BfR opinion on “Major pesticides are more toxic to human cells than their declared active principles” by Robin Mesnage, Nicolas Defarge, Joël Spiroux de Vendômois and Gilles-Eric Séralini

BfR opinion No 011/2014, 06 March 2014

1 Occasion for the opinion

The BfR has been asked to provide its expert opinion on the publication “Major pesticides are more toxic to human cells than their declared active principles” by the French task force led by Prof. Gilles-Eric Séralini.

It is hypothesized that many pesticides (= plant protection products, PPP) are more toxic to humans and animals due to their mixed-in additives than their actual tested and approved active substances. This hypothesis is supported by cytotoxicity testing results derived from tests with nine active substances (including glyphosate) and one formulation of each active substance.

2 Result

The new publication by Mesnage et al. (2014) provides further indications that some PPP may have higher toxicity than the active substances contained therein. However, the publication does not provide any new information for the toxicological assessment of glyphosate and no general regulatory consequences can be derived from it. The publication’s aspects regarding the toxicological assessment of mixed-in additives in PPP are already incorporated in the BfR´s draft assessment report, which was prepared during the re-evaluation process of the active substance glyphosate in December 2013. The BfR report describes in detail that the toxicity of glyphosate-containing PPP is higher than the toxicity of glyphosate itself. Therefore this new publication is of no relevance to the current re-evaluation process of glyphosate in the European Union.

The used methods are suitable for cytotoxicity testing of active substances and their formulations and the in vitro data seem plausible. Therefore the data are considered to be additional indications to the BfR draft re-evaluation report. However, the data are not sufficient for a comprehensive regulatory assessment of PPP. The data are also inadequately for the assessment of the transferability of the results to intact organisms including humans.
The publication by Mesnage et al. (2014) and the comments in the BfR´s draft assessment report highlighting the need for research in the area of combined toxicity to which the BfR will continue to contribute.

3 Statement of reasons

Assessing the cumulative toxicity of PPP for the purpose of determining the risk to human health is laid down in the relevant EU regulations and poses a great challenge for experimental and regulatory toxicology. Cumulative effects can, on the one hand, result from the interaction between the various active substances in different PPP and then from the interaction between active substances and mixed-in additives within the same PPP. The publication by Mesnage et al. (2014) refers to the latter case. Due to the large number of approved PPP it is not feasible to test all the theoretically possible combination effects through repeated exposure as part of conventional animal experiments. For this reason, it is very important to develop and validate suitable model systems which can be uesd as screening test battery prior to any animal experiments.

The French researchers led by by Prof. Séralini report in their publication (published in advance http://www.hindawi.com/journals/bmri/aip/179691/ but not available as print yet) on in vitro cytotoxicity experiments of nine active substances of PPP and one formulation for each active substance. As test systems three well characterized cell lines of human origin were used: HepG2 (liver), JEG-3 (placenta) and HEK293 (embryonic kidneys).
These cell lines are also often used by the BfR to investigate combination effects of PPP in vitro by using the following methods:

- The MTT test investigates the damage to cell organelles and hence, indirectly, to cellular respiration on the basis of a measurement of succinate dehydrogenase activity in mitochondria following 24-hours exposure.

- The measurement of adenylate kinase release as an indication of damage to membranes (necrosis markers).

- The determination of the apoptosis rate in “Caspase-Glo 3/7-Assay”.

The methods are often used in cytotoxicity testing and it seems that there are correctly applied. At least for the MTT test experimental experiences are available at BfR.

Using the same methods and the same cell lines, Mesnage and colleagues (Mesnage et al. 2013) had already demonstrated increased toxicity of glyphosate-containing formulations and / or adjuvants compared to the active substance glyphosate. Showing this, the findings of other groups were confirmed. The BfR have already taken into account these data in the German draft assessment report, which was prepared during the re-evaluation process of the active substance glyphosate in December 2013. For the current EU assessment of glyphosate the newly published data does not provide any new information.

The in vitro experiments cover a wide range of concentrations. It is to be assumed that the actual concentration levels after exposure to the active substances or its formulations in human tissue and organs tend to be lower due to kinetics (distribution, excretion) than actual tested. Moreover, it is not to be expected that the organism is affected consistently over 24 hours by the substances. These uncertainties can be removed by oral or dermal administration studies, where the concentration of the substances in liver and other organs were determined. The obtained range of concentration can than be used in follow-on in vitro studies. For the BfR´s assessment of the testing results by Mesnage and colleagues, only limited data are available. The BfR collected by itself in 2012 such data for some active substances acting as fungicide. In consideration of the BfR test results and the results of Mesnage et al. (2014), BfR can conclude that the tested concentration is almost realistic for the liver after exposure to the active substances epoxiconazole and prochloraz.

In eight of nine cases of the tests described by Mesnage et al. (2014), the tested active substances (fungicides: epoxiconazole, prochloraz, tebuconazole; herbicides: fluroxypyr as its methylheptyl ester, glyphosate; insecticides: acetamiprid, imidacloprid, and pirimicarb) were less toxic than their respective formulations. The herbicide isoproturon was poorly soluble like its formulation Matin, and therefore the results for both will not taken into account for the assessment. The major difference in toxicity between the active substance and the formulation in almost all tests systems and types of cell lines was found between the active substance glyphosate and its formulation Roundup GT+ with an ethoxylated ether alkylamine as its declared adjuvant. However, the active substance glyphosate showed especially low cyto-

toxicity in MTT tests compared to all other active substances. Nevertheless the difference was significant.

For the other test methods listed above, the differences, notably for fungicides, were not as pronounced as in the MTT test. The results were also depended on the cell line.

For one of the methods, the MTT test, the BfR has gained experimental experience with the three active substances epoxiconazole, prochloraz, and tebuconazole tested in the two of the cell cultures used by Mesnage et al. (2014), i.e. HepG2 (liver) and JEG-3 (placenta) cells. For epoxiconazole and prochloraz, cytotoxicity to placenta cells was in the same concentration range as described in the publication. In the case of prochloraz, this also applies to liver cells. Due to the different dosages chosen for epoxiconazole in liver cells a comparison between the tests of Mesnage et al. (2014) and BfR is not possible.
Regarding the active substance tebuconazol BfR suspects a dilution error in the testing series due to the very high LC50 values and the fact, that tebuconazol already precipitates at the concentration used by Mesnage and colleagues. Therefore, the effective cytotoxicity of tebuconazole can not be confirmed and BfR is questioning the statement that the formulation Maronee is 1056 times more toxic than the active substance tebuconazole.

In conclusion, the data for the MTT test are considered reproducible on the basis of the BfR's own experience. The findings of the apoptosis testing are not robust at least for the formulations because of using dosages in the range which is already cytotoxic.

In order to be able to derive generally valid consequences for regulatory purposes it would be necessary to determine first which in vitro tests are most suitable to show higher toxicity of PPP formulations compared to the active substance. The methods and cell lines used by Mesnage et al. (2013, 2014) can be taken into consideration. However, given the current state of knowledge, they are certainly not sufficient. What is required, rather, is a screening test battery made up of suitable in vitro tests. On the basis of these results it is then possible to decide which PPP formulations show higher toxicity. This could subsequently lead to systematic further experiments with repeated administration. This procedure is the only way to avoid unnecessary animal experiments and to use scarce resources efficiently. To achieve this, collective efforts on the part of government authorities, academic research institutions, and the applicant industry are of high impact and indispensable. The BfR will make a contribution to these efforts in the form of a workshop on the regulatory categorization of data which have been collected, by means of new bio-molecular methods, from cell cultures and animal tissue. The workshop is planned for the fourth quarter 2014.

For far-reaching regulatory consequences, for example in relation to the recently amended data requirements for pesticides and their active substances, the current data basis is not sufficient. The new publication can be seen as a stimulus for further discussion.

It is important to note, that there is strong evidence for the higher toxicity of glyphosate-containing herbicides than for the active substance glyphosate due to mixed-in additives in the PPP (see BfR´s draft assessment report). The new publication by Mesnage et al. (2014) provides further indications that this also applies for some other PPP. However, it remains still an open question what relevance these in vitro findings in the commented publication have for the health assessment of PPP.

Independently of this new publication, the BfR is planning to conduct further research into the combined toxicity of different active substances and the combination effects of active substances and mixed-in additives. A relevant draft for the project was presented to the Federal

Ministry of Food and Agriculture (BMEL) in December 2013. The need for more research in this specific area is of particular importance and is substantiated by the new publication of Mesnage (Mesnage et al. 2014).

4 References

Mesnage, R.; Defarge, N.; Spiroux de Vendômois, J. and Séralini, G.-E. (2014, in press): Major pesticides are more toxic to human cells than their declared active principles. http://www.hindawi.com/journals/bmri/aip/179691/

Mesnage, R.; Bemay, B. and Séralini, G.E. (2013): Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology, 313 (2-3), 122-128. (PMID:23000283)