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D O S S I E R

THE AMBASSADORS
THE WAYS OF THE GLYPHOSATE ARE INFINITE



S U M M A R Y

DOSSIER | THE AMBASSADORS | THE WAYS OF THE GLYPHOSATE ARE INFINITE

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The Ambassadors

The main characters in this artwork are two of the most influential personalities of their era. They are portrayed together, both with the most important and meaningful clothes and objects of their life. Social class, youth, culture and hopes of an era are represented in perfect proportions and symmetry aimed at creating a painting with functions and symbology that are typical of Renaissance. On the left, the French King's Ambassador to London; on the right, a high prelate and Ambassador of the Holy See: friends and key players within a balance of power that France (birth place for both) and its King are trying to maintain with difficulty, to oppose the Anglican Schism. Holbein paints the desire for a political and religious balance that his era and his commissioner are missing by depicting two men and friends, in their full blush of youth and in the pinnacle of their power, framed by a highly communicative geometry and symbology. Everything in the painting is young, cultured, strong and harmonious, with the exception of the strange oblique stain across the floor.

Lithuanian art critic Baltrusaitis' words on this image are enlightening. In his journal, he wrote: 'In Polisy Castle (...) the painting was definitely placed in

a big room, opposite a door and close to another, where each door coincided with one of the two points of view. (...) The first act starts when the visitor enters from the main door and sees, in front of him or herself, at a certain distance, the two men standing out on the background, as if they were on a stage. (...) A single point disturbs the visitor: the strange object at the feet of the two men. The visitor moves forward to look at things closer: the physical, almost material nature of the vision is enhanced the closer one gets to the painting. However, that singular object remains absolutely incomprehensible. The disconcerted visitor exits from the right door, that is the only open door, and the second act starts. When the visitor is about to enter the adjacent room, he or she turns his or her head for a last look at the painting, and in that moment everything is clear: due to the sudden visual shrinking, the scene disappears and the hidden shape comes out. Where before everything was secular magnificence, now the human skull appears. The two men, with their scientific equipment, vanish. In their place, the sign of Nothingness arises from the nothingness: end of the performance".



The painting technique is called anamorphosis and the skull in *The Ambassadors* is one of the most famous and effective examples of the technique: the painting changes its meaning, which is overturned, depending on the perspective it is observed from. Exactly like the glyphosate matter. The Ambassadors in our era, guarantors of Money and Health, show us the molecule of desire, respectful of Youth (allegory of the Ecosystem, in our case) and of the natural Balance of Things. Glyphosate information campaign seems to say: 'It makes you rich and it doesn't hurt', like in the front sight of Holbein's painting that fascinates for its symmetry and beauty, despite the strange image on the floor. However, the beauty and the perfect balance between Money and Health disappear if we change perspective: on the herbicides packaging the skull will then appear to symbolise the danger of the active ingredient. The anamorphosis holds sway within the information available on glyphosate. And this is particularly

true on the Internet, where Scientific Knowledge, often hidden and not for free, completely overturns the free and at hand information, like the one provided by Wikipedia. We are saying this not to demonise Wikipedia, which is a great source for the officially recognized knowledge, but to highlight the existing discrepancy between official and scientific information on glyphosate. **The analysis of this discrepancy will be useful for the assessment and understanding of EFSA's (European Food Safety Authority) recommendation with regards to the revision of the regulatory limits of the molecule residues in food (MLR), as well as SANTE's (EU Directorate-General for Health and Food Safety) that recommends a revision of honey LMR. In this Report, we will try to suggest a rational review to EU policies on glyphosate, highlighting the informative contradiction that puts the ecosystem life at risk.**

Glyphosate in honey, the end of an economic miracle

The first studies aimed at finding glyphosate residues in honey date back to 2016. Those investigations began after the molecule was detected in many, if not all, the matrices taken into account in various studies carried out throughout the world. Glyphosate was found in food, nappies, sanitary pads, human blood and sperm, placenta, baby's milk, water and beer, just to name a few amongst the examples that impressed the public opinion the most. The list could be endless though, as **the molecule is present anywhere, including honey.**

The news about the wide presence of the molecule in food and human metabolism spread out globally after IARC (International Agency for Research on Cancer) announced that glyphosate was likely to be carcinogenic. For the first time since 1974 (the year Roundup® was first commercialized), IARC's announcement classified the molecule as potentially harmful for human health. An unprecedented emergency had to be faced and that was even worse than the ones triggered by DDT, atrazine, dioxin and PCB. The most widely used herbicide was likely to cause cancer and it was present everywhere, including human blood! For a few months, the global health catastrophe was thought to be imminent and inevitable: the boundaries had already been crossed and

even a sudden ban of the molecule would not have had retrospective effects.

The studies commissioned by EFSA - aimed at assessing the risks involved in the use of the molecule - and then EFSA itself determined that the emergency was over, by confirming that the molecule was indeed not harmful and authorizing the use of glyphosate-based herbicides for another 5 years. According to the European scientific authorities, IARC's warning was exaggerated, as there was no evidence to confirm that the molecule was carcinogenic and there was no real reason to prohibit its use.

European Authorities' stance reduced the concern for the residues widely found in food and human matrices: the data were assessed in relation to

the regulatory limits; therefore the health problem no longer persisted.

While regulatory limits justify the presence of glyphosate in any - or almost any - farm product, honey falls within a specific category for which no residues are acceptable, with the exception of the margin of error caused by the instrument used in the analysis process (LOQ).

The root of the honey problem - with its minimal allowed residues, though still able to kill the beekeeping market - and that of the issue raised (or to be raised) to the Authorities lay exactly in the regulations that impose the residues legally tolerated in food products (MLR).

The norm is based on two incontrovertible and absolute assumptions. **According to the first assumption, glyphosate is not harmful for human or animal health.** The molecule has a very specific target and inhibits a biochemical process linked to the unique genetics of plants. Animals, who do not have the 'sensitive' gene, cannot be harmed, unless they swallow an excessive amount. All risk assessments carried out by the Authorities since 1974 confirmed that glyphosate-based herbicides are not dangerous. **Therefore, from a legal perspective, the presence of the molecule in food farm products is absolutely acceptable.** Basically, the logical and incontestable principle is that if glyphosate is used in cultivation, it is normal and inevitable to find it in food or, as the law defines them, final products.

The rationale of the law is very simple in this case: after using the amount of glyphosate indicated in the data-sheet, the final product is analysed. The residue found in this product becomes the reference value by law. For example, if we analyse the sugar extracted from beetroots cultivated using glyphosate and 15 mg of residue are found per kilo of sugar, then the maximum residue limit accepted by law will be 15 mg per kilo, or - expressed as absolute concentration value - 15 ppm (parts per million), that is 15,000 ppb (parts per billion).

The mechanism used to determine the maximum residues allowed is based on the amount of herbicides needed to obtain the standard crop production, not on the potential health issue that could arise. **The health issue is excluded a priori, while the precautionary principle is the only matter.**



¹ EFSA, *Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005*, EFSA Journal, 2018

As a consequence, if weed didn't die and the usual amount of glyphosate had to be increased to obtain the same standard crop production, then the maximum residue allowed could be raised. To continue with the example above, if a new crop production policy required the amount of herbicides to be increased with a consequent residue of 30 ppm in the final product, then, the maximum residue amount can be raised from 15 to 30 mg/kg, provided that it doesn't clash with the precautionary principle. **The mechanism of increasing the regulatory limits according to the needs is definitely in line with the rational of the law. It was widely used in the past and it is likely to be used in the near future, in case the new MLR proposed by EFSA in May 2018 are accepted.**

As there is no health risk involved, the maximum limits allowed express nothing but how appropriate is the food production process: within the limit, the farmer respected the rules; out of the limit, the farming method employed is considered excessive and therefore censurable. Although non compliant products represent a major problem for the farmer, they are by no mean linked to food toxicity: **at the end of the day, the excessive use of the molecule is censored more for its ability to cause drug resistance phenomena, than for its potential toxic effect.** As a matter of facts, weed drug resistance increases the risk for a modern farming essential instrument to lose its effectiveness. In short, with the regulation on residues, the farming system ends up protecting almost exclusively its own instrument.

The second absolute assumption on which the regulation is based is again connected to the risk assessments that have been carried out since 1974: **glyphosate is a molecule that degrades rapidly and, thanks to its ability to tie up with minerals in soil, it is not able to contaminate areas and crops where the herbicide is not used.** Basically, if I cultivate with the aid of glyphosate, the law considers as normal and acceptable to find the molecule in my final product and in the metabolism of those who ate it. On the other hand, if I produce honey, and glyphosate is not supposed to be used in my production process, then it is neither normal nor acceptable to find the molecule in it. **According to this rationale, bees cannot collect glyphosate in nature, because it is present exclusively in soil that was directly sprayed and in no other place. Furthermore, the beekeeper does not spray beehives with the herbicide; therefore the process to obtain honey is to be considered completely exempt from the contamination risk.** The maximum limit allowed is therefore zero.

Due to the sensitivity of the analysis tools though, this value is rounded up to 50 µg/Kg, or – expressed in absolute concentration - 50 ppb, which equals the technical limit of investigation of the officially recognized analysis tools.

Therefore, the paradox of having two comparable products on the nutritional point of view, like sugar and honey, with very different residue levels (sugar can be 300 times more contaminated than honey!) it's not actually a paradox for the law. The sugar production chain contemplates the use of glyphosate that is instead not allowed for the production of honey. That is how the rational of the law explains its consequences.

Finding concentrations in honey that exceed the margin of error of tools is therefore nonsense for the law. How can we find glyphosate where it has not been used? Who is wrong, the beekeeper or the law? Probably, neither one nor the other. **Contamination in honey calls into question something that is far more serious: the risk assessments carried out on the molecule, within a timeframe of more than forty years.** If glyphosate were quickly degraded by bacteria in soil and if it really seeped through the terrain in a stable and non-dispersive way, as claimed by scientists and the Authorities, there would not be any issue with honey and the law would be right. Unfortunately, that is not the way things are: glyphosate is present in honey and something ends up being wrong in the risk assessment process.

Aspromiele tried to challenge the 'truth' established throughout more than forty years of risk assessments carried out on glyphosate and that are at the basis of the regulation on pesticide residues. Aspromiele did that on a mere bees' point of view, by monitoring the environment through the bees.

Is it true that glyphosate is a non-dispersive and quickly degradable molecule? Is it true that it does not affect bees' health? In order to answer those questions, a meticulous fieldwork, and a constant update on the current scientific research throughout the world as well as a dose of good luck were necessary. Fortunately, the global attention on bees' health, extremely relevant in the past years, promptly pushed for researchers to investigate the effects of glyphosate on bees and beehives. Research results are peremptory and agree upon the fact that glyphosate is detrimental for bees and potentially devastating for beehives.

These studies show that the molecule represents a risk for bees' health while other studies prove that a normal herbicide treatment can contaminate a very wide area,

to the point that it could affect the honey of beehives apparently not exposed to the drift. **In the past two years, the truth on glyphosate was revealed by bees and their product that proved wrong – at their own expenses - the results of more than forty years of risk assessment.**

If the assessments on the molecule pervasiveness and its toxicity for bees were wrong, who can tell if they were correct on the absence of risks on human health?

The answer to this question clearly falls outside the aim of this report which will cross-read the data of scienti-

fic research and those gathered in the field **in order to claim that, for bees and beekeepers at least, what is known on glyphosate is a barefaced lie.** The aim of this Report is that of providing a snapshot of the end of an illusion that lasted for more than forty years and that allowed a proper economic miracle to happen, at least in the farm world where production was obtained on extended lands, at a low cost and (apparently) in a healthy way. This is a dream that may turn into a nightmare for beekeepers, unless beekeepers themselves demonstrate that everyone was wrong in the past forty years.

PRODUCT	ResiduE in mg/Kg (ppm)	ResiduE in mg/Kg (ppb)	New LMR proposed by EFSA 2018 (in ppb)
Honey	0,05	50	50
Sugar beet	15	15.000	15.000
Sugar cane	0,1	100	2.000
Potatoes	0,5	500	1.000
Corn	1	1.000	1.000
Non cultivated mushrooms	50	50.000	50
Pears	10	10.000	15.000
Sunflower seeds	20	20.000	20.000
Cotton seeds	10	10.000	60.000
Barley	20	20.000	30.000
Tea leaves	2	2.000	50
Meat (pork/bpvine/ovine)	0,05	50	200
Bovine kidney	2	2.000	7.000
Ovine kidney	0,05	50	10.000
Herbal infusions	2	2.000	50
Olives for oil	1	1.000	30.000
Table olives	1	1.000	50
Apples	0,1	100	50
Pears	0,1	100	50
Wine grapes	0,5	500	50
Oranges	0,5	500	50
Mandarins	0,5	500	50
Melons	0,1	100	50

The data in the table are those contained in the Regulation (UE) No 293/2013, which acts as a reference for the MLR for glyphosate (and other herbicides) in farm products. All values include both glyphosate and its metabolite AMPA. In May 2018, EFSA proposed a radical revision of the maximum residue levels allowed for glyphosate, in light of a new risk assessment, which became necessary in order to adjust the molecule profile within the regulations on pesticides residues. The

values proposed by EFSA are shown in the right hand column. What stands out is the increase of the residue limits allowed in animal products as well as the decrease in products whose cultivation requires the presence of bees; potatoes, that accumulate glyphosate, represent an exception amongst fruit and vegetables whose limits were increased. Herbaceous products derived from crop rotation 'benefit' from a level increase because during crop rotation glyphosate does not have

enough time to degrade, contaminating the following crop. Honey value does not change, as a specific assessment is being carried out. Olives represent a special case: table olives are 'zero residue' fruit, whereas olives for oil production (picked up off the ground) have a very high limit.

For more information

<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5263>

Monitoring 2017

2017 was a tough year for everyone and under all aspects in agriculture. April frost events and summer drought wiped out honey production, gave a hard time to bees and farms, decimated crops of corn, hazelnut and grapes, creating huge difficulties to fruit and vegetable farmers in some areas of the country. In Piedmont, one of the areas that suffered climate vagaries the most, 2017 was literally a year of famine. In April, frost hit field grass too, compromising spring haymaking. Summer turned the Southern areas of the State into a desert, where dust stirred up by the wind replaced the usual summer mugginess.

Weeds too had a very bad season in Southern Piedmont: frosted during the blooming season in April, they got prematurely dry by the end of June and in many cases they could not even flourish. Bees and beekeepers know something about that, as they saw summer pollen disappear, which in turn had a huge impact on the colonies' health and energy, not to mention that on the wallet.

By the end of summer, the entire territory in Southern Piedmont looked like it had barely survived a complete disinfestation, caused by frost first and by the sun later, twice during the same season. As evidence of that, there are satellite pictures of the Po Valley during summer, showing brown as the predominant colour, particularly in the monitored area.

In a similar situation, farmers crippled by climate, could only see the advantage of saving the money they would have instead spent for herbicides and crop spraying operations. The majority of farmers solved the weed issue with a single treatment at the beginning of May, while

only a few farmers crop sprayed beforehand at the beginning of April and very few decided to waste their time and money to treat the stubbles in August.

Starting with these premises, one would not certainly expect to find glyphosate in hive matrices, but in fact...

Aspromiele's bio monitoring control units, located precisely in Southern Piedmont, recorded the presence of the molecule in almost all the samples of honey and pollen taken (from hives) over a period of six months (Allais e Bergero, l'apis, n.2/2018). Only June pollen and honey, as well as August honey were found to be glyphosate-free. Conversely, **224 ppb of molecule were found in August pollen** which literally made technicians and beekeepers shiver in fear.

Although 224 ppb in pollen could be justified by bees feeding on flowers directly spread with the herbicide, it is much harder to explain honey values that showed punctual contaminations between 3 and 45 ppb, in the months of April, May, July and September. It does not matter that all values were below the legal threshold of 50 ppb as the real issue suddenly appeared to be different and much more serious: **the contamination immediately appeared to be like a sort of 'background noise', generated by a soft but consistent flow of glyphosate in bees' feed.** Basically, the area seemed to have an unlimited reserve of the molecule, as if the flow was fed by a real spring, ready to supply the bees pretty much anywhere, anytime.

One of the most concerning data is the ratio between the frequency of contamination of hive matrices and the amount of glyphosate actually used during the sea-



son. **How could it be possible that in 2017, a horrible year for glyphosate distributors, the contamination of hives was so frequent and meaningful?** The possibility that samples were taken during the few episodes of disinfestation was also considered, or that sampling was perhaps circumscribed and spread out over time and space, perhaps out of the normal farming procedures context. However, in a similar case, only an imponderable, though always vigilant factor as bad luck could account for such a 'background noise'. Bad luck cannot definitely be considered a meaningful scientific variable, especially when we are talking about a period of six months. But who knows? It might have played a role right in this occasion. However, if doubts still existed on its actual role, the bio monitoring in 2018 (unfortunately) excluded bad luck from the contamination factors categorically.

So where did glyphosate come from? **One of the first hypotheses saw water as a potential source of contamination.** Bees, great consumers of water, could have collected the molecule and could have taken it to the hive where it could accumulate in honey through contact. The hypotheses suddenly showed a point of weakness though, as it is very hard to explain pollen contamination through the influx of water into the hives. Testing the theory would not hurt though. Consulting the data on contaminated surface waters spread by Arpa Piemonte seemed to be a good starting point. At the end of 2017 only data related to 2016 were available, but at the time this report is being written, we also have 2017 data, used to confirm the conclusion drawn last year. In Piedmont surface waters, glyphosate is present though in concentrations always lower than 1 ppb (legal limit: 0.2 ppb, according to Directive 2013/39/EU). **It is therefore hard to think that bees could concentrate 45 ppb of glyphosate in honey by collecting water; and this is even truer in 2017 when water was present only in rivers.**

The hypothesis was then finally ruled out by AMPA, glyphosate metabolite produced in terrain by bacterial action. In surface waters, AMPA is highly present and with a greater concentration than glyphosate: peaks up to 15 ppb were recorded. AMPA, per se, is not produced exclusively by glyphosate, as it can be the result of domestic detergent catabolism. However, its presence in water is so high that it would be impossible not to find it in honey, if hives were really contaminated by water sources. **In fact, there were no AMPA residues in honey; therefore water was not the source of the residues.** That does not mean that ARPA's data cannot be useful and used to understand the contamination dynamics. As

it will be shown later, residues in waterways give us an interesting data to measure the level of contamination in the areas where bees operate.

After excluding water, **the aerial drift hypothesis was considered: possibly, disinfestation contaminated spontaneous flowers on the cropland field borders, leaving residues in pollen and nectar.** Also this hypothesis seemed weak since the beginning though. In order to account for the 'background noise', disinfestation operations would have been necessary throughout the whole season, but the existing climatic conditions excluded this possibility significantly. Moreover, even the monitoring control units seemed to exclude phenomena of aerial drift. Although colonies were placed in predominantly agricultural areas, a thick bush land barrier shielded them. Another possible source of contamination was the field dust scattered by the wind: glyphosate spread in cultivated fields throughout the years could have been lifted by the wind and dispersed on flowers in a wide area. This last hypothesis could be plausible if it was not **totally inconsistent** with what it is said and known about the molecule: once in the terrain, it has a very short half-life, immediately reduced by the bacteria in soil. **According to the glyphosate information leaflet, it is then impossible that disinfestations carried out before April 2017 could represent a source of pollution for the current season.**

The last hypothesis to be considered and able to explain everything was therefore the complete and pervasive contamination of the environment where bees had been operating. This was dreadful, and completely inconsistent with any result emerged from the risk assessments carried out during more than forty years. However, it perfectly fitted the phenomenon recorded by the monitoring units. The hypotheses was promptly reported to the Department of Agriculture of Piedmont that decided to actively take part in 2018 monitoring.

Luckily, glyphosate and bees have been topics of interest to scientists and public opinion in the past years. Thanks to this interest, some very interesting studies were carried out and published between September 2017 and October 2018. Those studies help to comprehend the contamination dynamics in hives and are fundamental to deeply question the protocol and the results of risk assessments on glyphosate. In the following chapter, through the analysis of the possible contamination pathways, we will account for those studies, trying to stress how theory is far from reality, especially when we are talking about glyphosate.

2 The possible contamination pathways

2.1 The molecule, a matter of doses

Glyphosate is a weed-killer systemic molecule able to penetrate the lymphatic circulation of plants through leaves. From leaves, it quickly and non-selectively reaches all the body parts of plants themselves, including roots. Through the direct inhibition of the photosynthetic process, the molecule is able to kill a plant down to its roots. This characteristic is empirically important for some varieties of crabgrass, characterised by a complex and well-structured root system, which basically makes any contact herbicide inefficient.

A fundamental concept to comprehend the effects of the various dosages of glyphosate is the FAR (Field Application Rate), which in case of non-GMO cultivation universally corresponds to 830 g/ha of active ingredient (at least in the context of the studies on the consequences of glyphosate drift). The FAR can reach 2500 g/ha for the disinfection of Roundup Ready® cultivation, but this is not the case for Italy.

Doses between 0.5 and 1 FAR produce a complete herbicide action on any plant. Doses between 0.014 and 0.1 FAR produce effects that are not lethal but biologically detrimental for the majority of plant species (Olszyk et al. 2009)². With doses lower than 0.001 FAR, no visible or short-term damage was found on plants. However, there are no studies on long-term negative effects.

The lack of studies on long-term negative effects of small doses of glyphosate is also due to the discovery, which is basically contemporary to the commercialization of the molecule, of the **hormetic effect of glyphosate on plants. Hormesis is a positive stimulating effect on biological cycles of plants caused by a chemical agent, which is toxic per se and is administered at sub-lethal doses.** Glyphosate hormetic effect is widely documented in scientific literature which proves how sub-lethal doses of the molecule can affect plant growth, induce shikimic acid build-up, enhance the photosynthesis and the opening of leaf stomata, increase the production of seeds and shorten the plant life cycle (Brito

et al. 2017)³.

Two opposite scientific studies published in the last 5 years are particularly meaningful in this context:

- Londo et al. 2014⁴.

Based on the assumption that numerous studies on glyphosate amply demonstrated the selective effect on non-target plants, this study has the aim of proving how the selection happens through a biological mechanism that penalizes the flowering of plants. One of the species examined in the study is the *Brassica* spp. because it is similar and sexually compatible with the rapeseed Roundup Ready®: the spontaneous varieties of *Brassica* spp. are subject to the drift effect on terrains that are not cultivated though contiguous to cultivated fields. Results indicate that exposing *Brassica* spp. to sub-lethal doses of glyphosate alters the phenology and the reproductive function of the plant. Doses of 0.1 FAR significantly delay the blossoming and suppresses the reproductive function, especially that of male parts.

Doses lower than 0.1 FAR are able to alter the form of the flower, withering their petals and causing their colour fade. Those doses cause the deformation and shortening of anthers that seem to become unable to ripen and release pollen. Pistils are underdeveloped too but they seem to maintain their fertility: manually pollinated flowers whose female part of flower was deformed were able to produce a fertile seed.

Basically, the sterilisation of *Brassica* spp. pollen helps the rapeseed pollen and, as a consequence, it helps the crossing of spontaneous plants with the glyphosate resistant ones, causing genetic drift phenomena of spontaneous plants themselves.

Referring to analogous phenomena observed on corn, cotton, ryegrass and other plants, the study postulates that sub lethal effects on sexual development could apply to all plants; not only to the seasonal and spontaneous ones that are naturally present near the cultivation sites.

Glyphosate related problems manifest essentially

² David Olszyk, Thomas Pfleeger, E. Henry Lee, Milton Plocher, *Pea (Pisum sativum) seed production as an assay for reproductive effects due to herbicides*, Environmental Toxicology and Chemistry, 2009

³ Laís de Brito Rodrigues, Rhaul de Oliveira, Flávia Renata Abe, Lara Barroso Brito, Diego Sousa Moura, Marize Campos Valadares, Cesar Koppe Grisolia, Danielle Palma de Oliveira, Gisele Augusto Rodrigues de Oliveira, *Ecotoxicological assessment of glyphosate-based herbicides: Effects on different organisms*, Society of Environmental Toxicology and Chemistry, 2017

⁴ Jason Paul Londo, John McKinney, Matthew Schwartz, Mike Bollman, Cynthia Sagers and Lidia Watrud, *Sub-lethal glyphosate exposure alters flowering phenology and causes transient male-sterility in Brassica spp.*, BMC Plant Biology, 2014



in flowering, which is the main biological element that explains the selection of herbicide-resistant plants. Useless to say, flowering is the essential element for honey and pollen harvesting and production. **Even sub-lethal doses of glyphosate, caused by aerial drift phenomena, directly affect the source of food:** pollen and honey contamination found in the monitoring process start to make sense.

- *Da Silva et al. 2016⁵*: in spite of Londo et al. 2014, studies on glyphosate hormetic effect continue and Da Silva et al. prove how sub-lethal doses of glyphosate dissolved in irrigation water can lead the common bean to a major resistance to water stress. The debate on the use of glyphosate as fertilizer is positive and current, updated only two years ago by the result of this study. It does not matter if the molecule is able to deform plant sexuality and cause a widespread resistance amongst weeds; the use of glyphosate as fertilizer is studied and supported by pharmaceutical companies. This piece of information, apparently useless for the purpose of this Report, can actually be of great value: **even though the Authorities decided to prohibit the use of glyphosate as herbicide, we could still find it on the market, sold as fertilizer. Therefore, the problem of pollen, honey and bees would not find its solution.**

In light of the information derived from the technical characteristics of glyphosate and from the studies on its hormetic effect, **given the close similarity of the molecule with the plants physiology, we cannot exclude that small amounts of the herbicide (in the order of ppm or ppb) might even appeal plants themselves,** thanks to the biological advantages they could bene-

fit from, in case it became available. From a different perspective, as the consequences on flowering emerge using sub-lethal doses of glyphosate, **we cannot exclude either that pollen sterility and pistil deformation can be accompanied by contaminated nectar secretion and honeydew, at least for plants located near rural areas (exposed to concentrations close to 0.1 FAR).**

2.2 Terrain

In theory, terrain is a big filter for glyphosate: minerals present in soil should tightly tie up to the herbicide, preventing the molecule dispersion into the environment, while the rhizosphere bacteria should cause the molecule to degrade in few days. **But is this really the case? The answer is yes, if glyphosate is used in a laboratory, within a risk assessment context, where the terrain analysed hosts limited amounts and varieties of bacteria, where terrain minerals stay where they should because they are not affected by weather conditions. How about in nature though?** Are we really sure that laboratory conditions are that close to those of agricultural soil?

The warning issued by European government agencies and scientists who study soil biodiversity⁶ tells us that that is not really the case. Intensive farming has considerably impoverished soil vitality, remarkably altering the so-called functional biodiversity of terrain (Nuti et al. 2007; Nuti 2014).

Functional biodiversity is an active process happening in soil where genetic variety and the quantity of germs living in the topsoil are able to guarantee:

⁵ Silva, J. C. da; Gerlach, G. A. X.; Rodrigues, R. A. F.; Arf, O. *Influence of low doses and application times on the hormesis effect of glyphosate in common bean*, CAB Direct 2016

⁶ • CEC, Commission of the European Communities (1977) European Community policy and action programme on the environment for 1977–1981. Official Journal of the European Communities C139, 13 June

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- 1) Soil fertility;
- 2) Plant resistance to water stress, pathologies, etc.;
- 3) Terrain stability against erosion, flood, wind, etc.

It basically guarantees soil resilience. **A soil that preserves an efficient mechanism of functional biodiversity is a soil that is able to metabolise glyphosate, to nourish and defend plants and to resist to weather conditions. There is a limiting factor** to this mechanism though: the quantity of organic matter in terrain. The critical threshold for the mechanism of functional biodiversity to be active is approximately 1.75% of organic carbon (Lynch et al. 2004) that **is about 3.5% of organic matter**. Below that threshold, environmental stress (pH, inorganic fertilisers, pesticides, asphyxia, etc.) cannot be compensated, unless significant intakes of organic and biologically 'active' matter are able to bring the level up to more than 3.5%. **Italian agricultural lands, as well as the majority of European ones, have an organic matter index that is lower than 2% (in some areas in the Po Valley this index goes below 1%) and lost, in fact, their functional biodiversity.** Terrains, particularly the Italian ones, are no longer able to supply anything: they are on their way towards sterility, they cause plant weakness and, above all, they are no longer stable. The draught during 2017 summer clearly proved how poor our terrains are, dried and ruined by the wind after only two months without precipitations.

Going back to glyphosate: **spraying it on terrains whose organic matter index is below 2% does not guarantee the molecule degrade induced by bacteria.** Therefore we cannot exclude that the herbicide can accumulate in soil from season to season. The ability of the molecule to tie up to soil minerals is no longer a guarantee with such low level of organic matter. **The terrain itself is no longer stable and this allows glyphosate to circulate through the environment. If it does not degrade and gets attached to a mobile element, easily removed and spread by water and the wind, then, the conclusions of the studies on the herbicide risk assessment lose their value.**

In fact, the risk assessments themselves and their implementation protocols have no value: the first studies on soil fertility, with the related warning on the

low organic matter index, date back to 1977; they were therefore published only 3 years after glyphosate was first commercialized. That does not mean that glyphosate was the cause of soil impoverishment. However, it means that, since the beginning, the risk assessments did not take into account of reality, as they re-constructed in the laboratory conditions that no longer existed in nature. Ironically, a risk assessment carried out in 2018, could give the same results obtained in 1974 as **the protocol requires the assessment to be set on a standard terrain, as per the biology textbook, with an active functional biodiversity, when in fact the herbicide is used on terrains that have completely lost their functional biodiversity.** That is how we get to the paradox: let's have the laboratory say that glyphosate is a completely stable molecule and then we find it in honey!

However, the soil has not only become a direct source of environmental contamination activated by weather conditions; first of all, it is home for plants and it is on plants that the herbicide performs its lethal and sub-lethal effects, through biological mechanisms that act in soil. **Glyphosate affects the key function of rhizosphere, the part of soil that surrounds tree roots, which is essential for the health and the ability of plants to absorb nutrients.** The effects on plants include their reduced absorption of essential micronutrients, their higher vulnerability towards diseases and the biological nitrogen fixation, with a paradoxically lower yield and with meaningful variations in the bacterial composition of soil. (Zobiolo et al. 2010⁷; Sheng et al. 2012⁸)

Pseudomonas fluorescens and the manganese-reducing rizosphere bacteria are suppressed by glyphosate, reducing the defence mechanism in rizosphoere that are normally available at the initial stages of plant growth in order to prevent pathogenic agents (Zobiolo et al. 2010). Plant pathogens such as *Gaeumannomyces graminis*, seedlings or root rot parasitic fungi (Huber 2007⁹) and soy 'sudden death syndrome' are encouraged by the modifications caused by glyphosate in soil biology and chemistry (Bithell et al. 2009¹⁰). Soil biodiversity (bacteria, detritivor fungi) gets seriously damaged too, with a negative impact on the ecosystem functionality.



⁷ Luiz Henrique Saes Zobiolo, Rubem Silvério de Oliveira Jr, Don Morgan Huber, Jamil Constantin, César de Castro, Fábio Alvares de Oliveira, Adilson de Oliveira Jr., *Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans*, Springer Nature, 2010

⁸ Min Sheng, Chantal Hamel, Myriam R. Fernandez, *Cropping practices modulate the impact of glyphosate on arbuscular mycorrhizal fungi and rhizosphere bacteria in agroecosystems of the semiarid prairie*, Canadian Journal of Microbiology, 2012

⁹ DM Huber, *What About Glyphosate-Induced Manganese Deficiency?*, Fluid Journal, 2007

¹⁰ Bithell, SL; Butler, RC; McKay, A; Cromey, MG, *Effect of Glyphosate Application to Grass Weeds on Levels of 'Gaeumannomyces graminis' Var. 'tritici' Inoculum*, Plant Protection Quarterly, 2009



A study carried out by Neumann et al. (2006)¹¹ goes beyond the statement about the damage of glyphosate on the rhizosphere, proving how the rhizosphere can become an actual transmission route itself via its own biological mechanisms. The authors claim that there is a widespread belief that glyphosate molecule easily degrades in and is absorbed by soil, and its use in farming is therefore harmless. **They were able to demonstrate that this belief is wrong and dangerous for farmers because the risk assessment on glyphosate did not properly take into account the behaviour of the molecule in the rhizosphere.** With such evidence questioning the validity of the risk assessment, we can tell why this study was 'concealed' to the public and indicated as a disreputable study by official science. **The study assesses a mechanism that belongs to systemically acting molecules, such as glyphosate, but that has never been considered in the past 45 years: the mobility of non-target organisms within the biological cycle.** According to the authors, the molecule can be released in soil by the roots of dead weeds or by the ones of Roundup Ready® plants that exude glyphosate. It quickly reaches the roots from the leaves and from the roots ends up into the rhizosphere. Here, besides altering the radical microbiota and the assimilation cycle of some minerals, it goes back into the biological cycle and is absorbed by the roots of non-target plants, that gets therefore contaminated. **As claimed by the authors, amongst the potential effects of this actual glyphosate cycle, there is also a meaningful reduction of honey production, caused by a limited synthesis of flavonoid (e.g. floral pigments) in contaminated plants.** Flowers are pale and bees literally cannot see them anymore!

A phenomenon studied in depth by science has contributed to make this transmission route potentially more efficient and sneaky. Thanks to popular publications such as *The hidden life of trees*¹² and *Plant Revolution*¹³, the phenomenon is now a matter of public record. Plants use the rhizosphere to mutually nourish and water themselves, even at great distance. In case of extreme draughts for example, trees whose roots are close to a watercourse, such as in a valley, are able to 'send' water

to their 'mates' who are far apart, even the ones located on the top of a hill and short of water. This is the way a bush, which is a real super organism, faces critical situations. **Unfortunately, we cannot exclude that this is the same way plants exchange glyphosate.** In these regards, the poverty of Italian soil might at least have limited the contamination level of the entire National Forest Heritage and the consequent damage to bees and beekeepers.

2.3 Water

The ability of glyphosate to tie up to soil mineral components should reduce or almost wipe out the possibility to find the molecule in surface water, but that is not the case. As we just saw, the impoverishment of soil made the terrain itself unstable and volatile, causing the ability of glyphosate to tie up to become useless, if not detrimental. It is soil itself to make the molecule volatile; therefore it is absolutely normal to find the herbicide in surface water. Rain, erosion, wind and farming operations, together with the huge quantity of glyphosate used become all contamination factors. Ispra and Arpa's monitoring data (just to mention Italian agencies) seem to confirm this phenomenon of surface water contamination, which results to be more complex and definitely more widespread than the contamination observed in the risk assessment on the molecule.

The studies on the effect of glyphosate on aquatic organism prove, regardless any other data, the high presence of the molecule into fresh water. If aquatic organisms can be harmed by glyphosate, and someone has verified that, it is clear that the molecule represents a risk for the aquatic environment. It is interesting **to quote some of those studies as their results successfully questioned a fundamental point raised by the risk assessment: the molecule does not represent a risk for animals. Thanks to these studies, its toxicity towards aquatic organisms is in fact the only reported and acknowledged risk:**

- Nešković et al. 1996¹⁴, validates the biochemical and histopathological effects on carps.
- Jiraungkoorskul et al. 2003¹⁵, validates the biochemical

¹¹ G. NEUMANN, S. KOHLS, E. LANDSBERG, K. STOCK-OLIVEIRA SOUZA, T. YAMADA, V. RÖMHELD, *Relevance of glyphosate transfer to non-target plants via the rhizosphere*, Journal of Plant Diseases and Protection, 2006

¹² Peter Wohlleben, *La vita segreta degli alberi*, Macro Edizioni, Milano, 2016

¹³ Stefano Mancuso, *Plant Revolution*, Giunti Editore, Milano, 2017

¹⁴ N. K. Nešković, V. Poleksić, I. Elezović, V. Karan, M. Budimir, *Biochemical and Histopathological Effects of Glyphosate on Carp, Cyprinus carpio L.*, Bulletin of Environmental Contamination and Toxicology, 1996

¹⁵ W. Jiraungkoorskul, E. S. Upatham, M. Kruatrachue, S. Sahaphong, S. Vichasri-Grans, P. Pokethitiyook *Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (Oreochromis niloticus)*, Environmental Toxicology, 2003

and histopathological effects on Nile Tilapia.

- Cauble et al. 2005¹⁶, proves the negative effects on the development and metamorphosis of amphibian.

- Kreutz et al. 2010¹⁷ e 2011¹⁸, validates the negative effects on metabolism, haematological and immune parameters of a species of fish cat, the silver fish cat.

- Reno et al. 2018¹⁹, proves the negative effects on population dynamics of fresh water plankton (cladocera).

In particular, the last research shows how **the effects of glyphosate on aquatic organism can be seen in reproduction dynamics, as it happens in plants**. Reno et al. 2018 is not the only study that follows this direction and that proves the effects of glyphosate on animals, in particular on their sexual sphere. **These studies are also at the bases of Brussels' request of more in depth studies on the effects of the molecule as an endocrine disruptive element on humans, within glyphosate extension assessment**. The problem is that the Authorities only questioned the allegation 'glyphosate is not toxic for animals' while they should have questioned the risk assessment and its procedure! **Toxicity on aquatic organisms is not an exception to the rule but the symptom of a rule formulated on a wrong assessment**.

Talking about bees and honey, we already saw how water contamination is not sufficient per se to justify the residues found in hives. However, water could have other routes to 'feed' bees with glyphosate. From this perspective, Pérez et al. 2017²⁰ is a very interesting study. It proves how an aquatic plant, *Ludwigia peploides*, could be used as a biomarker thanks to its ability to bio accumulate glyphosate in proportion to its effective exposition to the molecule. The anatomy of this plant facilitates the absorption of glyphosate as it is characterized by the absence of cuticles and anfistomatic leaves, which increases the exchange of its body with water. The absorption through the submersed root is favoured by the pneumatophores that facilitate the exchange of ions and molecules dissolved in water. **Amongst aquatic plants, *Ludwigia peploides* is the only one that can be used as a biomarker. However, it is not the only one that bio accumulates glyphosate. In fact, all**

aquatic plants bio accumulate it.

El Crespo, the basin of a 65 km long Argentinian river, is being considered for the study. The river flows from South to North and the first 30 kilometres of its riverbed flow through an agricultural area cultivated with corn and GMO soy. The second part of its riverbed flows through a completely natural area. As it does not have tributaries, it keeps the pesticides collected from the fields and spreads them along its riverbed. It is therefore the best place to study the pathway of molecules such as glyphosate. Samples of water, sediments and leaves were collected between the river source (Site 1) and its mouth (Site 8): the analyses were carried out on glyphosate and AMPA.

Glyphosate and AMPA were found in 75% of water samples and in 100% of sediment samples. The amount of glyphosate and AMPA in water vary between 0 and 1,7 µg/l, and between 0 and 0,10 µg/l respectively. In sediments, glyphosate values are included between 3 and 10,5 µg/Kg and AMPA values are from 3,5 and 93,5 µg/Kg. In leaves, results were positive in 94,12% of cases (Site 6, showing a result of 2 µg/Kg was excluded because the uncertainty of the instrument was superior to the measurement). The level of glyphosate vary between 4 and 108 µg/Kg. AMPA was not found, as the molecule is not compatible with the physiology of plants (the study tells us that once glyphosate reaches plant tissues, it does not degrade into AMPA but into glyoxylate).

Glyphosate was found in the plant in all the sites, including Site 8, which is more than 30 km far from the cultivated areas, as well as Site 1 and Site 2, where glyphosate was not found in water and that were located upstream with respect to the cultivated area. Although the cultivated area was limited if compared to the whole area considered for the purpose of the study, it was able to contaminate the water of a river from its source to its mouth. This was discovered thanks to a plant and its ability to bio accumulate glyphosate.

None of the plants taken into consideration presented clear external signs of herbicide contamination and *Ludwigia* flowers were not examined by the study, therefore

¹⁶ K. Cauble, R. S. Wagner, *Sublethal Effects of the Herbicide Glyphosate on Amphibian Metamorphosis and Development*, Bulletin of Environmental Contamination and Toxicology, 2005

¹⁷ Luiz Carlos Kreutz, Leonardo Jose Gil Barcellos, Ariane Marteninghe, Ezequiel Davi dos Santos, Rafael Zanatta, *Exposure to sublethal concentration of glyphosate or atrazine-based herbicides alters the phagocytic function and increases the susceptibility of silver catfish fingerlings (*Rhamdia quelen*) to *Aeromonas hydrophila* challenge*, Fish & Shellfish Immunology, Volume 29, Issue 4, October 2010, Pages 694-697

¹⁸ Luiz Carlos Kreutz, Leonardo José Gil Barcellos, Stella de Faria Valle, Tális de Oliveira, Silva Deniz Anziliero, Ezequiel Davi dos Santos, Mateus Pivato, Rafael Zanatta, *Altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate*, Fish & Shellfish Immunology, Volume 30, Issue 1, January 2011, Pages 51-57

¹⁹ U. Reno, S. R. Doyle, F. R. Momo, L. Regalado, A. M. Gagneten, *Effects of glyphosate formulations on the population dynamics of two freshwater cladoceran species*, Ecotoxicology, September 2018, Volume 27, Issue 7, pp 784-793

²⁰ Débora J. Pérez, Elena Okada, Eduardo De Gerónimo, Mirta L. Menone, Virginia C. Aparicio, José L. Costa, *Spatial and temporal trends and flow dynamics of glyphosate and other pesticides within an agricultural watershed in Argentina*, Environmental Toxicology and Chemistry, 2017



we do not know if the molecule was actually present in the flowering process, on pollen and in nectar. It would be really interesting to look into this possibility further. In light of this study, **the hypothesis that all plants behave as the aquatic ones and that water could be a contamination route for trees such as *Robinia pseudoacacia*, for example, or other beekeeping plants, cannot be overlooked. This is particularly true if these plants live and flourish in particularly humid terrains.**

Water could be a contamination route for honey through another way too: drift droplets could be a direct source for hive matrices, as they could enter the hive directly in case the spraying process took place in areas very close to the apiary, or they could deposit on flowers and contaminate pollen and nectar.

The way bees choose their source of food highlighted by Liao et al. 2017²¹ make this last contamination route probable. **The study proves how bees are statistically more attracted by a sugar solution contaminated by small doses of glyphosate (compatible with the doses found in verified contamination phenomena) rather than by a non-contaminated sugar solution.** The explanation that the authors give to this phenomenon is extremely inspiring: they claim that the paradoxical preference could be linked to the 'habit' developed by bees as a consequence of their very numerous contacts with those molecules (or other chemically related molecules) and those concentrations.

2.4 Air

US EPA (United States Environmental Protection Agency) defines drift as follows: the movement of pesticide dust or droplets through the air at the time of application or soon after, to any site other than the intended area.

The definition itself is able to discredit any supposed credibility of the risk assessments conducted on pesticides, and that is particularly true in the case of glyphosate. We must remember that the molecule, sold as a miracle pesticide that stays where sprayed and that dissolves in soil even turning into a fertilizer, is subject to drift in any application context, as any other molecule. Drift is, by definition, the phenomenon of aerial dispersion that affects any pesticide. Therefore, going back to

glyphosate, **our molecule stays where you put it and disperses in the environment at the same time: it sounds like a brainteaser at the Paradox Festival!**

However, the point is: To what extent glyphosate can be affected by aerial drift? The real nature of the paradox can be understood only by carefully answering this question. Researchers have been trying to find this answer for more than 30 years. They initially **started from what they thought it was the radius reached by the droplets produced by spraying machines: 6 metres.** In those 6 meters, the effects of the molecule on non-target plants were observed to finally understand, about ten years ago, that 6 meters were not enough to describe the phenomenon. But first things first.

The dose of aerial drift regarded as 'typical' is 0.1 FAR, which is the concentration found in a droplet produced by a standard spraying machine in standard conditions. The effects of droplets on native flora were studied for the first time by Siltanen et al. 1981²²: the Finnish law allowed the use of glyphosate on the brush, a native bush that naturally constitutes the borders of cultivated lands in Northern European countries. The study took into consideration the main components of the brush, that is, cranberry, blueberry and lichens. **All the examined plants showed glyphosate contamination effects. Particularly meaningful was the data on contamination persistence: lichens, that do not have roots, had noticeable signs of contamination after more than a year from the treatment.**

The evidence, produced by Siltanen et al., of a strong threaten to native biodiversity were examined and reiterated by the already quoted Olszyk et al. 2009. This study proves how glyphosate can threaten the survival of *Brassicaceae*, a natural competitors of rapeseeds, in areas cultivated with Roundup Ready® rapeseeds. In addition, it proves how glyphosate facilitates the selection of hybrids, which are molecule resistant.

On the bases of the collected data, the authors form a hypothesis, which is very interesting from our perspective: **in cultivated areas all native plants could be contaminated by doses equal to small FAR percentages. In 2009, for the first time, the following hypothesis was considered: non-target plants located even further from the 6-meter radius could be affected by the drift.** That was an important step as cultivated areas could be deemed entirely contaminated the molecule.

²¹ Ling-Hsiu Liao, Wen-Yen Wu & May R. Berenbaum, *Behavioral responses of honey bees (Apis mellifera) to natural and synthetic xenobiotics in food*, Scientific Reports volume 7, Article number: 15924, 2017

²² Hilikka Siltanen, Christina Rosenberg, Mikko Raatikainen, Terttu Raatikainen, *Triclopyr, glyphosate and phenoxyherbicide residues in cowberries, Bilberries and Lichen*, Bulletin of Environmental Contamination and Toxicology, July 1981, Volume 27, Issue 1, pp 731–737

The radical change of perspective happens with the publication of a study that investigates the effects of glyphosate drift on native plants of the Argentinian virgin forest, carried out by Ferreira et al. 2017²³. The Argentinian agricultural scene is characterized by a specific phenomenon, which envisages, in some areas of the country, the deforestation of virgin lands in favour to GMO crops. In these areas, some tropical forest remnants are still present among the arable lands, often clung to arduous slopes and North-facing. The study proved that the glyphosate drift jeopardises the biodiversity of this forest remnants. It should be also noted that some of the native plants, though only few of them, are not affected by the herbicide; and those plants are the only ones facilitated by the selection, at the expense of all others.

Therefore, not only the cultivated areas are affected by glyphosate, even the bordering forest remnants are. The study is Argentinian and the crops are GMO, therefore the peculiarity of the farming model may partly explain such a wide drift radius. However, the initial 6 metres radius became a few hundreds...

An Italian study confirmed and provided an even 'worse' picture than the one described by the Argentinians: Lucadamo et al. 2018²⁴ radically questioned the assumption on glyphosate and on the related drift phenomena. Through a study carried out in the province of Crotone, using lichens (*Pseudovernia furfuracea*) as bio-markers, the authors examined an area of 22 km² that includes an intensive farming area (63% of arable lands, 16% of vineyards, 21% of biomass crops) and a semi-natural bordering area.

The lichens were collected from a natural reserve and the preliminary examination of some specimens excluded that they could be already contaminated at that point. Transplanted on tree branches at 2.5 meters from the ground in 28 different sites spread all over the area, the lichens were collected after 3 months and examined with the aim, among others, of measuring glyphosate residues. Lichens were chosen because of the evidence produced by previous scientific studies that prove how they are excellent biomarkers as they bio accumulate in proportion to the extent of exposition (Vannini et al. 2015)²⁵.

The data of the study suggest that the entire monitored area is characterised by a process of contamination: thanks to the wind and the lack of barriers between the cultivated area and the semi-natural one, the glyphosate drift affects an area of 22 square kilometres! What the authors did not take into account, though obvious, is that **glyphosate can directly contaminate not only lichens but also tree leaves at a distance of 2.5 m from the soil**; and let us reiterate that all that happened within an area of 22 Km²!

The 6-metre radius has turned into a 2.6 kilometre radius, considering a 22-km² circular area. Getting back to the Paradox Festival, **glyphosate-based herbicides tie up to the spraying site spreading over a radius of 2.6 km: the extent of the blunder** - or shall we call it 'The Shell game'? - **(not) revealed by the risk assessment is striking**, and this is particularly true if we consider that the anti-drift technologies applied to spraying farm machinery since 1974 have considerably limited the damages (for real, this time).

2.5 Bees and the glyphosate cycle

The study Berg et al. 2018²⁶ finally drew the line at the conclusions that the glyphosate risk assessments had come up to. The study examined the hive and the environment matrices in a Hawaiian farming context, after a normal herbicide treatment. **All types of matrices were contaminated**, but we probably expected that. What is surprising though is the data of the molecule distribution in the environment around the hives: it was so homogeneous and broad that the authors could only explain it by admitting that **bees constitute a contamination route!**

Obviously, that does not mean that bees are the cause of pollution: **in this case, besides being the victims of contamination, bees are the direct and irrefutable evidence of the extremely high mobility of the molecule.** The American study provides a snapshot of **glyphosate biological cycle: the molecule is sprayed, it spreads in the environment, lands on flowers, contaminates bees and, through their bodies, it gets back to the environment, expanding and making its scope uniform. And that is not all: if a contaminated bee, instead of continuing with the pollen gathe-**

²³ Ferreira María Florencia, Torres Carolina, Bracamonte Enzo, Galetto Leonardo, *Effects of the herbicide glyphosate on non-target plant native species from Chaco forest (Argentina)*, *Ecotoxicology and Environmental Safety*, Volume 144, October 2017, Pages 360-368

²⁴ Lucio Lucadamo, Anna Corapi, Luana Gallo, *Evaluation of glyphosate drift and anthropogenic atmospheric trace elements contamination by means of lichen transplants in a southern Italian agricultural district*, *Air Quality, Atmosphere & Health*, April 2018, Volume 11, Issue 3, pp 325-339

²⁵ Andrea Vannini, Massimo Guarnieri, Martin Bačkor, Ivana Bilová, Stefano Loppi, *Uptake and toxicity of glyphosate in the lichen *Xanthoria parietina* (L.) Th. Fr.*, *Ecotoxicology and Environmental Safety*, Volume 122, December 2015, Pages 193-197

²⁶ Carl J. Berg, H. Peter King, Glenda Delenstarr, Ritikaa Kumar, Fernando Rubio, Tom Glaze, *Glyphosate residue concentrations in honey attributed through geospatial analysis to proximity of large-scale agriculture and transfer off-site by bees*, *Plos One*, 2018

ring, decides to go back to the hive, contaminated pollen and nectar will reach the hive. In turn, pollen will contaminate the larvae, whereas the residue in nectar will increase in the process of transformation into honey, which is a perfect glyphosate accumulator, ready to contaminate the future generations.

One third of the examined honey samples came back positive, with residues up to 342 ppb. The researchers were impressed by the data to the point that they found it necessary to specify that beekeepers had not sprayed glyphosate into their hives beforehand.

Once again, bees proved to be an extraordinary tool to snapshot how chemicals sneak into the ecosystem biological cycle: in this case, we really ought to say that glyphosate moves in mysterious ways. Once sprayed, it is able to take advantage of any type of 'support' or means to spread widely through the environment and into living organisms, whether they are hives, the body of living aquatic organisms or the human body.

Faced with such evidence and such a meaning of the word 'drift', it is really hard to understand EFSA's decision, which, by the way, was based on the risk assessment once again carried out on the molecule in 2015. At this point of the dissertation, we can understand how useless a laboratory risk assessment can be. It is also detrimental to subordinate the concept of drift to the one of laboratory risk assessment; which, in fact,

disqualifies the meaning of risk. Logically, the extent of the drift should be measured first and subsequently the risk should be assessed in all the examined area. However, that has never been the case.

Conversely, if, as usual, the risk assessment is combined with variable drift, which spreads the potential danger caused by the molecule through an unknown and purposely ignored area, we then end up protecting all but the ecosystem.

Therefore, beekeepers are twice victims of the warped government health system in this case:

a) Because they have to come up against a law based on the risk assessment and that therefore requires that residues must not and cannot be present where glyphosate is not used;

b) Because the animals they factory farm live in symbiosis with plants (later, we will see the actual implication of this symbiosis); plants, in turn, due to their physiological compatibility with the molecule, are the real victims of glyphosate. In particular, they are victims of a purposely ignored risk: the extreme mobility of the molecule through the environment.

Air, water, soil and humans end up to potentially distributing glyphosate to bees and beekeepers.

3 Monitoring reports 2017/2018

2017	Months of monitoring	Matrix	SITE 1	2018	Months of monitoring	Matrix	SITE 2	SITE 1
			U.m. µg/Kg (ppb) glyphosate				U.m. µg/Kg (ppb) glyphosate	U.m. µg/Kg (ppb) glyphosate
	APRIL	POLLEN	3,8		APRIL	POLLEN	68	10
		HONEY	5,1			HONEY	90	<1
	MAY	POLLEN	4,5		MAY	POLLEN	<1	<1
		HONEY	3,2			HONEY	26	<1
	JUNE	POLLEN	<1		JUNE	POLLEN	25	<1
		HONEY	<1			HONEY	790	55
	JULY	POLLEN	10		JULY	POLLEN	12	12
		HONEY	10			HONEY	16	10
	AUGUST	POLLEN	<1		AUGUST	POLLEN	98	<1
		HONEY	<1			HONEY	<1	<1
	SEPTEMBER	POLLEN	<1		SEPTEMBER	POLLEN	<1	<1
		HONEY	10			HONEY	<1	<1



The beekeeper who made one of his apiaries available for Unit 2, chose, for the monitoring, two hives close to each other, characterized by the same strength and with two 2016 white sister queens. The two hives were numbered 26 and 27, according to the beekeeper's personal progressive numbering system. Hive 26 was assigned the role of control unit, while hive 27 was assigned the role of witness. **As shown by the examination data, two peaks of honey contamination were found in hive 26: 90 ppb and 790 ppb of glyphosate. To make sure the reader does not think we made a typing mistake, we write the second amount in words: seven hundred and ninety.**

Now, why focussing on hive 26, leaving aside the other unit and the data related to the other area? Because the season of hive was highly significant for the purpose of this Report. For this reason, we asked the beekeeper to tell us how his beehive lived during the glyphosate-contaminated year 2018.

4.1 The season of hive 26

Hive 26 had a very good start during spring, in spite of the fact that the apiary did not come very well out of 2017 and winter. Hive 26 and its neighbour Hive 27 then had a disruptive development in March, with extra brood honeycomb and the climb to the shallow super already before mid April. Spring flowering in the area was good but not excellent for nectar production. It was sufficient though to let the two hives build three comb foundation sheets each and ensure a great pollen flow, a guarantee for healthy and solid broods.

Hazel honeydew, which had filled up the shallow super in the same area in 2017, was abundant but late. Its harvest started only after April 10th and lasted no more than three days, finishing before it started to rain. The three days were sufficient though for the bees to obstruct the hive. The shallow super remained empty and during the rainy days, bees sealed the side honeycombs and the hive crowns.

Aspromiele's technician collected the honey sample and the pollen cells from these honeycombs, and send them to the laboratory. A few days later, the lab results were a real shock: **90 ppb of glyphosate in hazel honeydew and 68 ppb in pollen.** In the meantime, acacia bloomed and shallow supers were full... But full of what? Was it

acacia only, or part of that honeydew ended up in the shallow super? Was acacia clean or was it contaminated as well?

The beekeeper decided to request a self-monitoring analysis on a sample collected from shallow super 26: a few days later the result made everybody breathe a sigh of relief, as **the residue value found was 'only' 10 ppb.** Had the honeydew residue diluted with acacia? Was the honeycomb where the sample had been collected the only one being contaminated? Those questions remained unanswered, as many more samples and analysis would have been necessary to try to formulate any plausible hypotheses. We had to make do with the sigh of relief: the production of acacia was safe, at least before the law.

The early acacia flowering lasted no more than four days and was spoilt by rain. A few days after the shallow supers were removed, **the beekeeper reported the need for a change of queen bee in hive 26.** The white queen, that up to that point proved to be a great queen, suddenly quit laying eggs and bees started to grow emergency queen cells. **The way the hive was preparing the queen bee change was definitely to be considered unusual for the month of May.** The beekeeper promptly added a new queen and hive 26 reached the pace of the witness hive in a very short time.

We obviously wondered whether all that glyphosate could have caused the death of the queen. The two events, contamination and queen change, seemed interconnected but there was little or no evidence to prove it. Glyphosate LD50 (the dose that causes the death of 50% of the population the substance is administered to) **is > 100 µg for bees:** according to the glyphosate information leaflet, a bee has to swallow at least 100 µg of herbicide to have a 50% chance to die. **With the concentrations found in the hive in honey and pollen, the queen should have eaten at least 1.5 kg of pollen or 1.2 kg of honey in order to die or show serious health problems. And that is impossible. Was that a random event then? That is possible, though strange.**

Because of the short-lasting harvest and the late flowering of tilia, the sampling in May was carried out with an almost empty hive. There were still some sealed crowns and the two-coloured honey left no space for doubts:

honeydew cells were mixed to acacia cells. The residue appearance in honeydew was therefore to be expected and the 26 ppb found confirmed the forecast. In return, pollen was clean.

In the meantime, Hive 26 started to benefit from the presence of the new queen: extensive, solid and healthy broods filled up 8 frames. Hive 26 was about to exceed the strength of his neighbour witness hive when *tilia* bloomed, together with *Amorpha fruticosa*, a widely present plant in bare terrains along the Tanaro River.

Multi flower harvest was really good and finished at the time of June sampling. The beekeeper had already removed the shallow supers and you could see the signs of an imminent abundance of Metcalf honeydew. **As a precaution, he collected a sample from hive 26 shallow super, in case a check on the product would become necessary.**

The analysis results left everyone speechless: **790 ppb of glyphosate in honey and 25 ppb in pollen: a disaster.** The sample collected by the beekeeper became fundamental to understand the extent of the contamination. **It was sent to the laboratory together with a sample of the batch of honey extracted from the apiary and gave unexpected results: the residue was lower than 10 ppb. The same result was obtained from the apiary batch.** Everyone breathed a liberating sigh of relief again.

The phenomenon had already happened though: like in April, a high level of contamination was found in the hive and little or no residues appeared in the shallow super. That could not be a coincidence.

Considering the short harvest period in April and the fact that it took at least the first two to three days of multi flower harvest for bees to fill up the hive, the following hypotheses was proposed, which quite likely can be considered plausible or at least relevant: **contaminated nectar was harvested in a very short period of time and, after that, either the molecule 'disappeared' or bees changed pasture. In two to three days, nectar with high concentrations of glyphosate was substituted by clean nectar, which filled up the shallow super, when the hive was already full.**

The hypothesis of 'flash contamination' is considered plausible also in light of what has been said about the molecule so far: as glyphosate is extremely mobile throughout the environment, after the initial phenomenon of direct drift on bee foraging plants, it quite likely spread through the environment, distributed and diluted by atmospheric agents and by bees themselves up to a point where it was no longer detectable.

On the one hand, this hypothesis may be comforting because a crop will probably never be entirely contaminated, unless more than likely build-up phenomena happened over time. However, at the same time, it is very concerning: if the phenomenon occurs halfway through or at the end of the harvest, the contaminated honey ends up directly into the shallow supers, which may potentially cause very serious consequences. There is nothing else we can say: 2018 was a really good year for the owner of hive 26.

Where all that glyphosate came from still had to be explained. The beekeeper had his own opinion: the late seeding in a nearby cornfield quite likely caused a delayed disinfestation. Recently yellowed brambles only 20 meters far from the apiary witnessed that. Although hives were shielded by a high and solid hedge, that was not enough to avoid the risk of direct contamination.

The yellowed bramble branches were populated by thousands of metcalfs that had already ejected a fair amount of honeydew. Bees had indeed already started to collect it and they can be seen on the brambles leaves too. The fear of a new contamination was automatically triggered.

As agreed with Aspromiele, **an extraordinary sampling plan** was arranged only 10 days after the previous one. **The analysis focussed on: recently collected honeydew, yellowed bramble branches, metcalfs and a branch of 'witness' acacia collected 70 m away in the opposite direction from the cornfield. All the samples came back negative for glyphosate: the molecule had already dispersed.**

4.2 Goodbye honeydew

During the extraordinary sampling a 'weird' phenomenon occurred: **every three days, 500 to 600 dead bees were found in the nets contained in the cage positioned in front of the hive with the purpose of collecting dead bees. This mass death of bees was slow but consistent and lasted for almost the whole month of July. What was weird is that the dead bees were young** and, on closer inspection, they were spread on the grass further from the area covered by the cage. In twenty days time, thousands of bees died in front of hive 26. The phenomenon was way less obvious in front of the 'witness' hive, but similar to the one occurred in front of other 4 hives of the apiary.

Looking at the deep super, hive 26 did not seem to have any problem, showing extensive and solid broods and a fair amount of fresh honey. **However, the shallow super was nearly empty**, and that was the case for the whole duration of the harvest period.

The brood interruption carried out by the beekeeper seemed to have a positive impact on the colony that had a good restart at the end of August. There was very little varroa and there were no signs of viruses. The colony has gone through winter very well so far, with a high number of bees and abundant pollen supply, while abundant feeding of sugar supplies was required.

In a 40-hive apiary whose average honeydew production exceeded 20 kg per hive, the production of hive 26 and other 4 hives was close to zero. In the same apiary, only these 5 colonies required feeding during winter, while the others had abundant supplies.

Was the phenomenon linked to the glyphosate peak recorded in June? Again, referring to LD50, the two events seemed not to be interconnected: each dead bee should have eaten 125 g of contaminated honey, which was impossible this time too.

Nobody was convinced of the fact that the two episodes were not related but with the data available, there was one explanation only: no connection. **In addition, the phenomenon was very similar to depopulation episodes recorded by the beekeeper during other seasons, and not only in that same apiary. He himself admitted that he would not have paid much attention to it, if the monitoring plan had not provided additional data.** At the end of the day, the routine would have suggested that the event was a normal 'beekeeping' episode, if it was not for the analysis.

4.3 Literature

In autumn too, the doubt that hive 26 had been damaged by glyphosate was instilled in technicians and beekeepers' thoughts and arguments. However, glyphosate datasheet is clear: LD50 for bees is 100 µg per bee. **Questioning the LD50 was not that simple; a well-equipped laboratory was necessary and the impression was that it would be difficult to obtain meaningful results. The queen and the bees died – in May and July respectively - after and not during the contamination. It was therefore hard to think about direct poisoning.**

We then decided to refer to scientific literature. In the past years, studies were published on the effect of glyphosate on bees' biology, with doses compatible with the ones found in the environment after drift phenomena. Besides the already mentioned Liao et al. 2017, interesting studies are Herbert et al. 2014²⁷ and Balbuena et

al. 2015²⁸.

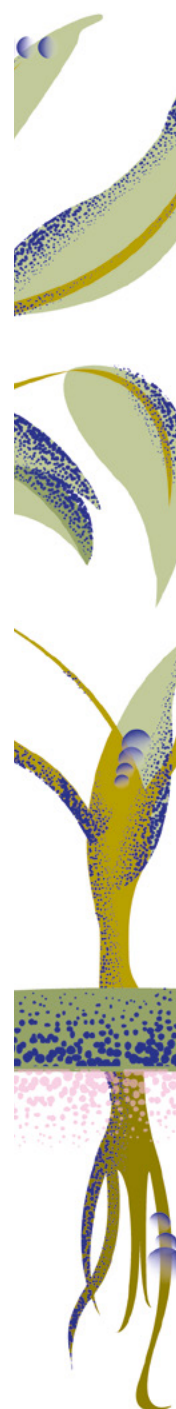
The first study examined the molecule influence on the ligule ability to extend and the consequent effect on pollen-gathering behaviour in bees exposed to small doses of glyphosate. A reduced ability to recognize the sugar syrup and a reduced ability to learn were observed in bees: their memory, that is, their ability to remember the response to a previously received stimulus, diminished considerably. **Despite the strong influence on bees' memory, the molecule did not strongly affect the pollen-gathering behaviour under semi free flying conditions.** However, the normal pollen-gathering activity recorded may have a potentially dangerous consequence. **As pollen-gathering bees can go back to the hive, they could become a continuous source of contaminated nectar which could be distributed into the deep super, stocked in honeycombs and have long-term negative effects on the entire colony.** The monitoring results confirmed this last theory.

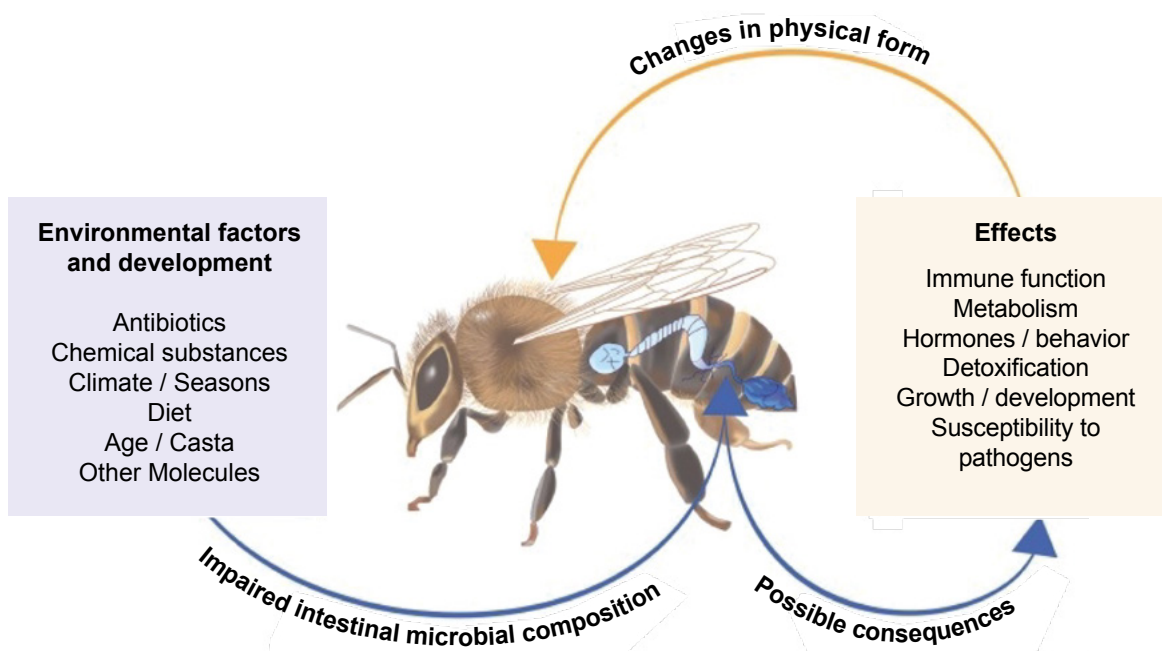
In the second study, Balbuena et al. come to the conclusion that **the exposition to levels of glyphosate similar to those present in agricultural areas (between 2.5 and 10 mg/kg) can alter bees' ability to integrate space information to efficiently retrace the way back to the hive.** These effects could have long-term consequences on the ability of the colony to find food. However, that was not the case for hive 26: all the bees returned back but died in front of the hives.

The turning point arrived in autumn, with the publication of a study carried out by Motta et al. 2018 and whose title was unequivocal: Glyphosate alters bees' gastrointestinal microbiota²⁹. Among the study authors there is also Nancy Moran, already known by l'apis readers, as she was often quoted in articles on bees' microbiology. **Proving the detrimental effects of the molecule on intestinal bacteria, the study hypothesises that altering the microbiota balance can expose bees to pathogenic attacks.** What is the extent of the risk and what are the pathogenic agents involved, though? The study answers these questions too, 'discovering' characteristics of the bees' microbiota that should not surprise us. We will try to simplify the researchers' complex argument by saying **that one of the main bacteria in bees' intestine has a gene that makes it very similar to plants.** That is not surprising as we are aware of the symbiosis relationship between our insects and the plant world. The gene is called EPSP and if it were for the

²⁷ Lucila H. Herbert, Diego E. Vazquez, Andres Arenas, Walter M. Farina, *Effects of field-realistic doses of glyphosate on honeybee appetitive behaviour*, Journal of Experimental Biology 2014

²⁸ María Sol Balbuena, Léa Tison, Marie-Luise Hahn, Uwe Greggers, Randolph Menzel, Walter M. Farina, *Effects of sublethal doses of glyphosate on honeybee navigation*, Journal of Experimental Biology 2015





gene only, *Snodgrassella alvi*, one of the essential bacteria for bees' metabolism and immune system, would definitely be a plant.

Well, the gene EPSP is the glyphosate only target! The molecule inhibits the cell biochemical process produced by the gene, interrupting a vital and essential process in all plants, and in *Snodgrassella*. Having such a selective target, glyphosate is considered non harmful for animals because animals do not have this gene: this theory is at the basis of the herbicide risk assessment. Bees, due to their symbiosis relationship with plants, are the exception to the rule. Or maybe not, considering that a 2018 Italian study²⁹ provides scientific evidence of the alteration of the gastrointestinal microbiota in rats too, and therefore, in mammals.

For the moment, the only clear exception to the rule is highly significant for the purpose of this investigation: we all breed the only insect known for being susceptible to glyphosate... **But what are the practical consequences to *Snodgrassella's* sensitivity to the molecule? The exposition to pathogenic bacteria such as *Serratia marcescens*, which is highly present in nature and**

it is a regular guests in bees' intestine. They are pathogenic agents that are not normally able to catch on, but without the biofilm protection produced by *Snodgrassella* on bees' intestine walls, they find fertile ground on which to develop. Bees, particularly the young ones whose gastrointestinal microbiota is still being developed, die for septicaemia, even several days after being exposed to glyphosate.

Like in hive 26, we cannot affirm with certainty that the phenomenon recorded during the environmental monitoring is fully attributable to a damage caused by glyphosate and occurred as per the process described in the study. Microbiological analysis on dying bees' intestines would have been necessary. However, unaware of the possibility to solve the rebus, nobody collected useful samples. However, the researchers' theory still provides a clear and plausible explanation of the phenomenon recorded with hive 26. This is confirmed by the duration of the mass death phenomenon: 3 weeks. Researchers highlight the potential damage that glyphosate contamination can cause to the entire hive over time: **bees that were contaminated by the molecule and had their microbiota**

²⁹ Erick V. S. Motta, Kasie Raymann, and Nancy A. Moran, *Glyphosate perturbs the gut microbiota of honey bees*, PNAS October 9, 2018

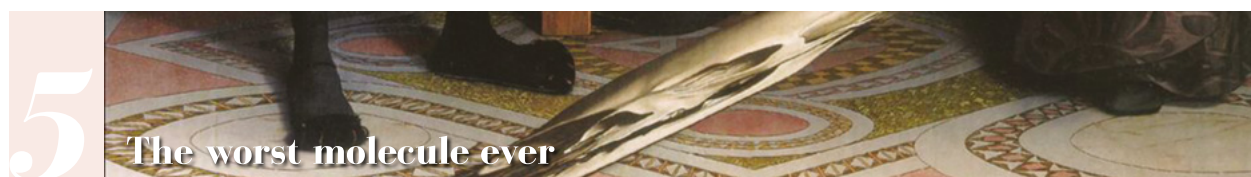
³⁰ Qixing Mao, Fabiana Manservigi, Simona Panzacchi, Daniele Mandrioli, Ilaria Menghetti, Andrea Vornoli, Luciano Bua, Laura Falcioni, Corina Lesseur, Jia Chen, Fiorella Belpoggi and Jianzhong Hu, *The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome*, Environmental Health 2018

damaged would breed, before dying, sisters that are microbiologically maimed. The damage is therefore transmissible for at least one generation!

To conclude, there is a matter still to be discussed: **what is the meaning of LD50 in this case? It has no meaning: bees do not die for poisoning, unless they drink the herbicide directly from the bottle. They die for septicaemia, days or weeks after taking the substance. It is a perfect murder, supported by a perfect alibi: LD50. Once again, an important element of the risk assessment ends up invalidating the assessment itself.** However, in this case, some extenua-

ting circumstances exist: molecular analysis tools and genome mapping of bees and their symbionts are very recent acquisitions. In 2015, year of the last assessment, those instruments and that knowledge were indeed not yet available.

That does not mean that bees were not able to question even the last apparently well-founded piece of information related to glyphosate. After the truth about LD50 collapsed, we can say that everything is thought to be known and is told about glyphosate is false.



Extremely mobile and pervasive, easy to bio accumulate in honey and able to cause sneaky and potentially catastrophic consequences, glyphosate is undoubtedly the worst molecule ever for bees and beekeepers. It is indeed able to jeopardize hives health and the integrity of honey as a product, at the same time. It is present in a systematic and pervasive way in the environment and is completely available to pollen-gathering bees. In addition, it bio accumulates in bees food, and that is where it can cause the most serious damage to both bees and beekeepers. **We already observed how, in agricultural areas, honey production that is completely free from residues is purely a matter of luck.** In order to avoid having their honey rejected by the market for non-compliance, beekeepers that do not want or cannot move from agricultural areas (provided that it serves the purpose), can do nothing but turn to the Lord and pray. It is really a Russian roulette: **the variables involved in the contamination mechanisms and procedures and that can make the difference between 10 and 790 ppb are numerous and unpredictable.**

Conversely, on the bees' perspective, residues in honey are a high and certain risk factor in any case: for example, wintering on highly contaminated supplies can make the difference between the life and the death of the colony. Even during peak season, despite the fact that weather and food conditions are advantageous for the bees, glyphosate can still open the door to pathogenic agents, and it can do it anytime.

Once again, bees are the second most exposed living

beings – after plants – to the effects of a chemical molecule; and once again the information that these insects are able to provide to humans have absolute value. The bio monitoring experience, accompanied by a meticulous and in-depth consultation of scientific literature, allowed us to reveal what had never been said or written on glyphosate. **Bees and beekeepers were able to prove an environmental contamination that has few precedents and that was publicly denied, no more than three years ago. In particular, what has no precedents is the discrepancy between the official information on the molecule and its actual behaviour in the environment. This discrepancy was already very obvious in other contexts but it was impossible to unmask in a systematic way without the bees' help.** Bees radically denied the information that was spread out by the pharmaceutical industry and the Authorities – that used suspiciously similar terminology - to publicly guarantee the health of all of creation. This should make the whole world pause for thought. **Bees woke us up from an illusion that lasted more than forty years.** In addition, this Report proves **how the public health and the environment safety system is inappropriate and misleading, because it is based on the wrong interpretation of the concept of risk assessment.**

In front of the fall of the safety system, **the regulation on residues itself, which risks to seriously harm our industry, has no value, as it is based on a false risk assessment. The law ignores and validates a fact: the agricultural environment, where food is produced, is widely contaminated by glyphosate. From this perspective, honey is not a product to be penalized**

but a warning light on the environment health. And that light is telling us that it has been a while since the borders were crossed and they were crossed in a remarkable way. Beekeepers have to be aware that they do not have to apologise for not being able to always guarantee clean honey to the market. On the contrary, they should expect apologies and compensation from those who were to forecast the phenomenon and avoid this situation, as well as from those who took and still take huge advantages from the sale and use of the molecule.

The only bright spot – and here the concept of positive has to be broadened to include our spot – **is the knowledge acquired that will allow us to shed new lights on episodes of colony depopulation (with the related pathologies), that would otherwise remain a mystery.** In this respect, the words of the owner of hive 26 were highly meaningful: if it was not for the analysis data, he would have considered a behaviour he was used to at that point, as a routine phenomena. To continue with the question marks we are getting used to, the same mysterious phenomena occurred in the Asti area in 2015 could validly be identified as glyphosate damage. At that time, nobody thought about the possibility to look for the molecule in honey. However, in light of the knowledge acquired, the lead can by no means be excluded. **Even the nosemosis caused by *Nosema ceranae*, in its various forms, could be favoured by the intestine microbiological alteration caused by glyphosate.** There is no scientific literature in this regards but the hypothesis is not to be excluded.

To conclude, we appeal to beekeepers and the Au-

thorities. We recommend that beekeepers make micro batches of honey from a specific area or the apiary, using self-monitoring procedures particularly when the production occurs in intensively cultivated areas. The warning is even more compelling for those who produce using certified organic methods. As their honey cannot go past 10 ppb of glyphosate, they are more exposed to the problem, and the attention they have to pay to their environment and their product, without control over them, has to be commensurate to the risk. We also ask that the Authorities take note of what has been said so far which, we are convinced, gives more than a valid reason to immediately stop the authorization to use the molecule. This way, we intend to give an answer to EFSA and SANTE as, we are confident, their recommendation protects the products but not the bees. And without bees we would not have any product or consumer to protect. The decision to acknowledge the presence of glyphosate residues in animal products seems to be a quite risky move, especially from a microbiological perspective as it actually justifies the contamination of the food chain and therefore, of the gastrointestinal microbiota. While we hope that glyphosate era will come to an end, a contemporary and radical review of the risk assessment methods is required for any chemical molecule used in agriculture. We are confident that a precise, complete and serious risk assessment would forever protect bees and beekeepers from poisoning and contaminations.



FORMULA CHIMICA GLIFOSATO



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The EPSP gene and the EPSPS enzyme are common to plants and microorganisms. In addition to *Snodgrassella alvi*, glyphosate is capable of affecting many strains of *Lactobacilli* and *Bifidobacteria*, essential life forms for the health of soils, bees, animals and human beings. Recognizing it and accepting it, the European Authorities have de facto legalized the damage from glyphosate to the digestive and immune systems of the entire planet.



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