

Clomazone: one of the latest really low toxic multi-crop herbicides?

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ABSTRACT

To reduce pollution and risks of pesticides, numerous studies and research are carried out to identify new degradable compounds, identify more effective and less dangerous formulations. For this reason, hundreds of new compounds and formulations are continuously available every year. Use, programs and controls of these chemical compounds are not uniform all over the world. In some poor or developing countries, means to control exposures and risks, associated with these substances, are not available or limited. The main active components of herbicides, registered in the world in 2019, were clomazone and isoproturon, used as single agents or mixed with other components. The use of Clomazone, in particular, has grown in many countries, but in China and other emerging economies in the recent past there has not been the expected expansion, despite the growing world demand. In fact, many Chinese producers have ceased the production of this compound for its higher production costs than other substances. However, analysts expect an increase, for the next years, of clomazone consumption for various reasons, first of all, because it is considered much less toxic than other products. However, in the absence of indications deriving from long periods use, it seems evident the need to avoid recourse to the massive use of this herbicide by carefully observing the indications and precautions provided by the appropriate international bodies (US EPA, EFSA, etc.). As regards the studies on the effects on the reproductive functions of both terrestrial and marine animal organisms, it is evident the need to increase their number, until now completely insufficient.

Keywords: properties; toxicity; animals; health, risks, environment, plants.

Journal of Economic Literature (JEL) Classification Number: Q01, Q15, Q56.

1. INTRODUCTION

About a sixth of the world's population, about a billion of people, constantly growing, does not have access to adequate food. Then, there is a strong trend to prepare more advanced methods and technologies to increase agricultural production. This situation is leading to the development of various seed treatment methods and to a continuously increasing demand for pesticides to eliminating weeds, controlling parasites and fighting plant diseases, saving yield loss and providing great

economic benefits (Mariyono, 2009; Popp et al., 2013; Leoci and Ruberti, 2020). At the same time, however, the increasing use of these compounds, mostly containing toxic and highly persistent substances, can lead to environmental contamination, health risks for farmers and consumers and potential toxicity to non-target plant organisms and animals (Arias-Estevez et al., 2008; Logaswamy and Remia, 2010; Whitacre, 2014; Kim et al. 2017).

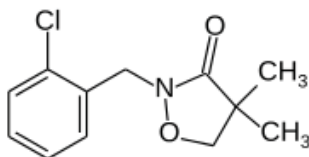
Currently, European Commission (2021) lists more than 500 regularly permitted active chemicals, very different from each other, grouped into 60 classes of herbicides, 18 classes of pesticides, 22 of fungicides, 20 of insecticides, 8 of acaricides, 6 of nematocides, 5 of plant growth regulators and 1 of repellents. According to the European Pesticides Database, these classes included a total of about 1500 substances. The three main product categories registered in 2019 were still pesticides, fungicides and herbicides, with a number of registrations, respectively, of 592 (55.64%), 179 (16.82%) and 110 (10.34%).

The main active components of herbicides, registered in the world in 2019, were clomazone and isoproturon, used as single agents or mixed with other components (glyphosate, ammonium glufosinate, fluoroglyphophene).

The use of Clomazone, in particular, has grown in many countries, but in China and other emerging economies in the recent past there has not been the expected expansion, despite the growing world demand. In fact, many Chinese producers have ceased the production of this compound for its higher production costs than other substances. However, most of the clomazone produced in China is exported to countries having a high degree of agricultural mechanization (principally Europe and North America) and where the relative demand is constantly increasing. Analysts expect an increase, for the next years, of clomazone consumption for various reasons, first of all, because it is considered much less toxic than other products, especially against harmful herbs of rice and orchard crops (Transparency Market Research, 2020).

2. CHEMICAL CHARACTERIZATION

Clomazone is the active ingredient of various herbicidal formulations, available on the market under various names, as we will see below. The well-known formula is as follows:



Clomazone appears, if pure, in a white crystalline solid state, melts at about 33 °C and boils at 281.7 °C (Alvarez, 1993; Brachet, 2003). It is moderately volatile (19.2 mPa of vapor pressure). Its vapors produce various types of alterations visible to sensitive plants. Clomazone is very soluble in water (1102 mg per liter at 20 °C) without decomposing, and aqueous solutions are stable with a pH

of 9.78 (de Ryckel, 2006; Dziedzic, 1982; Cao et al., 2013). The partition coefficient is independent of the pH. It is not explosive and it is not very flammable: it has a flash point of 110 °C. It is soluble in various organic solvents (methanol, dichloromethane, ethylene acetate, toluene, acetone and n-heptane) (National Center for Biotechnology Information, 2021). It is not an oxidant (Extoxnet, 1993).

As can be seen, the compound is formed by chloro-benzene bonded to a heterocyclic ring or, according to IUPAC name, 2-(2-chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one. According to Chemical Abstract (CA), it would be: 2-(2-chlorophenyl)-methyl-4,4-dimethyl-3-isoxazolidinone). It is a selective systemic agent belonging to the Isoxazolidion family herbicides (Maffioli, 2014).

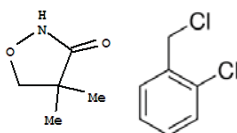
It was registered as herbicide in 1986 (Van Scoy - Da Silva and Tjeerdema, 2014), approved by US EPA in 1993 and marketed by FMC Corporation, based in Philadelphia (USA). It is applied, in pre-emergence, for broadleaf weed control in various crops (Tomlin, 1997). About the development of resistance by the treated plants, two cases have been reported until now: one in the USA and another in Australia. No cases in Europe (European Commission, 2018). Liebl and Norman (1991) investigated weed tolerance of clomazone. They noted that “velvet leaf” (plant of the genus *Verbascum*) was 256 times less tolerant to this substance than soybean seedlings, while maize and “smooth pig” (*Chenopodium album*) were, respectively, 66 and 13 times less tolerant. Furthermore, a reduction in the weight of fresh shoots and leaves of these plants was observed.

Currently, this compound is mainly produced by FMC Corporation, by ADAMA Agan Ltd., based in Ashdod (Israel) and, in Europe, by Albaugh Europe Sàrl, based in Lausanne (Switzerland), by Oxon Italia SpA, based in Pero (Milan), by Sapeac Agro S.A., based in Setubal (Portugal). Its trade names are “CENTIUM® 36 CS” in addition to “FMC-Clomazone 360 CS”, “Clomate” (produced by Albaugh Europe Sàrl). For use, it is sold in solution (about 360 g/L). Other trade names are mostly reserved for different formulations commercially available in EU countries (European Commission, 2021).

3. PREPARATION

To date, there are no simple methods for the synthesis of clomazone with a high degree of purity. The different procedures are almost all covered by patents. US patent 4,405,357A of September 20, 1983, which partially modifies that previous one (No. 15,535,380A of June 2, 1980), later abandoned, provides various steps (Bristow, 2013).

The manufacturing process normally involves the reaction between 4,4-dimethyl-3-isoxazolidinone (Zhang et al., 2005)



in aqueous environment, in the presence of a base, in particular an alkali metal hydroxide. A method for preparing fairly pure clomazone is also reported, which involves: (a) the crystallization of the clomazone present in solution in an organic solvent and (b) the separation of the resulting crystals. N-benzene is a particularly suitable solvent.

3-isoxazolidinone, which is a very weak base, belongs to a class of organic compounds known as oxazolidinones (HMDB, 2020), derives from hydroxamic acid (Yale, 1943) which in turn is prepared from the reaction between some esters and hydroxylamine salts. In turn, 2-chlorobenzyl chloride is a liquid which boils at 225 °C, which is prepared from the reaction between 2-chlorotoluene and sulfuryl chloride (SO₂Cl₂) (NCBI, 2020).

However, the process envisaged by the aforementioned US patent 4,405,357A provides for the use of methanol and dimethylformamide as solvents, which are not without drawbacks. In addition to being flammable, methanol is also susceptible to forming peroxides. The polar aprotic solvents, in turn, such as di-methyl formamide (DMF), are miscible in water and are generally recycled as azeotropes containing large amounts of water. The subsequent patent US 4,742,176 therefore brings various improvements to the process envisaged by the previous US 4,405,357A. However, there is a need to identify more efficient methods for the preparation and purification of clomazone, which overcome the drawbacks and shortcomings of the known methods.

An important limitation of the use of clomazone in herbicidal formulations is its high volatility. Due to this characteristic, the application of clomazone on the soil surfaces by common agricultural spraying techniques involves the possibility of its migration in adjacent areas, causing undesirable damage to the organisms and plants present in these zones. In order to avoid these risks, restrictions on the use of clomazone have been imposed. It is necessary, then, to take into account the meteorological conditions, the volumes to be sprayed, the distance from the nearby fields to be protected, etc. In order to overcome these limitations, various systems to reduce the volatility of clomazone in erbicide formulations have been prepared. Among these, the most convincing one seems to be the microencapsulation of the compound. In 1981, US patent 4,280,833A was published for the packaging of the herbicide in microcapsules (Monsanto, 1981).

In 1989, Chang and Baum patented another improved variant for the preparation of clomazone. In 1990, Monsanto also published US patent 4,936,901A for the formulation of a product in water-soluble granules. Other patents from Ciba-Geigy Corporation and other companies followed with other improvements. One of these (Steinbrenner et al., 2015) is based on the fact that the volatility of clomazone can be reduced by mixing it, preferably unencapsulated, with other nitrogen-containing compounds. In such formulations, the effective volatility of clomazone is surprisingly reduced by the presence of the second component, thus allowing the production of herbicidal formulations based on clomazone which do not have to rely on sophisticated encapsulation technologies.

To reduce mobility, other scholars have proposed the addition of some types of alginates to the compound (Włodarczyk and Siwek, 2016).

4. PERSISTENCE IN SOILS

Generally, the leaching of pesticides through the soil is a particularly important aspect for a number of resulting environmental and agronomic problems. It is considered the main cause of groundwater contamination, largely determined by the physic and chemical properties of the active substances of pesticides, soils (physical composition, organic matter, soil moisture, pH) and rainfall (Figge, 1993).

Clomazone is generally poorly absorbed by soils and the absorption rate would depend on the carbon content (Gunasekara et al., 2009). According to several studies (Liu et al., 1996; Antonious, 2000; Bhattacharyya et al., 2014), it undergoes biological degradation, showing a half-life in the soil of 1-4 months, depending on the various types of soil (Mervosh et al., 1995). In ventilated soils, clomazone degrades regardless of pH, without the formation of important metabolites. Available data suggest that the metabolism of clomazone in aerobic soils involves the cleavage of the isoxazolidinone ring and the consequent loss of carbonyl carbon as CO₂, with the formation of minor, short-lived metabolites.

The toxicity of clomazone seems to be caused by 5-ketoclomazone, a metabolite of plants (US EPA, 2007), which depends by ability of a plant to oxidize the parent compound. Transport, in the form of vapor, and microbial degradation are the main ways of dissipation. Clomazone does not undergo hydrolysis in solutions and soil/water photo-degradation. Carbon dioxide seems to be the major product of the degradation process.

Under anaerobic conditions, clomazone degrades rapidly (in ½ - 120 days) forming CLZ-M01 as the main metabolite or *N-[(2-chlorophenol)methyl]-3-hydroxy-2,2-dimethylpropanamide*, stable under these conditions (US EPA, 2007). The metabolic pathway in aerobic or anareobic soils has been described by the European Commission (2018). Soil photolysis experiments show that sunlight irradiation does not cause significant degradation of clomazone, forming metabolites above 5%.

Helbling et al. (2010) were among the first to study the biotransformation of clomazone. Mono- and di-hydroxylated clomazone were identified as transformation metabolites in sludge wastewater treatment enriched with a 100 µg/L concentration of this substance (Van Scoy-Da Silva and Tjeerdema, 2014). Liu et al. (1996) subjected, to the action of clomazone, bacteria of *Cunninghamella echinulata* and fungus of *Aspergillus niger* (UI-X172), resulting in dehydrogenation and formation of various clomazone-based metabolites (Van Scoy-Da Silva and Tjeerdema, 2014).

However, as a result of these characteristics and its persistence in water, this herbicide could cause contamination of groundwater. Residues of clomazone were in fact detected in water samples taken from numerous wells (50-70%) and from many rivers (90%) located in the rice growing areas where the herbicide was used. Clomazone residues, from 0.20 to 0.82 µg per liter, were found in 70% of the sources analyzed (Caldas et al., 2010). However, it seems that the adsorption of the herbicide into the solid components of the soil slows down both its degradation and volatilization (Mervosh et al., 1995a). Clomazone volatility and damage to sensitive off-target plant organisms are remarkably reduced by its encapsulation (Mervosh et al., 1995b).

In a recent study (Włodarczyk and Siwek, 2016), the influence of the herbicide formulation on its mobility in soils was determined. In another study (Tropaldi et al., 2019), it was studied and compared the clomazone formulations effects in various conditions of application. Of great interest are the studies conducted by Zanella et al. (2008) about the clomazone degradation in distilled water and irrigation water of rice fields, subjected to the action of UV radiation or natural effects. Clomazone degraded faster in distilled water (half life of 3.2 days) than in natural surface waters (20 days). The identification of numerous by-products (i.e. 2-chlorobenzaldehyde and 2-chlorobenzene methanol) shows that the concentration of intermediates initially increases and then decreases as a result of degradation. During the research, in addition to the aforementioned compounds, 3-hydroxy-2-chloro-

benzaldehyde, benzaldehyde, 2-chloro-oxime and benzonitrile-2-chlorine were also identified as degradation products.

In conclusion, due to its characteristics, clomazone is catalogued in Class III – “Slightly toxic”. Formulations containing clomazone must have, on the label, the “caution” indication.

5. EFFECTS ON PLANTS

“Command® 36 CS” acts on weeds in germination (anti-germinal) and by root absorption in the early stages of seedlings in moist soil conditions, causing bleaching of leaf cells. However the precise way in which this happens is still unknown.

A simple relationship, between the chemical structure of a herbicide (its family to which it belongs) and its biochemical behavior (its mode of action), has never been identified. At first, a classification based on structural formulas was used, but later, for the increasing number of new molecules, the use of the chemical structure was no longer used.

However, clomazone is a herbicide, of the systemic type, that is absorbed by the shoots and roots and of plants to transfer into the xylem (US EPA, 2007), inhibiting or reducing the formation of carotenoids, chlorophyll and other photosynthetic pigments (Storzer, 2002; Gara et al., 2011). It does not transfer from the upper to the lower parts or from leaves to leaves. It is not absorbed by other plants. Clomazone can cause, as mentioned, bleaching of treated crops, but these symptoms seem temporary and rarely involve the loss of plants or yields. In tolerant crops such as rapeseed, clomazone is absorbed by roots and shoots, but the acropetal translocation is reduced and the compound is rapidly metabolized into active non-herbicidal forms by oxidation, hydroxylation and conjugation.

Clomazone blocks the synthesis of carotenoids, chlorophyll and other photosynthetic pigments in plants by inhibiting the biosynthesis of the enzyme 1-deoxy-D-xylulose-5-phosphate synthase (DXP). The mode of action was investigated, in particular the pathway of methylerythritol-4-phosphate (MEP), deriving from clomazone, of 5-hydroxyclozomazone and of 5-ketoclozomazone. A study (Ferhatoglu and Barrett, 2006) stated that 5-hydroxyclozomazone and clomazone, while not blocking the MEP pathway, they cause plant bleaching. Furthermore, metabolite and hydroxylate do not inhibit the production of 1-deoxy-D-xylulose-5-phosphate synthase within this path, unlike 5-ketoclozomazone. The same scholars conclude that the subsequent toxicity and bleaching of plants derive from 5-ketoclozomazone. However, the exact location of the attacked plants is up for debate. Suggested points are: (1) prenyl transferase, (2) isopentenyl pyrophosphate isomerase and (3) enzymatic phytylation of chlorophyll (Duke and Kenyon, 1986; Sandmann and Boger, 1987).

In a paper (TenBrook and Tjeerdema, 2006) it is documented the primary way of phase II detoxification in glycoside conjugation, identifying the metabolites in *Oryza sativa* and the conjugated glycosides in *Echinochloa spp.* While others (Norman et al., 1990) had not identified the particular clomazone metabolites. However, the same authors noted that less than 5.9% of the residues recovered in cotton (*Gossypium hirsutum*) and soybeans (*Glycine max*) were composed of β -glucosides.

Ferhatoglu and Barrett (2006) used spinach chloroplasts to ascertain whether clomazone, clomazone 5-keto or clomazone 5-OH inhibit the chloroplastic pathway for isoprenoid formation. Their study demonstrated that neither clomazone 5-OH nor clomazone, but only clomazone 5-keto inhibits chloroplastic pathway for isoprenoid synthesis.

Because clomazone is widely used for controlling rice crops weeds, many scholars have studied its influence on rice cultivations. Clomazone, in microcapsules, was applied by Bollich et al. (2000), at different doses (0.28-2.2 kg/ha), on fields with water and on seeds sown in holes, observing slight bleaching, although increasing doses augmented the bleaching effect, reducing grain yield. Production in holes had a greater impact than aqueous production. Others (Webster et al., 1999) detected rice injuries 7 days after the emergency; nevertheless, post-emergence treatments resulted in fewer injuries.

With regard to the European Community, EFSA (2011) issued a series of recommendations on the use of clomazone and its inclusion in the Annexes of Regulation (EC) No 396/2005. The characteristics of the herbicide, the list of European States that have approved its use, metabolites, environmental impact, ecotoxicology, human health protection have been summarized in a document from the University of Hertfordshire (2019).

6. EFFECTS ON ANIMALS

Burdett et al. (2001) studied clomazone effects of on “non-target” aquatic animals. By subjecting the larvae of midges (*Chironomus tepperi*) to 0.288 mg/L or to the same treatment concentration used in practice in the fields (0.576 mg/L), no substantial adverse consequences (emergence changes, development times or length of wings, etc.), with respect to thiobencarb (S-(4-Chlorobenzyl) diethylcarbamothioate), were observed.

Wong et al. (1993) considered the effects of clomazone on the early life stages of some organisms. Soy cyst nematodes (*Glycine max*) were subjected to clomazone concentrations of 50-500 µg/mL (in the dark, for 24 days, at 25 °C) to evaluate consequences on egg hatching. The results showed the absence of negative effects.

Studies conducted by the California Department of Pesticide (2003) on toxicity for bees, birds and rats have revealed that clomazone is moderately non-toxic for these non-target species.

Regarding the effects of pesticides, and clomazone in particular, the environmental systems with the highest risks are those near the areas where chemicals are used: agricultural fields and water bodies through currents, volatilization or drainage. Organisms that may be involved include mammals, birds, fish, amphibians, invertebrates and plants. Data on acute toxicity indicate that clomazone is non-toxic for birds and small mammals, slightly toxic for freshwater fish, moderately toxic for freshwater invertebrates, slightly toxic for fish and highly or moderately toxic for estuarine/marine invertebrates.

Chronic toxicity studies (US EPA, 2007) provided the subsequent NOAEL values: 50 mg/kg/day for small mammals, 2.2 ppm for freshwater invertebrates, 2.29 ppm for freshwater fish and 1020 ppm (diet) for birds. EPA had not issued Ecological Incident Information System (EIIS) reports about effects for wildlife and fish attributable to clomazone. EIIS, instead, reported 110 cases of injury to non-target plants.

Fagundes et al. (2015) reported the first mass poisoning by clomazone to 103 sheep (134 mg/kg b.w.), some of which (20) exhibited respiratory problems and neurological signs. Clomazone was found in muscles, liver and kidneys of these animals as well in the vegetation samples and soil. Clinical problems (neuropathy, anorexia, tachypnea, weakness, ataxia and somnolence) were detected.

Accidental poisonings have been recorded for freshwater fish (Crestani et al., 2007; Moraes et al., 2009; Cattaneo et al., 2012); these poisonings were experimentally reproduced on fish and mice (Dos Santos et al., 2004; Miron et al., 2008), with damage to teleost fish (*Rhamdia quelen*) (Crestani et al., 2007) and to silver catfish (Jonsson et al., 1995; Dos Santos et al., 2004), inhibiting *acetylcholinesterase*.

The result of the first studies of the effects on dogs, rats and mice, fed for 28 days with doses of clomazone, is reported in a 1983 US EPA document. The risks for human health were resumed later, in 2007, again by the US EPA, in addition to the summary of the effects on plant products and the permitted doses. Subsequently, some scholars (Kobel et al., 2010) have pointed out that the duration of studies and tests on dogs should extend for no more than three months.

7. EFFECTS ON REPRODUCTIVE FUNCTIONS

About the effects of pure clomazone on sexual functions and fertility, the studies conducted by the manufacturing companies were reported by the European Commission and published in 2018.

In a study on two generations of rats, no toxicity conditions were detected on reproductive activity. However, the two higher dosages administered had slight consequences on adult rats such as weight reduction or, on the contrary, weight gain and an increased consumption of food. No meaningful differentiations between the control groups and those undergoing treatment, on both the number and vitality of pups. The observed effects on pups body weight were not pondered to be dose related. A reduction in the fertility index was observed only in one group (letter "b") for the higher dose and had no effect on the other population data. No other effects were observed on the fertility index, the mating index and the gestation index. The parental NOAEL was 75 mg/kg bw/day and the progenies NOAEL was 314 mg/kg bw/day.

In concomitant toxicity studies on rats (European Commission, 2018), performed since 1984, with administration of pure clomazone doses (100-600 mg/kg bw/day) to 15 gestations, it was observed diminishing of maternal food feeding and clinical disruptions, only with the highest doses. The toxic effect was slight: decreased fetal b.w. of females at high dose, delayed ossification of fetuses with high doses and hydroureter only at high-doses. A value of 89 mg/kg bw/day was the NOAEL.

In a subsequent study on the effects of the herbicide on the development of toxic effects on rats, they were treated with doses of 250-750 mg/kg/day for 6-19 days. A reduced increase in body weight at medium and high doses and a diminution in food intake was observed for all groups of guinea pigs. At elevated doses a mother died after clinical signs of lethargy and slight salivation were observed. Symptoms that were observed in high-dose mothers on single occasions (5 of 28 mothers had lethargy, each for only one day; 22 mothers had mild salivation with a duration of 1-5 days). There was no effect on the number of corpora lutea, early or late resorptions, pre- or post-administration losses or blockages with resorptions. Mean fetal weights were statistically significant but lower than

expected for high doses, while litter size and total number of fetuses were less than expected. The results of examinations of the external parts showed the protruding tongue and anasarca in one fetus as effects of the medium dose and in two fetuses the anasarca and forelimbs flexing due to the high dose. The fetus in the high-dose group that presented anasarca also had several visceral malformations. A fetus subjected to the high dose had deformed forelimbs after skeletal examination (short humerus and folded ulna). Under high doses, the dose-response relationships were statistically significant.

In other studies (Frey et al., 1998), two reproductive toxicity tests conducted with technical clomazone are reported for Bobwhite quail. NOEC (No Observed Effect Concentration) values, reported at the highest doses, indicate that clomazone does not cause reproductive toxicity. Because no clinical signs and deaths were detected in these studies with doses up to 94 mg/kg bw/day (1000 ppm) inclusive, it is still considered acceptable to consider the EU-agreed NOEC for long-term risk assessment for birds. In reproducing risk, a NOEC value of 76.5 mg/kg bw/day was detected as an effect on reproduction. In addition, the EU accepted NOAEL level of a maximum of 88.8 mg/kg bw/day is always considered valid for long-term risk assessment for mammals.

From a study by Salamon et al. (1984) on two generations of rats, each generation fed with the addition of clomazone equal to 5-200 mg/kg/day (for 11 weeks), no effect on reproductive performance of the same guinea pigs was observed. According to the same scholars, no alterations were found in the offspring of rats treated with 600-700 mg/kg/day.

To evaluate the potential for toxicity on reproductive function and the effects on neonatal growth and survival, Dæhli et al. (2011) subjected albino rats to experimental diets containing doses of clomazone with concentrations of 0 (comparison group), 100, 1000, 2000 or 4000 ppm, during the entire experimental period up to the weaning of the litters (pre-mating, mating, gestation, birth and weaning). In the higher dose groups (2000 and 4000 ppm) various statistically significant alterations were observed, such as reduced body weights of premature babies, body weight gains of preterm babies and changes in total weight. Reduced final body weights, not statistically significant, were observed in females of both generations in the higher dose groups. No significant changes were observed in the calculated reproductive parameters (mating index, gestation index and fertility index). However, the calculated fertility index (percentage of copulations leading to conception) was statistically lower in the high-dose group (65.6%) than in the comparator group (95.5%).

8. CONCLUSIONS

Although clomazone has not yet had a use and diffusion similar to that of glyphosate, the researches and studies conducted seem to indicate that it is less dangerous both for human health and for the environment, and for some organisms, animals and reproductive functions. However, in the absence of indications deriving from long periods use, it seems evident the need to avoid recourse to the massive use of this herbicide by carefully observing the indications and precautions provided by the appropriate international bodies (US EPA, EFSA, etc.). As regards the studies on the effects on the

reproductive functions of both terrestrial and marine animal organisms, it is evident the need to increase their number, until now completely insufficient.

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