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The Need for a Closer Look at Pesticide Toxicity during GMO Assessment

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Summary

Public policy is regularly shaken by health crises or unexpected discoveries; future directions in toxicology assessment are therefore urgently needed. This chapter focuses on agricultural genetically modified organisms (GMOs) because they are essentially pesticide-plants, designed to tolerate and/or produce new pesticide residues in food and feed. Moreover, the usual concepts of regulatory toxicology become erroneous or insufficient with regards to endocrine or nervous disruption, or epigenetic effects. Most pollutants affect cell-cell communication systems in the same way as unsolicited spam emails, and may promote chronic and environmental pathologies. We also describe how formulated pesticides are mixtures which have not been investigated for their long-term toxicities. They contain adjuvants that are even more toxic than the supposedly active principle. Finally, long-term and multigenerational testing *in vivo* often appears essential. This can be accomplished within two years on rats, independently of the biotechnology industry, with raw data being transparent to the scientific community to allow healthy debate before the next health crisis.

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10.1 Purpose, aim and scope

In the matter of health issues, public policy is regularly shaken by health crises or unexpected scientific discoveries. Late lessons are learned from early warnings of toxicity of pesticides and pollutants in general (European Environment Agency, 2013). There is an inescapable lag between scientific discoveries and advances in regulation, but this may be amplified by private, political, or economic interests. History has taught us that environmental hazards could be avoided by proper risk assessment and the use of active preventive research, according to the precautionary principle (European Environment Agency, 2013). Unfortunately, several decades ago this was not the case for health risk management of thalidomide, diethystilbestrol, asbestos, organochlorine pesticides, plasticizers, and heavy metals, which were all believed to be safe and used at a large scale. These substances were regulated after several crises, and sometimes only prohibited or limited in their use after decades of contradictory debates as was the case for the ban on tobacco smoking in public areas or the proper labeling of risks. The lack of scientific knowledge in these matters had huge costs for public health and past, present, and future issues of practical food safety. It is now time to study this challenge for food and feed based on genetically modified organisms (GMOs) and the specific pesticides used or produced during their growth (see in particular Section 10.3 on pesticide plants).

As one of the research teams who published the most on the toxicity of edible GMOs and their associated pesticides, we were involved in an international scientific maelstrom when we published the first lifelong toxicological assessment in mammals of a Roundup-tolerant GM maize (NK603) and of the whole Roundup formulation (Séralini *et al.*, 2013). This GM maize was designed to tolerate (and thus to contain without dying) the Roundup formulation. Almost all agricultural GMOs cultivated at a large scale are pesticide plants, accumulating unusual levels of pesticide residues. Our long-term test was similarly criticized by health agencies and the biotech industry promoting the commercial authorizations of these products without conducting lifelong tests. We carefully answered all critics (Séralini *et al.*, 2013).

In particular, we noticed that pesticide toxicity was overlooked in these files. For instance, the modified insecticidal *Bt* toxins, known to be produced by *Bt* maize or cotton, were never tested on human cells between 1995 (origin of these commercial GMOs) and 2012, when our group tested them (Mesnage *et al.*, 2012a). By contrast, the main toxicological assessment of these GMOs is the establishment of the substantial equivalence (relative to gross chemical composition) with their natural counterparts or the closest isogenic lines of plants for controls. It has become obvious that more rigor in GM research is necessary (Domingo, 2000; Graef *et al.*, 2012; Séralini *et al.*, 2009, 2011; Spiroux de Vendômois *et al.*, 2010). This is true in particular on their potential toxicities as pesticide carriers (the reason why they were genetically modified), or due to unexpected genetic or metabolic disruptions caused by the technology itself.

10.2 A silent pandemic

Pesticides alone in non-GM treated plants, in the food chain or in the environment are already believed to be responsible for a silent pandemic, as described in Sections 10.2.1 and 10.2.2.

10.2.1 First observations on animal and human reproduction

Pesticides are formulated toxics, supposed to be specific to plants (herbicides), insects (insecticides), or fungi (fungicides). However, non-target effects are very often described in the literature (Colborn *et al.*, 1993; Androutsopoulos *et al.*, 2012; Mrema *et al.*, 2012) because they act as disruptors of the universal cellular metabolism (quite often on the respiration chain), or disruptors of cell–cell communications highlighted by hormonal or nervous disruption *in vivo*. Pesticides are widely used, not only in agriculture but also in the environment (public or private

parks, gardens, along roads and railway tracks, etc.) or even indoors for domestic use (insecticides, acaricides) or medical use (human head lice products).

These products have been used on a large scale for decades, and about GBP 5.2 billion are used worldwide every year (US EPA, 2012). Their spraying was intense after the Second World War, until it was noticed how detrimental they were to wildlife and then in mankind (Carlsen *et al.*, 1992; Colborn *et al.*, 1993). One of the first people to sound the alarm was Rachel Carson with her book *Silent Spring* (Carson, 1962) which was widely read.

It is noteworthy that early warnings concentrated mostly on the reproduction problems of wild populations of animals. An important remark is that the physiological function of reproduction initially appears more sensitive to environmental pollutions, rather than other physiological vital functions maintaining the homeostasis of the body, for instance blood composition and circulation, heartbeat, respiration, digestion, or elimination. Reproduction is non-essential for the individual life, but essential for the survival of the population as a whole. It is therefore one of the first phenomena to be affected by a physiological stress of an individual. Moreover, the meiosis at the origin of sexual cells and pregnancy are fragile phenomena which are very sensitive to environmental toxics. It also demands more energy than basic physiology. It is therefore understandable that early warnings discovered on the effects of pollutants in wildlife always concerned reproductive problems in different species.

One of the first to be reported was in 1952, when the ornithologist Charles Broley noticed a change in the behavior of bald eagles in Florida and discovered abandoned nests. Eighty percent of the birds appeared to be sterile and the number of juveniles declined (Broley, 1958). This symbolic eagle associated with the flag or US seal had an increase in blood levels of organochlorine pesticides (Coon *et al.*, 1970). Alligators of Lake Apopka, near a factory releasing large amounts of dicofol and DDT, developed an atrophy of the penis which led to the decline of the population (Guillette *et al.*, 1994). The reproduction of Florida panthers was impaired when male panthers became feminized as a result of prenatal or postnatal exposure to endocrine-disrupting chemicals such PCBs and pesticides such as DDT or methoxychlor (Facemire *et al.*, 1995).

Male reproductive health was also affected in the meantime. A decline in sperm quantity and quality was first noticed by Carlsen *et al.* (1992) and later confirmed by Jorgensen *et al.* (2001). A rising incidence of testicular cancer (Jacobsen *et al.*, 2000) and an increase in birth defects such as hypospadias and cryptorchidism was observed and grouped under the term 'testicular dysgenesis syndrome' by Niels Shakkebaek (Skakkebaek *et al.*, 2001).

10.2.2 Endocrine and nervous disruptions due to the aromatic structure of pesticides

Pesticides and plasticizers as well as large number of industrial products (paints, inks, etc.) are often synthesized from petroleum chemistry. The fossilization of plant aromatic compounds takes millions of years of sedimentation to make aromatic stable petroleum compounds. Plant aromatic compounds help sexual plant reproduction by attracting insects, and also have pheromonal activities for mammals in perfumes since they structurally resemble animal sex steroids with aromatic cycles. They are in general part of sexual signaling systems. The petroleum molecules chemically processed and pesticides have for this reason aromatic or pseudo-aromatic structures (Figure 10.1), like plasticizers. For instance, phytoestrogens are structurally and physiologically close to animal estrogens. This is why we can observe a crossed reactivity between these cyclic compounds in plants or petroleum and steroid hormones. Their endocrine-disrupting activities, including pesticides, are hardly surprising, and may be at the origin of reproductive disorders in the last 50 years since large stable aromatic compounds began to be spread in the environment by industry.

Most pesticides are usually stable and lipophilic because of their petroleum origin. As a consequence, they persist in fat tissues and can bioaccumulate in organisms, increasing their levels in the food chain

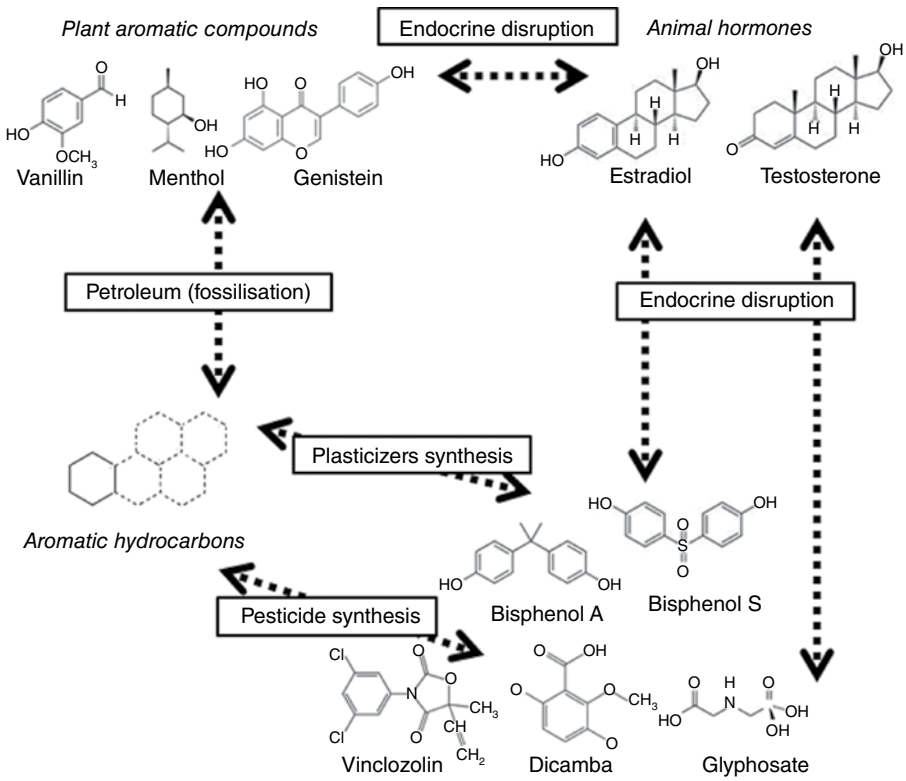

Figure 10.1 The origin of pesticides and plasticizers from petroleum and plant aromatic compounds. The fossilization of plant aromatic compounds, in particular, takes millions of years of sedimentation to make aromatic stable petroleum compounds. Plant aromatic compounds structurally resemble animal sex steroids with aromatic cycles, and this explains their sexual effects and pheromonal activities. For this reason, chemically processed petroleum molecules and pesticides have aromatic or pseudo-aromatic structures, like plasticizers. This is why we can observe a crossed reactivity between these cyclic compounds in plants or petroleum and steroid hormones.

cycle (Smith, 1999). Moreover, breastfeeding allows the transfer of xenobiotics ingested from the mother to the baby (Bouwman *et al.*, 2012). As a consequence, xenobiotics or their effects can be transmitted from one generation to the next.

Their lipophilicity also makes them act as electric insulators. This has been particularly researched for plasticizers surrounding food or feed, to prevent microbiological organisms from breaking in. This is particularly a concern for plastic baby bottles that allow the contamination of infants by plasticizers (Kubwabo *et al.*, 2009). Pesticides can also modulate neuronal activity however because the brain, physiologically dependent on neurosteroids, is very sensitive to aromatic compounds such as estrogen, catechol-estrogen or catecholamines (Gonzales *et al.*, 2007). They may even modulate sexual behavior (Balthazart *et al.*, 1990) and can also initiate Parkinson's disease (Gaikwad *et al.*, 2011). In some cases, pesticides trigger neurological impairment (Rosenstock *et al.*, 1991; Rohlman *et al.*, 2007). Alzheimer's or Parkinson's neurodegenerative diseases are observed after both chronic and acute intoxication by pesticides (Elbaz *et al.*, 2009; Weisskopf *et al.*, 2010; Parron *et al.*, 2011).

All these links make it possible to understand the endocrine or nervous disruptions, and in general cell–cell communications interactions, linked to the general structure of aromatic pesticides resembling hormones.

10.3 Link between pesticides and agricultural GMOs

Here we describe more precisely the links between agricultural GMOs and pesticides. The application of genetic engineering in agricultural practices was advocated as the most important recent advance in plant protection for the last decades (Van Montagu, 2011). The industry claimed to reduce the use of pesticides by introducing genetically modified (GM) plants (Monsanto, 2013). This is not yet established however, since an increase, in particular in Roundup use, is documented (Benbrook, 2012). However, it is clear after 15 years that pesticides are the keystone of the agricultural GM characters. Almost all commercially cultivated agricultural GMOs are pesticide plants since their origin. For instance in 2011, 160 million hectares of GM crops were cultivated worldwide, with 59% of herbicide tolerance only (mainly Roundup), mostly in soybean, maize, canola, and cotton. They promote the use of the main herbicide of the world, Roundup, on edible plants. Another 15% of GM crops had for their GM character insecticide production only; 26% had stacked traits combining both characters (James, 2011). For example, SmartStax maize has six traits of different insecticide production (*Bt*) and two traits for herbicide tolerance, and therefore may contain up to 8 kinds of pesticide residues in addition to those which can be conventionally applied such as fungicides.

This method of pest management was agronomically criticized. These GM crops were first promoted as new tools for Integrated Pest Management (IPM). However, these practices cannot fulfill the main ecological principle of IPM, generally considered as a protection measure which should be synchronized only when pest damage exceeds a critical level (Székács and Darvas, 2013). In fact, the cultivation of GM plants is necessarily accompanied by the intensive use of glyphosate-based herbicides, and even more so when glyphosate-resistant weeds develop (Owen, 2008). This is amplified by the systematic insecticidal production at high levels in *Bt* plants. However the use of additional insecticide sprays (Tabashnik *et al.*, 2008) increased, explained by a raise in insect resistances (Devos *et al.*, 2012).

For the cultivation of GM plants, Roundup may be sprayed several times in almost 80% of cases during the plant growth, simply because the plant can tolerate it. In fact, Roundup kills almost all normal plants except GM plants, and this facilitates the growth and harvest of some GM crops. GM plants therefore accumulate Roundup residues during their growth, while residue contents increase with the number of sprays (Arregui *et al.*, 2004). Because of the high content of GMOs in these residues, the Maximum Residue Limits (MRL) of glyphosate and aminomethylphosphonic acid (AMPA) (a compound of Roundup and its main metabolite used as markers) have been considerably increased with the development of these GMOs. MRLs represent the maximum level of residues expected when applying a pesticide according to 'good' agricultural practices. When other pesticides are generally found in edible plants at levels around 0.01–0.1 ppb (Sanco, 2013), glyphosate and its metabolite AMPA have among the highest MRL levels with up to 500 ppm authorized in GM feed (Sanco, 2013). Even 2 ppm of glyphosate and AMPA were authorized in bovine kidney, for instance (EFSA, 2009), since cattle are increasingly fed with transgenic Roundup-tolerant soya. The MRL in transgenic soybean, a major edible GMO grown for farm animals, has been set to 20 ppm.

For the second character, around 20 ppm of *Bt* toxin can persist in the transgenic plant (Székács *et al.*, 2010). This content can differ according to the GM variety and environmental conditions (Then and Lorch, 2008). Note that standardized guidelines to assay *Bt* toxins that are reproducible are lacking (Székács *et al.*, 2011). Herbicide-tolerant plants and *Bt* corns are regularly consumed not only by mammals but also by humans (on a regular basis in America), and their residues are even found in maternal and fetal cord serum (up to 93 ppb of glyphosate and around 0.2 ppb for *Bt* residue; Aris and Leblanc, 2011), but this does not take into account the possible bioaccumulation in the tissues. Roundup residues are not only found

in the urine of farmers spraying glyphosate-based herbicides but also in the urine of their children living far from the fields (Mesnage *et al.*, 2012b).

We consider as pesticides in GM plants not only the new modified *Bt* toxins alone or in combination, but also the herbicide residues that they tolerate, mostly glyphosate, with the adjuvants that go with these. As a matter of fact, the active principle of a pesticide is never used alone. Its toxicity is technically amplified by the choice of adjuvants, as cell penetrant helpers and stabilizers (Baynes and Riviere, 1998; Marutani and Edirveerasingam, 2006). Some of these, such as polyethoxylated (15) tallowamine (POE-15), are even more toxic on human cells than glyphosate (Mesnage *et al.*, 2013).

A minority of GM plants may also tolerate other herbicides such as glufosinate, bromoxynil, or the most recent ones such as 2,4-D and dicamba. Gufosinate ammonium is structurally related to glutamate which acts in neurotransmission in mammals. This other GM pesticide can induce spatial memory impairments, hippocampal magnetic resonance imaging modifications, and glutamine synthetase activation after chronic exposures (Calas *et al.*, 2008). Bromoxynil disrupts the development of the axial skeleton in mammals (Chernoff *et al.*, 1991; Kawanishi *et al.*, 2003). The 2,4-dichlorophenoxyacetic acid (2,4-D), a major ingredient of Agent Orange, is a synthetic auxin, a class of plant hormones. Its effects on health vary from a suspected carcinogenicity (Zahm *et al.*, 1990) to developmental effects such as a higher rate of morphologic and skeletal abnormalities in fetuses exposed during pregnancy to 2,4-D (Mazhar *et al.*, 2012). Dicamba is known as being genotoxic (Gonzalez *et al.*, 2009), but its role in the incidence of some cancers is debated (Weichenthal *et al.*, 2010).

10.4 Focus on Roundup toxicity in GMOs

The regulation of GMOs and the study of their health effects as pesticide plants is a hot topic of research.

10.4.1 Adjuvants: glyphosate is not the major toxicant in Roundup

Glyphosate is the most-used herbicide active ingredient in commercial formulations of pesticides worldwide, including insecticides and fungicides. Its formulations are made up of 36–48% of glyphosate, water, salts, and adjuvants such as polyethoxylated tallow amines or polyethoxylated etheralkylamine (POEAs), isobutane, sodium benzoate, sodium salt of o-phenylphenol, light petroleum distillate, methyl p-hydroxybenzoate, and 5-chloro-2-methyl 3(2H)-isothiazolone (Cox, 2004).

We have tested more than 10 glyphosate-based formulations on 10 mammalian cell types and 3 microorganisms. These included placental cell lines and fresh placenta (Richard *et al.*, 2005), embryonic human kidney cell lines HEK293 (Benachour *et al.*, 2007), umbilical cord primary cells (Benachour and Séralini, 2009), hepatic cell lines HepG2 and Hep3B (Gasnier *et al.*, 2010, 2011) and freshly isolated testicular cells (Leydig, Sertoli, germ cells, cocultures of Sertoli and germ cells; Clair *et al.*, 2012b), and 3 milk microorganisms *(Geotrichum candidum*, *Lactococcus lactis subsp. cremoris* and *Lactobacillus delbrueckii subsp. Bulgaricus*; Clair *et al.*, 2012a). All formulations were highly cytotoxic in all cases, of 10–20 ppm by contrast to glyphosate alone, which was not that toxic in comparison. Roundup causes dose-dependent total cell death within 1 h through an inhibition of the mitochondrial succinate dehydrogenase activity and mostly membrane damages, measured by the leakage of cytosolic adenylate kinase.

Glyphosate is a herbicide supposed to be specific to plant metabolism. Its adjuvants are generally considered as inert diluents. We always observed a greater toxicity of Roundup in comparison to glyphosate alone, and thus side-effects of all its ingredients have been claimed (Benachour and Séralini, 2009). We studied potential active principles for toxicity on human cells for 9 glyphosate-based formulations. The 3 less-toxic formulations were demonstrated

to contain no ethoxylated adjuvants by mass spectrometry, and are around 10,000 times less toxic on mitochondrial activity than POE-15 alone, the major adjuvant. All the other formulations were toxic proportionally to the dilutions of POE-15 or other ethoxylated adjuvants in the formulations. These can be considered as new active principles for human cell toxicity. This is even pointed out in the reviews sponsored by pesticide manufacturers (Williams *et al.*, 2012). Adjuvant toxicity appears to be a general feature of pesticide toxicology, a feature that we studied extensively for this major model of pesticides (Mesnage *et al.*, 2013), but which is also described for other pesticides (Eddleston *et al.*, 2012). We found that for eight major pesticides (out of a total of nine analyzed), the commercial formulation is up to 1000 times more toxic than the active ingredient assessed for safety by regulators (Mesnage *et al.*, 2014). Generally, indications of this problem concerning the considerable increase of toxicity of the supposed active principles of pesticides in commercial formulations by the addition of adjuvants by manufacturers, has already been suggested (Brausch and Smith, 2007; Krogh *et al.*, 2003; Tsui and Chu, 2003).

This does not exclude cellular endocrine disruptions below the levels of cytotoxicity that may not be due to POE-15 alone (or other ethoxylated adjuvants), but that occur at least due to glyphosate (Richard *et al.*, 2005).

10.4.2 Glyphosate action in non-target species

Interestingly, glyphosate tolerance in GMOs is obtained by an overexpression of a transfected and mutated 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) insensitive to glyphosate inhibition. This is because the wild EPSPS responsible for aromatic amino acid synthesis is normally inhibited by glyphosate, which explains its properties as a weed killer. Glyphosate is structurally almost half of an aromatic cycle. It binds to aromatic-recognizing active sites and this is the way it acts (Boocock and Coggins, 1983). In mammals, glyphosate also inhibited steroidogenic enzymes which have steroid aromatic binding sites, or steroid aromatic receptors (Gasnier *et al.*, 2009); it may therefore act as an endocrine disruptor (Richard *et al.*, 2005). Its adjuvants may help to enter cell membranes including endoplasmic reticulum where aromatase sits (Figure 10.2).

We have measured androgen to estrogen conversion by aromatase activity and mRNA on placental extracts, and demonstrated that glyphosate interacts with the active site of aromatase (Richard *et al.*, 2005). We also observed a human cell endocrine disruption from 0.2 ppm on the androgen receptor in transfected cells, and then from 2 ppm for both estrogen receptors (Gasnier *et al.*, 2009). In freshly isolated rat testicular Leydig cells, non-cytotoxic concentrations of Roundup and glyphosate induced a testosterone decrease by 35% (Clair *et al.*, 2012b).

These results were obtained *in vitro*; cellular cultures are used instead of animal experimentation when possible (Hartung, 2009). Our studies are generally performed over 24 h and do not anticipate the elimination of xenobiotics, their possible bioaccumulation, or long-term combined effects. The human cellular effects of Roundup indeed increased with time (Benachour *et al.*, 2007) and radio-labelled glyphosate accumulated in cells within 48 h, suggesting a bioaccumulation of low concentrations of glyphosate (Gasnier *et al.*, 2011). Roundup adjuvants may also form adducts and link to DNA, avoiding direct elimination (Peluso *et al.*, 1998).

Results were also observed *in vivo*; Roundup altered the spermatogenesis in rats exposed *in utero* to 50 ppm per day (Dallegrave *et al.*, 2007). In a study performed by Yousef *et al.* in 1995, Roundup-treated rabbits presented a decline in body weight, libido, and sperm quality and count (sperm concentration and volume, semen osmolality and also semen initial fructose). Structural and functional testis and epididymides alterations were found in drakes after a 15-day exposure (Oliveira *et al.*, 2007). In rats, the same team showed changes in the progression of

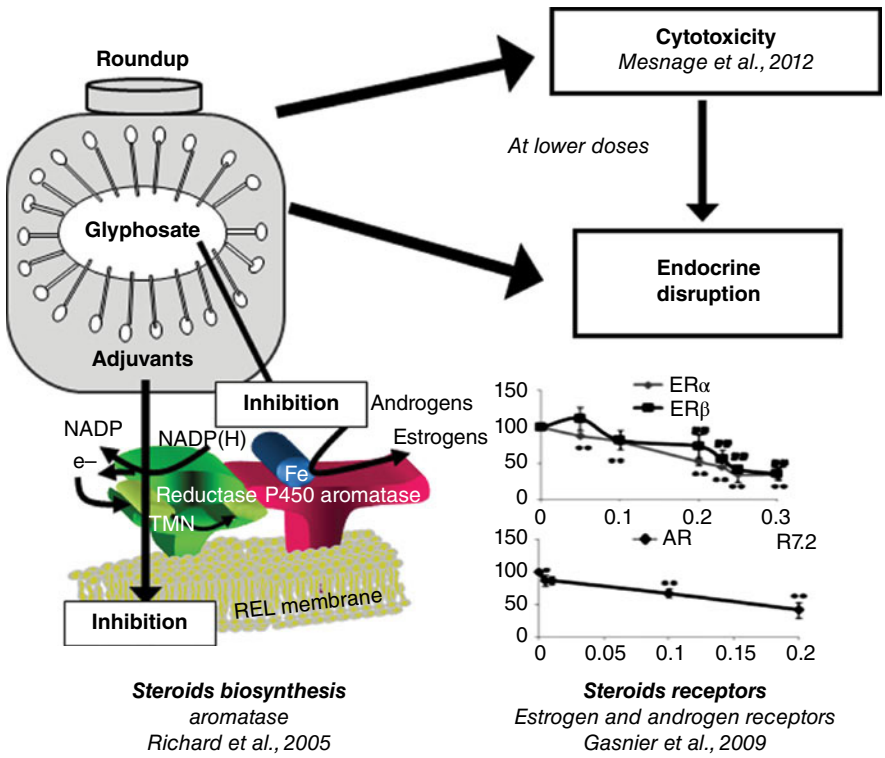

Figure 10.2 Glyphosate and Roundup are cytotoxic and endocrine disruptors at lower levels in human cells. Roundup is highly cytotoxic on human cells because of the non-specific actions of its adjuvants on cell membranes. At lower levels, Roundup is an endocrine disruptor. This is because glyphosate is structurally almost half of an aromatic cycle. It binds to aromatic-recognizing active sites and acts as an endocrine disruptor on aromatase and sex steroids receptors (androgen and estrogens receptors). Its adjuvants may help to enter cell membranes including endoplasmic reticulum, where aromatase is located.

puberty, function and structure of testis in prepubertal wistar rats after a 30-day exposure (Romano *et al.*, 2010) as well as on male offspring reproductive development (Romano *et al.*, 2012).

10.4.3 Long-term effects of Roundup or its residues in GMOs

No regulatory authority currently requests mandatory chronic animal feeding studies to be performed on edible GMOs and formulated pesticides. This is why we have recently studied the health effects on rats of a Roundup-tolerant genetically modified maize after a 2-year exposure (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.1 ppb in water) (Séralini *et al.*, 2012). Our work is the most detailed study involving the life-long consumption of any agricultural GMO and especially on NK603, for which only a 90-day safety test was previously conducted using the same rat strain (Hammond *et al.*, 2004). It is also the first long-term detailed research on any mammal exposed to a highly diluted pesticide in its total formulation with adjuvants (Séralini *et al.*, 2012). We have therefore replicated, extended, and improved the experiments conducted by Hammond and colleagues (Hammond *et al.*, 2004) or other manufacturers for other GMOs in two ways: (1) by measuring outcomes from 3 instead of

2 feeding doses and, more crucially, for a period 8 times longer in duration (2 years versus 90 days) and from (2) 11 blood and urine measures of around 50 parameters (34 organs instead of 17). We have compared the statistical findings that we precisely described (Spiroux de Vendômois *et al.*, 2009) with the results obtained in 90 days by the manufacturer (Hammond *et al.*, 2004) to check if they were biologically relevant or not in the long term.

We biochemically measured 10 rats per sex and group as performed by Monsanto. Even for a study of up to two years, it is indicated in OECD guideline 452 (for chronic toxicity testing) that biochemical effects should be monitored on at least 10 animals per sex per group, even if 20 rats are observed per group/sex.

We emphasized statistically discriminant biochemical effects at the 15th month, when most of animals were still alive (in treated groups 90% males, 94% females, and 100% controls). The significantly discriminant biochemical hepatorenal and sex steroid markers disrupted do correspond to the organic markers linked to the pathologies in a blinded analysis for the pathologists, who in turn linked them to the deaths. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed on GMOs. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls and the pituitary was the second most disabled organ; the sex hormonal balance was effectively modified by GMO and Roundup treatments. These unexpected effects were found at the lowest dose tested, from 0.1 ppb in water.

Within a week after the release of the publication, two international debates had begun. One debate was scientific, while the other was largely composed of insults and errors. Interestingly, the arguments were mixed in scientific journals. An example was a paper by Arjo *et al.* (2013) published in the journal *Transgenic Research*, a co-author of which was editor-in-chief Paul Christou. The quantity of insults and defamations in this paper is excessive. For instance, Arjo and co-authors suggest that by practicing 'flawed science' we are working against 'progress towards a better quality of life' and are 'actively working to make life worse'. Co-author Christou was previously employed by Monsanto during 1982–1994 and is the inventor of patents used to develop GM Roundup-tolerant maize (Christou, 1996). However, these interests were undisclosed in the published paper. Robinson *et al.* (2013) describe how conflicts of interest are biasing scientific opinion, eroding public trust and scientific integrity, and giving precedence to economic interests over public health.

In a recent development, Elsevier have announced the retraction of the Séralini *et al.* (2012) article (Séralini *et al.*, 2014). After the analysis of all our raw data, the editor-in-chief has stated that there was no fraud, no incorrect data, and no intentional misinterpretation. According to the Committee of Publication Ethics (http://publicationethics.org/files/retraction%20guidelines.pdf), retraction is only justified in the case of error or fraud. In our case, the Editor-in-chief writes that the data are inconclusive because of the rat strain and the number of animals used. These criticisms have however already been answered (Séralini *et al.*, 2013). The decision to retract the paper was reached after the appointment of a former Monsanto employee as Associate Editor for biotechnology in Food and Chemical Toxicology, the journal that published our study. In sharp contrast to our study which provides evidence of toxicity, a study published in the same journal which claims to prove the safety (Hammond *et al.*, 2004) using *the same strain and measuring the same number of rats for serum biochemistry*, is not being subject to the same controversy. According to an editorial in Environmental Health Perspectives (Portier *et al.*, 2014): the decision to retract a published scientific work by an editor, against the desires of the authors, because it is "inconclusive" based on a post hoc analysis represents a dangerous erosion of the under pinnings of the peer-review process, and Elsevier should carefully reconsider this decision. These double standards highlight how economic and political issues are endangering science and public health.

We encourage other research teams to replicate such chronic experiments and to perform, as would be quite logical now, carcinogenesis and developmental studies after our long-term general toxicology study. What is now urgently required is to re-check the burden of experimental proofs of safety for other food/feed GMOs in studies that should be conducted independently of industry. GM NK603 and Roundup cannot be regarded as safe to date.

10.5 Agricultural GMOs producing *Bt* are new insecticidal plants

We recall that the second category of agricultural GMOs synthesizes modified insecticidal toxins out of *Bacillus thuringiensis* (*Bt*) modified transgenes. All natural *Bt* toxins are pore-forming proteins in insect cell membranes (Then, 2010). Since *Bt* toxins have long been used, even in organic farming, their modified counterparts are often compared to them. However, the latter derivatives are truncated, adapted, and contain modified synthetic sequences; consequently their activity is possibly quite different from the natural sequences (Séralini *et al.*, 2011). When a transgenic plant has been modified to produce its own insecticide, most often there is no side-effect-testing, but there is some on bacterial-*Bt*-produced toxins *in vitro*. These can be folded or glycosylated differently in transgenic bacteria and plants, for instance. Moreover, they are used essentially to measure their *in vitro* digestion and stability, and not directly their toxicity on non-target cells. Modified *Bt* toxins are also assumed to be safe through theoretical *in silico* considerations when compared to immunogenicity databanks. The structural and activity comparisons are not scientifically sufficient to predict toxicological effects or safety. For instance, these considerations were unable to predict the toxicity of the pathological prions, hormones and venoms, which are also proteins and are now well known as being far from innocuous. We are back to the same problem as discussed for herbicide tolerant GMOs: the full product is not tested in the manner it is used.

It must be highlighted that the modified *Bt* toxins produced by the GM plants are in soluble forms and thus already biochemically activated, while those produced by *B. thuringiensis* are secreted as inactive precursors (Hilbeck and Schmidt, 2006). The importance of *Bt* toxin activation is clearly shown by membrane actions in human erythrocytes. Their alterations were detected after exposure with solubilized *Bt* toxins, but not in the intact form (Rani and Balaraman, 1996).

In another of our recent studies, we tested for the very first time Cry1Ab produced by MON810 and Cry1Ac *Bt* toxins (10 ppb to 100 ppm) on the human embryonic kidney cell line 293 within 24 h on biomarkers of cell death (Mesnage *et al.*, 2012a). Cry1Ab caused cell death from 100 ppm; this was measured by membrane alterations. This occurred at relatively high concentrations (100 ppm) in comparison to the concentrations produced in GM plants (1-20 ppm; Székács *et al.*, 2010), but this is only in 24 h and does not anticipate chronic effects or combined effects with other factors that have already been demonstrated to play a role on *Bt* toxin toxicity (Then, 2010). Also, this does not anticipate implications of long-term effects.

After *in vivo* consumption by pigs of the transgenic maize MON810 producing Cry1Ab, the results were interesting to interpret. The group of Walsh *et al.* extensively studied MON810 effects. One of their studies revealed the persistence of the modified *Bt* protein during digestion. However, no evidence of Cry1Ab gene translocation or protein penetration in the organs from blood of weaning pigs was observed (Walsh *et al.*, 2011). The authors revealed alterations in the immune responses of the animals, however. In another study by the same group, serum biochemistry differences with controls were found, but they were dismissed because the changes were not accompanied by parallel histological lesions (Buzoianu *et al.*, 2012). However a delay is commonly observed between biochemical markers disruptions and histological lesions. Organic dysfunctions were even revealed at a later stage within chronic periods. We observed differences in biochemical hepatorenal markers after 90-day feeding trials of MON810 in rats in our reanalysis of Monsanto data (Spiroux de Vendômois *et al.*, 2009).

Other short- or mid-term experiments during animal MON810 consumption (short relative to the lifespan of the species concerned), but independent of companies, revealed adverse effects on the immune system in various species including Atlantic salmon (Sagstad *et al.*, 2007), pigs (Walsh *et al.*, 2011), or mice (Finamore *et al.*, 2008). However, the study of the immune system is not mandatory in regulatory tests for biotech firms, and potential effects of GMOs on the immune system cannot be detected by application of the current guidelines.

After all these arguments, it must be highlighted that regulatory and mandatory protocols to obtain commercial-release authorizations ought to be adapted according to state-of-the art scientific knowledge. Protocols must be implemented by the study of intestine histology after GM and/or pesticide consumption, by the measure of digestive and gut wall enzyme activities, and also of the cellular stress and immune responses or allergenicity markers. In general, studies cannot be fully conclusive if the animals are not fed during their whole life while the maize is conceived to be chronically consumed.

10.6 Side-effects of the genetic modification itself

The focus on pesticide residues means that other effects due to the transgene itself or inherent to the technique of genetic engineering are neglected. Genetic engineering is claimed to be an improvement of traditional plant breeding, but in facts consists most often of random and imprecise multiple truncated or abnormal DNA insertions (Uzogara, 2000; Rosati *et al.*, 2008).

Current assessment also relies on the notion of substantial equivalence, meaning that the nutrient composition of the transgenic plant is compared to its isogenic counterpart. This concept has failed to prove GMO food safety and is not supported by recent scientific evidence (Perry *et al.*, 2009). Substantial equivalence has never been precisely characterized, and is more of a commercial and a political judgment rather than a scientific concept (Millstone *et al.*, 1999).

10.6.1 Specific side effects of the transgene expression

In our chronic study similar effects with respect to enhanced tumor incidence and mortality rates were observed in the groups of animals fed with the NK603 transgenic maize without Roundup application. A possible explanation of this finding is the production or alteration of specific compound(s) in the GM feed by the new mutated EPSPS, which could either be directly toxic and/or the cause of an inhibition of pathways that in turn generate chronic toxic effects.

An example of these effects is that the NK603 GM maize used in this study is engineered to overexpress a modified version of the *Agrobacterium tumefaciens* EPSPS (Hammond *et al.*, 2004) allowing Roundup tolerance. The modified EPSPS is not inhibited by glyphosate, in contrast to the wild enzyme. This enzyme is known to drive the first step of aromatic amino acid biosynthesis in the plant shikimate pathway. In addition, estrogenic isoflavones and their glycosides are also products of this pathway (Duke *et al.*, 2003). In our study, it was found that they were not disturbed. However, the levels of caffeic and ferulic acids in the GM diets, which are also secondary metabolites of this pathway but are not always measured in regulatory tests, were significantly reduced. This may lower their protective effects against carcinogenesis and even mammalian tumors (Kuenzig *et al.*, 1984; Baskaran *et al.*, 2010). Moreover, these phenolic acids and in particular ferulic acid may modulate estrogen receptors or the estrogenic pathway in mammalian cells (Chang *et al.*, 2006). This does not exclude the action of other unknown metabolites.

This is despite the fact that the variety of GM maize used in this study was judged both by the industry and the regulators as being substantially equivalent to the corresponding non-GM closest isogenic line. As the total chemical composition of the GM maize cannot be measured in detail, the use of substantial equivalence is insufficient to highlight potential unknown toxins and therefore cannot replace long-term animal feeding trials for GMOs.

10.6.2 Insertional mutagenesis or new unexpected/unexplainable metabolism

Hazards could arise from the consumption of the GMO itself. The result of the genetic modification is not a clean insertion and is very imprecise. In fact, the insertion of the transgene can induce wide metabolic changes, for instance, in the MON810 maize where the transgene indirectly introduced 50% changes in osmolytes and branched amino acids (Manetti *et al.*, 2006). This may be due to insertional mutagenesis or a new metabolism. In the case of MON810 maize, 43 proteins were up- or down-regulated with respect to their isogenic lines (Zolla *et al.*, 2008).

The transgene is often altered and other fragments can be introduced elsewhere in the host genome. These changes have been characterized following the insertion of the EPSPS in the Roundup Ready soybean event 40-3-2 (Windels *et al.*, 2001). As a result, new 'fusion genes' from the plant's neighboring DNA sequences of the insertion site can be synthesized (Rosati *et al.*, 2008). The insertion of the transgene may activate, inactivate, under- or overexpress a nearby gene. Very strong promoters indeed are used to overexpress the transgene. The read-through of the new sequences inserted downstream of the EPSPS was processed in four different RNA-variants which might code unknown EPSPS fusion protein (Rang, 2005). Other potentially active compounds such as micro RNAs can be synthesized (Zhang *et al.*, 2012). This is exacerbated by the instability of the transgenic DNA that tends to break, rearrange, delete, or insert elsewhere in the genome, which can be promoted by environmental changes (Matthews *et al.*, 2005), in a transposon-like mechanism of DNA rearrangement.

10.7 Limits and difficulties of interpretations in toxicity tests

All pesticides made from synthesis chemistry are tested on laboratory animals, such as invertebrates or fish, before being marketed. These *in vivo* toxicity tests are supposed to reveal potential adverse effects on mammals, most of the time rats, whose physiology is similar to that of humans; rats also serve as models for other mammals exposed to this feed. An understood side-effect in one mammal should exclude the consumption by others even from an animal ethical point of view. The chronic experiments are based on analyses of blood and organs (after autopsy) and such experiments are conducted for periods ranging from six months to two years, the average lifespan of a rat for the active principle only (at the most). Biotech firms are making different types of tests following very specific standards that they helped develop within the Organization for Economic Co-operation and Development (OECD), which manages international trade. The results, classified as confidential for the public at large as well as for the scientific community, are presented to expert panels in governments, food safety agencies from various countries, or directly to the European Union. This section debates the interpretation of possible side effects.

Doull *et al.* (2007) indicated their general criteria needed to classify the observed significant effects during 90-day toxicological tests on mammals as biologically relevant. The example taken was a GMO, a *Bt* maize called MON863, producing in its cells a new kind of modified insecticide. The authors claim to have applied the same criteria to other products such as pesticides and drugs. Below is the list of criteria used for all commercialized products. They were applied in the reanalysis of the German Health Agency for the renewal of glyphosate approval (German Federal Agency CPFS, 1998). They were strongly criticized because their application has led to the dismissal of glyphosate teratogenic effects (Antoniou, 2012).

1. Many authors make comparisons with historical data of control rats, both within the laboratory and the breeding company from which animals are sourced. However, this clearly enhances control variability and heightens the risk of false negative findings (Yoshimura and Matsumoto, 1994; Cuffe,

2011). It is now established that this concept should be used with caution. There are several reasons for this. Control diets for rats are contaminated with pesticides (Hayes, 2004) or chemicals leaching from cages (Howdeshell *et al.*, 2003); this artificially enhances background effects. The rat suppliers even recognize that their historical data come from rats potentially fed on GMOs (G.E. Séralini, personal communication, 2012). Last but not least, the occurrence of some spontaneous neoplasms in historical controls data is not stable over time and subject to positive or negative time trends (Tennekes *et al.*, 2004). Statistical comparisons therefore cannot be considered as relevant when they are performed between different experimental conditions such as in historical norms. They should focus on controls of the same experiment.

2. The effects have to be plausible. The authors reserve the right not to consider an outcome if they find it unconvincing. This was the case when some doses used in glyphosate precommercial testing that reported adverse effects were considered as too low to elicit a relevant effect. This is not a scientific method.
3. A major gap in some toxicological assessments is the lack of measurements investigating endocrine-disrupting effects (Birnbaum, 2012). The central dogma in toxicology is that effects vary linearly with dose. This is true for standard poison intoxication. However, toxins with endocrine-disruptive properties can give response curves that are U-, inverted-U-, or J-shaped, as frequently observed in the case of exposures to environmental pollutants (Vandenberg *et al.*, 2012). These effects were acknowledged more than a decade ago, and have been extensively reviewed by an expert panel upon the request of EPA confirming non-monotonic dose/effect relationships of endocrine disruptors (Kaiser, 2000). Low-dose effects cannot be invalidated because of the lack of high-dose effects (Myers *et al.*, 2009b). Even if food safety agencies today acknowledge that endocrine-disrupting effects at low doses do occur, they do not change their opinions on commercialized endocrine disruptors such as bisphenol A because they only performed a so-called 'validated' study (Fagin, 2012), meaning studies following international guidelines such as those of OECD. Advice to industrial chemists on how to screen chemicals for endocrine-related effects have now been published (Schug *et al.*, 2013).
4. The occurrence of similar effects in both sexes is an important criterion of toxicity for Doull *et al.*, but is not for us. Sex-dependent differences in chronic diseases resulting from chemical intoxication are well established (Lu *et al.*, 1991; Sissung *et al.*, 2006). This is a major mistake because many organs are sex differentiated; moreover sex-specific chronic diseases are a well-known feature (Kobliakov *et al.*, 1991). We have developed this topic in a review including experimental evidence (Séralini *et al.*, 2009).
5. Chemical, pesticide, drug, or GMOs companies often assumed that biochemical parameters disturbed could be rejected if no association with lesions in histopathology was evidenced. In short-term and mid-term studies, metabolic changes often precede histological changes which may only be detectable in long-term studies.
6. The effects must be always reproducible. This is a very important criteria, but one which must be used cautiously. Toxicities cannot be simply dismissed because they were not consistent between various experiments from different teams to conclude on safety. By contrast, this should be a reason to bring more effort into replicating the experiments and into finding the reason(s) for the lack of reproducibility. In a review of 121 replicate rodent carcinogenicity assays from both sources, only 57% of concordance was found between two classifications (Gottmann *et al.*, 2001). Rodent carcinogenesis bioassays are therefore not easily reproduced and the final decisions for risk assessment have to come from converging evidence at different levels

(*in vitro* assays, structure-activity relationship models, in-cell studies, animal lab studies, farm and wildlife observations, medical reports of workers in the factories where the potential toxic compounds are produced, socioeconomic considerations, etc.). Most of the time, all these data exist but are disregarded by agencies. Data provided by companies with OECD norms are often the main, if not the only, data considered.

7. The assessment of the quality of a toxicological test by agencies often refers to the Good Laboratory Practices (GLP) guide. Generally, it is standard practice that a regulatory agency does not take into account research studies, because they are not conducted under GLP conditions (Myers *et al.*, 2009a). By its very nature, a research protocol is rarely compatible with GLP agreements. A GLP agreement is a good tool to normalize regulatory assessment, but research studies need a greater degree of freedom in test protocols, models, etc. Experimental evidence of the hormonal disturbance induced by bisphenol A have been rejected because they were not conducted in a manner consistent with the GPL.
8. Statistical thresholds are perceived as an absolute truth. In our opinion, statistics do not tell the truth, but may help us in our understanding of experimental outcomes. Biological interpretations and the crossing of methodologies are key (Cooper and Kavlock, 1997).

10.8 The relevance of *in vivo* findings and length of the nutritional tests

10.8.1 Insufficiencies of *in vitro* tests

The usual manner in which agricultural GMOs and formulated pesticides such as Roundup are commercialized with short-term or no *in vivo* testing, as well as keeping raw data hidden for reasons of commercial confidentiality, is certainly not scientifically rigorous. Moreover, the first focus of regulatory assessments of agricultural GMOs should be the toxicity of pesticide residues. Current practice overlooks the fact that all GM crops are genetically modified to contain pesticide residues. These pesticide residues are new elements in our diet, both in type and quantity. The proteins usually compared (modified *Bt* toxins and wild toxins) are not identical, and the tests on human cells of *Bt* proteins are not performed or requested by authorities. Their stability has been assessed *in vitro*, and GM insecticidal toxins are never fully digested *in vivo* (Paul *et al.*, 2010). If some consumers suffer from stomach problems or ulcers, the new toxins will possibly act differently; digestion in children could also be affected. However, these GMOs could be eaten anywhere and all proteins would never be fully decomposed in amino acids by the digestive tract.

We were the only researchers to test modified *Bt* toxins produced in GMOs on human cells (Mesnage *et al.*, 2012a), as regulatory authorizations depend on more theoretical assessments of safety. We also found evidence of toxicity of adjuvants in Roundup-type pesticides, that is to say glyphosate-based herbicides. Adjuvants are routinely assumed to be inert and are not tested for long-term toxicity as part of the regulatory process (Mesnage *et al.*, 2013). In future, these practices may even be extended to pesticides or chemicals in general. This should not be the case within the framework of a rigorous health or environmental risk assessment.

An increasing number of scientists claim that *in vitro* testing and the use of computational models are the future of toxicology, therefore avoiding the use of animal models, with a view to providing a fast answer to the challenge of testing the 10,000 to 30,000 chemicals currently marketed for which the hazard data is insufficient for assessing potential health risks (Betton *et al.*, 1994). The use of physiology-based pharmacokinetic (PBPK) models is advocated to estimate the chemical bioavailability for tissues in order to assess the relevance of *in vitro*

toxicities. Applied to the prediction of *in vivo* embryotoxic effect levels, it cannot fully replace animal studies and sometimes underestimates embryotoxic potential because embryonic stem cell tests do not perfectly reproduce embryotoxicity (Verwei *et al.*, 2006).

The adult human is composed of about 400 cell types (Vickaryous and Hall, 2006), and we are far from fully understanding their responses to chemical exposures and the systemic consequences for a whole organism. In our opinion, these methods may be a promising tool for the search of therapeutic candidate molecules during the research and development approach; they cannot fully replace animal studies for risk assessment, however.

10.8.2 Limitations of 90-day-long tests

The framework for GM food toxicity testing is not precisely defined and there is a huge gap to fill. Some scientists consider that it is not possible to test the long-term toxicity of a GMO. In chemical testing, short- and mid-term tests provide information on acute and sub-chronic toxicities, often the Lethal Dose 50, used to set up the protocol for longer tests. This is not possible for GMOs which are food and feed. However, that does not prevent at all the comparison between the GM feed and its closest isogenic counterpart in the long term.

The acute toxicity approach (less than a month of investigations on rodents with high doses) may yield results which are more proportional to the dose, as it might correspond to a rapid poisoning of the animals (generally with force-fed experiments). However, for many pesticide studies in the scientific literature, some long-term side effects of pesticides at environmental doses are described, but they are not visible in short-term experiments (Hernandez *et al.*, 2008). Sometimes, a 90-day feeding trial is performed. A meta-analysis of the studies performed by the US National Toxicology Program showed that 30% of the toxic effects were neither seen nor predicted during 3-month-long subchronic tests (Betton *et al.*, 1994). They therefore appear insufficient to ensure food safety.

The physiological interpretations of 90-day-based effects are indeed somewhat limited. It is obvious that the 90-day-long trials on mature animals performed today cannot scientifically replace the sensitivity of developmental tests on neonates. A good example is the gene imprinting by drugs that will be revealed only at maturity; this is an important subject of current research, and many findings have been reported for some chemicals such as bisphenol A (Braun *et al.*, 2009; Braniste *et al.*, 2010).

Trans-generational effects occur after epigenetic imprinting by a pesticide (Anway *et al.*, 2005). These effects cannot be detected by standard 90-day feeding trials; instead, they will be visible after many decades by epidemiology in humans if there are any, as illustrated in the case of diethylstilbestrol, which induced female genital cancers among other problems in the second generation (Wise *et al.*, 2005). The F3 multigenerational study is too rarely performed in regulatory tests. This is why, because of the number of parameters disrupted in adult mammals within 90 days in GMO feeding trials performed by the petitioner, it was necessary to independently reanalyze the raw data obtained by Court order afterwards (Séralini *et al.*, 2007; Spiroux de Vendomois *et al.*, 2009). These studies demonstrated possible signs of hepatorenal toxicity that were confirmed after long-term in vivo testing of the same GMO given at similar levels to the same rat strain, with a comparable number of animals (Séralini *et al.*, 2012). Additional tests including long-term periods should be systematically performed to protect the health of billions of people that could directly or indirectly consume these transformed products.

10.8.3 The need for additional tests including long-term tests

In order to take the debate forward, more data are needed. First, our long-term assessment (the only one of this kind) should be repeated with more animals, even if ten animals per sex per group (as we used in our experiment) allows

powerful statistical analysis of biochemical data according to OECD protocols 452 and 453. Some agencies recently admitted, in contrast to their previously published opinion (EFSA, 2008), that there was a need for long-term studies on GMOs (EU Food Policy, 2012).

It has also been acknowledged that no other long-term study has examined the effects of regular consumption of a pesticide on blood parameters. Even data on the short-term effects of Roundup consumption on blood parameters are lacking (CRIIGEN, 2013). Concerning the regulatory assessment of glyphosate, the presumed active ingredient of Roundup, it appears that several agencies accepted the product as safe despite the fact that they were not in possession of the raw data, which remains commercially confidential (Antoniou, 2012).

The outcome of the debate relies on the scientific community having access to the raw data that allowed the commercialization of Roundup and NK603. When this is made public, our raw data (Séralini *et al.*, 2012), having already been given to a notary, will be published on a website making comparisons possible. The Monsanto toxicological data on NK603 maize recently made public by EFSA (January 2013) is not in a statistically usable format, and an agreement with Monsanto is requested. Moreover, the data examined for Roundup authorizations are clearly abnormally lacking. Indeed, data with implications for public health are not related to manufacturing patents and should not be kept confidential. Finally, we would like to suggest a system where companies fund independent research on their products, commissioned by food safety or research agencies and provided to the scientific community online, with long-term testing for all products to which we are likely to be exposed in the long term.

10.8.4 Unraveling the effects of mixtures

In toxicology, unraveling the effects of chemical mixtures is a huge challenge. As a matter of fact, the first mixtures to be scientifically studied should be the formulations of the pesticides containing adjuvants with an active ingredient, which are always used in that form. These formulations are designed to stabilize and enhance the cell penetration of the so-called active ingredients. However, pesticides are administrated as single molecules in *in vivo* chronic tests. Such chronic tests are used to calculate acceptable daily intakes (ADI) which are considered as robust and regulatory objective values, even to predict other combined effects with different compounds such as the estimation of the Hazard Index (HI). This is a major conceptual gap. Indeed, the fact that an ingredient of a mixture (glyphosate in the formulation) is active in plants does not mean *a priori* that this ingredient is the most toxic of the mixture to non-target species, or more toxic by itself than the mixture. However, there is an unexpressed, widely believed hypothesis that the active principle against plant metabolism (glyphosate) is the most toxic compound in a formulation to non-target species. The differential effects between the major formulated herbicides of the world and their active principle glyphosate certainly invalidate this hypothesis.

We have even highlighted ethoxylated adjuvants as new active principles for human cell toxicity, definitively invalidating the use of glyphosate alone as the only active principle in chronic tests. ADI is therefore miscalculated and, to fit with reality, its definition has to be improved by up-to-date peer-reviewed knowledge. This was requested by the CE 1107/2009 regulation. A working group of the European Commission indicated that ADI should be recalculated anyway to offer a proper level of protection (SCCS, 2011), in particular because of co-exposures to chemicals. This is in accordance with our conclusions, in particular for combinations of formulated pesticides. However, any exposure to a single formulated pesticide is at first to be considered as a co-exposure to an active principle and adjuvants. This should be assessed as a priority; otherwise, the theoretical risk assessment of several active ingredients together would be nonsense if these have been underestimated by neglecting adjuvants. The regulatory two-year chronic tests on mammals should be systematically performed with the formulated pesticide to calculate a more realistic ADI and thus to better estimate health risks.

10.9 Conclusions and future outlook

In general, little attention is brought to formulated pesticide effects although this is the keystone of GMO agricultural management. Lessons have to be learnt from early warnings. We are never exposed to single compounds and this is even truer when stacked traits are designed to accumulate residues of different pesticides. Their combined actions have to be assessed. We recognize that all the combinations cannot be tested, but the relevant combinations such as the combined effects of pesticide residues at the level where they co-occur in GM plants should be tested to ensure a proper risk assessment. We call for a public, independent, transparent, and multidisciplinary assessment of GM food and pesticide formulations. Social and economic issues must be considered in light of the fact that over 400 scientists and experts challenged the contribution of GMO-based farming to food security (McIntyre, 2008). As molecular biologists, we are in favor of genetic engineering used as a research tool in closed laboratories, but we are more concerned about public health than economical interests.

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