperplastic changes (they are grouped together).

Statistical analysis

The statistical analysis used in the study has been criticised on several different fronts.

The sample size was small:

• The numbers are so low they do not amount to substantial evidence.

• Ten rats per group is a small number. For example, is the death of three out of ten controls compared to five out of ten males in the treated group statistically significant?

• Although 10 animals per group do not appear unusual in animal studies, statistical analysis should have more data points for robustness in the conclusions.

• The control group is inadequate to make any deduction. To show how variably tumours appear there should be more controls compared to test rats.

• Sprague-Dawley rats appear to have a high probability of health problems after 2 years. And when there is a high probability of health problems, there is a high probability that just by chance you will find differences between treatments, especially if your sample size for each treatment is only 10 individuals.

The statistical analysis used is unorthodox and unclear:

• The statistical methods are unconventional, there is no clearly defined data analysis plan and probabilities are not adjusted for multiple comparisons.

• How was the analysis technique described in the paper applied to the data?
• Regarding the statistical methods: they're not just "unorthodox", they’re exclusively devoted to secondary analysis. No mention at all of what the investigators did with the mortality and tumour data.

• For a paper with such potentially important findings, it would have been more satisfying to have seen something with a more conventional statistical analysis. A comparison of each measured parameter, which took into account the variance throughout the experiment, which would have been revealed using a multiple range test, would have provided better evidence for the concluding remarks and the abstract.

• Some of the effects are presented in a way that makes it difficult to evaluate their significance. For example, there does not appear to be a statistical analysis of the mammary tumours. These occur quite often in untreated animals. One would usually also take into account the historical controls in the testing lab, in reaching a conclusion. The pesticide itself has been subject to long term studies in rodents by others.

• No confidence intervals or p-values have been calculated. These would have been able to show whether the results were statistically significant, for example, the pituitary sentence is indicative of the problems - why not calculate confidence intervals on the whole groups - which are too small anyway. [For all R treatment groups, 70–80% of animals presented 1.4–2.4 times more abnormalities than controls in this gland].

Data

Many people have called on the researchers to publish the full data set. This could have been done as per the journal’s guidelines as supplementary files. Sense About Science first requested the data on 19/09/2012 and is waiting for a response:

• The findings reported in the paper do not contradict previous findings that genetic modification itself is a neutral technology, with no inherent or environmental risks.

• Without access to the full data, we can only say that these results cannot be interpreted as showing that GM technology itself is dangerous.

Mechanism of action

The mechanism by which GM maize and the herbicide could lead to increase in tumour number is unclear:
• The herbicide risk is not convincing, especially with respect to cancer. There is no consistent pattern in deaths with dose of either Roundup or GM maize: this is not just showing a threshold, as the authors suggest, since in all six of their comparisons the highest-dose group has lower mortality than lower-dose groups. The hypothesis of hormone-related cancer differences is not supported by the multivariate biochemical analysis, which found differences in salt excretion but not in testosterone or estradiol. The strongest conclusion that could be drawn from this study is that it would be worth studying a larger group of controls than just 10 and (since there is no sign of dose-response) just a single low dose of Roundup or GM maize.

• The authors cite hormonal disturbance as the probable overriding cause. I would have thought that if this is so they should have used more than one breed of rat.

• How is the gene overexpression related to these tumours? Is it all by one mechanism?

Conclusions

The conclusions the researchers draw in the paper and the points made in the publicity of the paper lead to several questions:

• This was a study of one event with one gene. How can it be extrapolated from this that all genetically modified crops are dangerous?

• The difference in rates of tumours between the control and test groups are no different from what may have occurred by chance. Surely this means that the paper does not show a link between GM, Roundup and tumour growth?

• The results in the paper seem so extreme, why has nothing emerged from epidemiological studies in the countries where GM has been in the food chain for decades?

• Why have no previous published, peer reviewed studies come across results such as those described in this paper?

• How can this study be used as the basis of a call for a change in EU policy when the study does not fit the OECD 451 guidelines, so could not be used in regulation (too few animals per group)?