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Human cell toxicity of pesticides associated to wide scale agricultural GMOs

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Agricultural genetically modified (GM) plants are essentially plants which contain pesticides, because they were designed to tolerate or produce pesticides. In 2011, GM crops reached 160 million hectares, with 59 % of herbicide tolerance (mainly Roundup) mostly in soybean, maize, canola, cotton, 15 % of insecticide producing varieties and 26 % combining both traits (James 2011). We characterized cellular side effects of these pesticide residues on non-target human cells. We summarized here our findings:

Glyphosate-based herbicides toxicity

Roundup (R) was highly toxic on human cells, from 10-20 ppm, far below agricultural dilutions. This occurred on hepatic (HepG2, Hep3B and embryonic (HEK293) as well on placental (JEG3) cell lines, but also on human placental extracts, primary umbilical cord cells (HUVEC) and freshly isolated testicular cells (Benachour & Seralini 2009; Benachour et al. 2007; Clair et al. 2012; Gasnier et al. 2010; Richard et al. 2005). All formulations cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, through the release of cytosolic adenylate kinase measuring membrane damage. They also induced apoptosis through the activation of enzymatic caspases 3/7 activities. Most importantly, the R commercialized formulation is always more toxic than the active principle alone, the glyphosate (G). These effects were more dependent on the formulation and thus adjuvants content than on the G concentration. We recently measured compositions and effects of 9 G-based formulations and identified ethoxylated adjuvants (commonly called POEA) as the active principle of cytotoxicity. However, these are considered as inert diluents in international regulations and are not taken into account for chronic effects which are insufficiently tested, and only with G in pre-commercial testing. We previously underlined this loophole (Mesnage 2010). Long term feeding and reproductive trials with pesticides are the only tests long enough to reveal a potential endocrine disruption which was consequently never studied for R, however it was for G by itself.

We investigated it by measuring androgen to estrogen conversion by aromatase activity and mRNA on placental human cells and showed that G interacts with the active site of the purified enzyme (Richard et al. 2005). Both parameters were disrupted at sub-agricultural doses within 24 h. We also observed a human cell endocrine disruption

from 0.5 ppm on the androgen receptor in transfected cells, and then from 2 ppm the transcriptional activities on both estrogen receptors which were also inhibited (Gasnier et al. 2009). Aromatase transcription and activity were disrupted from 10 ppm on HepG2. On freshly isolated rat testicular cells, low non-toxic concentrations of R and G (1 ppm) induced a testosterone decrease by 35 % (Clair et al. 2012). This is expected to occur in human cells which are fitted with the same steroidogenic equipment.

G-based formulations are claimed to have been extensively studied by industry and regulatory agencies and are considered as one of the safest pesticides (Williams et al. 2000). This allowed the establishment of high maximum residue limits (MRL) for GM food/feed (up to 400 ppm). For instance, 20 ppm of G are authorized in GM soy and this MRL is in the range of concentrations typically found in a GM soy harvest. In the light of our results, the safety of these thresholds is clearly challenged.

Insecticidal toxins (Bt) toxicity

Modified toxins from Bacillus thuringiensis are Cry proteins forming pores in insect cell membranes (Then 2010). They are claimed and believed to be inert on non-target species. We have tested for the very first time Cry1Ab and Cry1Ac modified Bt toxins (10 ppb to 100 ppm) on the HEK293 cell line, as well as their combined actions with R, within 24 h, on three biomarkers of cell death: measurements of mitochondrial succinate dehydrogenase, adenylate kinase release by membrane alterations and caspases 3/7 inductions (Mesnage et al. 2012). Modified Cry1Ab caused cell death from 100 ppm. For Cry1Ac, under such conditions, no effects were detected. In vivo implications should be now assessed, as Cry1Ab does not appear to be proved as an insect specific toxin.

Combined toxicity

In the new growing generation with stacked traits, G-based herbicides (like R) residues are present in the R-tolerant edible plants and mixed with modified Bt insecticidal toxins that are produced by the GM plants themselves. However, the toxicology of mixtures cannot be fully understood without knowing the combined toxicity of the various compounds of the formulations. In some in vitro conditions, G and its adjuvant synergistically damage cell membranes in a similar manner to R (Benachour & Seralini 2009). R adjuvants change human cell permeability and amplify toxicity induced already by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation. For the mixtures of Bt toxins and R, the only measured significant combined effect was that modified Cry1Ab and Cry1Ac reduced caspases 3/7 activations induced by R; this could delay the activation of apoptosis and impact on necrosis. There was the same tendency for adenylate kinase activity and succinate dehydrogenase

activity measures. Pesticides have to be tested together, 26 % of agricultural GMOs are indeed stacked events.

We also reviewed 19 studies of mammals fed with commercialized GMOs (Seralini et al. 2011). Meta-analysis of all biochemical disruptions indicated liver and kidney problems as end points of GMO diet effects. These are the major reactive organs in case of food chronic intoxication, and several contingent factors suggested that pesticide residues may be involved in the pathological features. All together, our results raise new questions in the risk assessment of food and feed derived from genetically engineered plants.

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