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DEBATE

The Brazilian hazard-based cut-off criteria for pesticide registration: a critical appraisal

Critérios de exclusão baseados em perigo adotados no Brasil para registro de pesticidas: Uma avalição crítica

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ABSTRACT

Brazil, the world's top consumer of agricultural pesticides, adopts a unique hazard-based cut-off approach to pesticide registration. Cut-off criteria for mutagenicity, carcinogenicity, teratogenicity, hormonal disturbances and damage to reproductive organs were introduced by the Pesticide Law enacted in 1989. As far as health is concerned, law enforcement is additionally regulated by rules issued by the federal health authority (National Agency for Health Surveillance – ANVISA). Contrasting to the European Union's hazard-based cut-off criteria for pesticides, Brazilian rules do not make an exception for "negligible" exposures. Moreover, Brazilian regulations have shortcomings (e.g. no reference to relevance of Mode of Action to humans) that make cut-off criteria difficult to be put into effect. The deficiencies of regulations and difficulties to consistently apply the hazard-based cut-off criteria are appraised in this article. Adoption of a risk assessment approach or cut-off criteria based on classification into the Globally Harmonized System's hazard categories 1A and 1B is suggested.

KEYWORDS: Risk assessment; Hormonal disturbances; Teratogenicity; Carcinogenicity; Mutagenicity

RESUMO

O Brasil, líder mundial do consumo de agro-químicos, adota uma singular abordagem para registro de agrotóxicos que é baseada em critérios de exclusão quanto à periculosidade. Critérios de exclusão para mutagenicidade, carcinogenicidade, teratogenicidade, distúrbios hormonais e dano a órgãos reprodutivos foram introduzidos pela Lei de Agrotóxicos promulgada em 1989. Em relação à saúde, a aplicação da lei é também regulada por portarias publicadas pela autoridade sanitária federal (Agência Nacional de Vigilância Sanitária – ANVISA). Em contraste com os critérios de exclusão baseados na periculosidade que a União Européia usa para agrotóxicos, a regulamentação brasileira não faz exceção para exposições insignificantes. Além disso, a regulamentação brasileira apresenta deficiências (e.g., não faz menção à relevância do modo de ação para seres humanos) que tornam difícil a aplicação dos critérios de exclusão. As falhas dos regulamentos e as dificuldades para aplicar consistentemente os critérios de exclusão baseados na periculosidade são examinados neste artigo. Sugere-se a adoção da avaliação de risco ou de critérios de exclusão baseados na classificação quanto a periculosidade (categorias 1A e 1B) do Sistema Harmonizado Globalmente.

PALAVRAS-CHAVE: Avaliação de risco; Distúrbios hormonais; Teratogenicidade; Carcinogenicidade; Mutagenicidade

Introduction

The notion that the magnitude of a toxic reaction depends on the amount of substance to which someone is exposed - or that "*the dose makes the poison*" - is a cornerstone principle of toxicological science. A direct corollary of this principle is the idea that there should always be a dose (exposure level) below which no toxicity occurs. Both notions are conveyed by the famous maxim "*All things are poison and nothing is without poison; only the dose makes a thing not a poison*" ("*Alle Dinge sind Gift und nichts ist ohne Gift; allein die dosis machts, dass ein Ding kein Gift sei.*"), enunciated by Paracelsus almost five centuries ago[1]. It should be highlighted that Paracelsus used the negative ("*..the dose makes a thing not a poison*") instead of the affirmative form ("*..the dose makes a thing a poison*") to convey his ideas [1,2]. Taking a top-down view of dose/exposure-toxic effect relationships, as modern toxicologists do for risk assessment, Paracelsus seemed to be far ahead of his time [2].

To assess the risk of a chemical substance, today's toxicologists use data from animal and human studies to identify the hazard and uncover dose/exposure – toxic response relationships. The risk assessment process provides a rational basis for interventions (including regulatory decisions) aimed at protecting the population from health hazards posed by thousands of natural and man-made chemicals we have to live with in our increasingly technology-driven society [2,3].

Pesticide toxicity, hazard and risk

Although hazard and risk are terms commonly used by toxicologists and public health scientists, the distinction between them is not always clearly understood by non-experts.

Hazard is a source of potential harm or adverse health effect on someone that may or may not occur depending on certain conditions. Sometimes, however, the word hazard is employed to refer to the harm or adverse health effect produced rather than to its source. For instance, although a chemical carcinogen is generally considered a hazard, or a hazardous agent, its effect – cancer - might be at times called a hazard as well.

Risk, on the other hand, is a probabilistic concept. As far as toxicity is concerned, risk is the chance or probability that a person or animal will experience an adverse health effect if exposed (under certain conditions) to a chemical.

Therefore, while *hazard* is something or a set of circumstances that can potentially harm a person's health, *risk* is the likelihood (or probability) that a person will be harmed by a particular hazard.

The likelihood of developing an illness or getting injured after being exposed to a chemical agent depends on the level of exposure (actually, the internal dose received) as well as on some other factors. For instance, if exposure to a toxic substance is negligible, so is the health risk associated with it. A less hazardous chemical, on the other side, may pose a significant health risk if anticipated levels of exposure in real world scenarios are high [2,3].

In Brazil - since 2008 the world's top consumer of agricultural pesticides -hazard-based cut-off criteria are adopted for pesticide registration. Only if active ingredients and other ingredients pass cut-off criteria for mutagenicity, carcinogenicity, teratogenicity, damage to reproductive organs and hormonal effects, are their risks subsequently assessed for risk management purposes, such as establishing an acceptable daily intake and imposing restrictions on use. The Brazilian hazard-based approach to pesticide registration is a unique example among the world's most important regulatory scenarios. This article critically appraises potential usefulness and disadvantages of adopting such unique hazard-based cut-off criteria, and technical difficulties in consistently applying them.

Legal and regulatory framework for pesticide registration in Brazil

According to Brazilian Pesticide Law (Federal Law No 7.802, July 11th, 1989) "*..pesticides, their ingredients and the like …shall only be manufactured, exported, imported, commercialized and used, if previously registered by a federal agency in accordance with the guidelines and requirements of health, environment and agriculture federal authorities*"[4]. The law also establishes that (Art. 3rd § 6): "*..it is forbidden to register pesticides, their ingredients and related products…*" "*b) for which there is no antidote or effective treatment in Brazil*", "*c) that have teratogenic, carcinogenic or mutagenic properties revealed by updated results from experiments conducted by the scientific community*", "*d) that produce hormonal disturbances and damage to reproductive organs, as demonstrated by updated procedures and experiments of the scientific community*" [4].

The executive Decree No 4.074 (January 4th, 2002), which regulates the enforcement of Pesticide Law, additionally states (Art 31st) that "*…studies on mutagenesis, carcinogenesis and teratogenesis, performed in at least two animal species, should be conducted in accordance with criteria accepted by recognized national or international technical-scientific institutions*", and also clarifies what is to be considered a mutagenic compound: a substance "*…able to induce mutations, noted in at least two tests, one to detect gene mutations, carried out also with the use of metabolic activation, and the other to detect chromosome mutations*"[5].

A regulation issued by *Secretaria Nacional de Vigilância Sanitária do Ministério da Saúde* (SNVS-MS - National Secretariat of Sanitary Surveillance of the Ministry of Health,

[i] Paracelsus was a pseudonym adopted by Theophrastus Phillipus Auroleus Bombastus von Hohenheim (1493–1541), an irreverent Swiss-German physician who mocked at the ideas – shared by most of his contemporaries – on the treatment of illnesses based on statements made by Galen, Avicenna and other classic authors. The Paracelsus maxim cited in this article is found in one of his works: The Third Defense ("Die dritte Defension").

regulation no. 3, January 16th, 1992) provides additional information on what is to be considered a "*carcinogenic*" or a "*teratogenic*" substance, or yet a substance with "*hormonal actions*", for putting into effect the Pesticide Law's hazard-based cut-off criteria[6].[ii] The aforementioned health authority regulation states that:

> "*1.3.2 To evaluate the carcinogenicity of pesticides, criteria used by the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) will be adopted, and substances will be considered as carcinogenic if there is: a) scientific evidence based on valid epidemiological studies of human carcinogenicity, …. b) scientific evidence based on valid data of carcinogenicity in at least two laboratory animal species, showing increased incidences of malignant tumors : - of the same type at a determined body site or organ; - in different tests, preferably using different routes of administration and several doses; - at unusual degrees with reference to incidence, site, type of tumors, or age at which it appears. The evidence is strengthened if there is a direct relationship between number of animals bearing tumors and increase in dose. It should be understood as an unusual degree a statistically significant difference compared to control group animals*" [6].
>
> "*1.3.4 A pesticide is to be considered teratogenic when there is scientific evidence of teratogenesis based on valid data in humans or in studies (undertaken) with at least two laboratory animal species. Tests on teratogenicity should include a dose high enough to produce maternal toxicity and a low dose with no effect on the dam or on the offspring*" [6].
>
> "*1.3.5 A pesticide is to be considered as having hormonal action which prevents it to be registered, when the no observed adverse effect level remains undetermined in experiments conducted with laboratory animals or in humans, (when) the hormonal change occurs at all tested doses and when the effect is not reversible upon discontinuation of administration, or exposure to the substance*"[6].

Endocrine disruption is one of the most controversial issues of modern toxicology and this topic and related matters, such as low dose effects and non-monotonic dose response curves, have been on the stage during the last two decades[9]. The starting point of this long and heated debate was the Wingspread Conference held in Racine, Wisconsin, USA, in 1991, during which the term endocrine disruptor was coined[9]. It is an astonishing fact that the cut-off criterion for "hormonal actions" was introduced by the Pesticide Law two years before the Wingspread conference. It is also amazing that health authority rules issued in January 1992[6] clearly stated that a substance would trigger the cut-off for hormonal action if the "no observed effect level remains undetermined in experiments", which is consistent with the idea of "low dose effects", one of today's most controversial topics in toxicology.

Recently, the Brazilian regulatory agency (ANVISA) announced its plan to undertake an extensive revision of SNVS regulation no. 3, which was introduced nearly 20 years ago. Actually, a draft proposal of a new regulation is currently on the table for public comments[10]. According to this draft proposal, which has undergone a public consultation, hazard-based cut-off criteria for pesticide approval for commercialization and use, particularly those regarding teratogenicity, mutagenicity and hormonal actions, would become far more restrictive.

On the cut-off criterion for teratogenicity, for instance, the draft proposal (Chapter III, Art 38) states: "*A pesticide is to be considered teratogenic when there is scientific evidence of teratogenesis based on valid data in humans or in at least one laboratory animal species*"[10].

The new criterion for characterizing a pesticide compound as mutagenic (Art 39) is also more stringent: "*A pesticide is to be considered mutagenic when there is scientific evidence (along this line) based on results from at least one in vivo study on the induction of chromosome aberrations*"[10]. Therefore, in both cases (teratogenicity and mutagenicity) positive results of a test on a single animal species are considered to be sufficient for preventing registration, without any additional comment on whether or not the mode of action in these cases is relevant to humans. It is noteworthy that, as far as the mutagenicity cut-off criterion is concerned, the new regulation proposed by ANVISA (in its Art. 39) comes into conflict with the Government Decree No. 4.074 (Art. 31), a higher ranking law, which clearly states that a pesticide is to be considered as mutagenic when it is "*..able to induce mutations, noted in at least two tests, one to detect gene mutations, carried out also with the use of metabolic activation, and the other to detect chromosome mutations*"[5].

Nonetheless, if the new rules proposed by ANVISA are put into effect, the most draconian cut-off criterion for pesticide registration will be that for substances suspected of having hormonal actions or causing injury to reproductive organs. According to article 41 of ANVISA's draft proposal, "*A pesticide is to be considered as causing hormonal disturbances and/or damage to reproductive system (organs) if the adverse effects, irrespective of being reversible or not upon discontinuation of substance administration or exposure, are demonstrated by valid data*"[10]. Although the term "*adverse effect*" is used in the aforementioned statement, what is to be interpreted as "adversity" for characterizing an endocrine and/or reproductive hazard is not entirely clear. It should be noted that

[ii] The Brazilian regulatory agency, Agência Nacional de Vigilância Sanitária (ANVISA - National Agency for Health Surveillance), was established in 1999 (**Law** 9.782, January 26th, 1999)[7]. Until then, the Secretariat of Sanitary Surveillance – with far less resources and staff than today's agency - was the Department of the Ministry of Health responsible for enforcing the Brazilian Health Surveillance Law (**Law** 6.360, September 23rd, 1976) at federal level. ANVISA's duties include, but are not limited to, regulating and approving for sale: drugs, medical devices, food products, pesticides, tobacco, cosmetics and other consumer products[8].

transient changes of hormone levels are produced by stressors and by a number of stimuli and physiological conditions. They may result from adaptive (homeostatic) alterations of the organism and are not necessarily harmful. Moreover, "*hormonal disturbances and/or damage to reproductive system*" at times occur secondarily to other target organ effects, particularly if they give rise to severe systemic alterations. Since no reference is made to the context in which the "adverse effect" occurs (e.g., dose level and selectiveness towards endocrine/reproductive systems), a plain and uncritical enforcement of the foregoing new regulation statement (Art. 41)[10] may prevent registration of a number of pesticides the primary toxic targets of which are organs other than those of endocrine and/or reproductive systems.

Consequences of hazard taking precedence over risk in decision-making

Since the likelihood that a person will be harmed by a particular hazard depends upon exposure (and some other conditions), in principle, risk (probability) and not hazard (potential to harm) should be used for guiding public health interventions. A great contribution along this line was the systematization of the risk assessment process by the US National Academy of Sciences (NAS) in the early 1980s[11]. Therefore, when the Brazilian parliament passed the Pesticide Law in 1989[4], a systematized approach to risk assessment was already available and was being used worldwide to support regulatory decisions on chemicals. Although the word "risk" ("*risco*") appears a couple of times in the law (e.g., "health and environmental risks"), it remains obscure whether it was used to refer to risk or hazard, a confusion that is often made by non-experts. At any rate, it is unclear why legislators ignored "risk assessment", a systematic, scientifically-based, and more rational approach to decision-making than the adopted hazard-based cut-off criteria.

It is more or less obvious that pesticides that pass the hazard-based cut-off criteria established by the law may pose a higher health risk than those that failed to do it. A number of relevant health hazards are outside the set of hazards for which cut-off criteria were introduced. More importantly, however, is that, depending on the level of exposure, a slightly to moderately hazardous chemical may turn out to be a significant health risk, and vice versa, i.e., a very hazardous substance may turn out to be a negligible risk to people's health.

Some supporters of the current hazard-based cut-off criteria for pesticide registration have argued that this would never happen in Brazil because ANVISA subsequently undertakes a risk assessment for the substances that passed the initial cut-off criteria based purely on hazard. According to them, Brazil's unique regulatory approach would in fact offer greater protection against harmful effects of pesticides on people's health because, after excluding the substances that potentially cause some important adverse effects (regardless of their risks), exposure would be taken into account for risk management of the remaining ones. This is a misleading argument because it omits an important fact regarding our society's dependence on the use of pesticides and how their market is regulated. Pesticides are needed for agriculture, to control insect-borne diseases, to exterminate domestic and urban pests, to protect wood from termites, and so on. If a particular pesticide is removed from the market (or not registered in the country) another product inevitably takes its place to fulfill existing agricultural or other needs. Therefore, a pre-selection based on hazard limits the number of substances available for a further selection based on risk and for choosing the best health risk management alternatives. Owing to this fact, a hazard-based selection, irrespective of whether it is followed by a risk assessment approach or not, is a bad option. To accomplish their mission to protect people's health against harmful effects caused by pesticides, regulators have to keep one eye on the risk and the other on society's need for a particular pesticide and alternatives.

Besides not consistently excluding the pesticide compounds that pose the highest health risks, hazard-based cut-off criteria are also difficult to implement. The Brazilian current regulations have a number of shortcomings that make implementation of hazard-based cut-off criteria even more difficult, error-prone and unpredictable.

Some additional shortcomings of Brazilian hazard-based cut-off criteria

No remark on the relevance of Mode of Action to humans. As far as human health is concerned, the extent to which animal data can be extrapolated to humans is a key question for using them to put into effect a hazard-based cut-off criterion for pesticide registration[2]. There are many examples of substances that are carcinogenic or teratogenic to laboratory rodents but not to humans due to species-specific modes of action. The artificial sweetener saccharin and d-limonene, a monoterpene found in a variety of plant essential oils (e.g. citrus peel oil), are examples of rat bladder and kidney carcinogens, respectively, that were shown not to cause cancer in humans[12,13].

The chronic treatment of male rats with high doses of d-limonene produced an increased occurrence of tubular cell hyperplasia, adenomas and adenocarcinomas of the kidney[12]. Similar harmful effects were not observed in female rats or in male and female mice. Further investigations demonstrated that d-limonene1,2-oxide, a metabolite of d-limonene, binds to a protein ($\alpha_{2\mu}$-globulin), leading to a progressive accumulation of "*d-limonene1,2-oxide+$a_{2\mu}$-globulin*" complex within the tubular cells (as hyaline droplets) that evolves to cause tubular cell death, which in turn stimulates a compensatory cell proliferation (restorative hyperplasia) that eventually results in renal tumors[12]. Since humans and mice (and female rats) do not synthesize appreciable amounts of $\alpha_{2\mu}$-globulin or similar proteins, d-limonene nephropathy and renal cancers do not occur in these species (and gender)[12].

Some rodent studies, including a two-generation experiment in rats, showed that sodium saccharin caused a statistically significant increase in bladder tumors in F_1-generation animals, a carcinogenic effect that was greater in males than in females[13]. It was also found that chronic treatment with sodium saccharin induced a hyperplastic response that apparently is needed for the development of epithelial bladder tumors in treated rats. Several nicely designed experiments by Samuel Cohen and coworkers indicated that hyperplasia was secondary to mild cytotoxicity in the superficial layers of the bladder epithelium. Injury to epithelial cells was caused by formation of a cytotoxic calcium-phosphate containing precipitate in the urine after administration of high doses of sodium saccharin[13]. A further long-term (beginning at birth and continuing throughout life) exposure of monkeys to saccharin found no indication of increased urothelial cells proliferation or tumors and no evidence of formation of a calcium phosphate-containing urinary precipitate[13]. The difference between species was attributed to a much lower concentration (a 100 to 1000-fold difference) of protein in the primate urine compared to the rat and mouse urine. Additionally, rat and mouse urine is much more concentrated than primate urine[13].

In both cases, further mechanistic studies have clearly demonstrated that the mode of action by which d-limonene and saccharin caused cancer in rats does not occur in humans and non-human primates. There are a number of other examples of substances that produce carcinogenic effects in rodents by a mode of action that is unlikely to occur in humans. The other way around is also true. Owing to interspecies differences, a human carcinogen may remain undetected by studies conducted in laboratory rodents.

The uncertainty regarding possible interspecies differences also holds true for teratogenicity, mutagenicity and hormonal actions. Thalidomide, for instance, is a potent human (and non-human primate) teratogen, but causes no birth defects in rats and is only a weak teratogen in rabbits[14]. Phenobarbital and aspirin, to cite only two of many examples, are teratogenic to rats but apparently not to humans (both are among the medicines that are most often used by pregnant women; therefore, clinical information is available regarding human prenatal exposure).

At any rate, whenever feasible, kinetic similarities/dissimilarities between test species and humans should be investigated to strengthen the predictive value of in vivo animal tests for teratogenicity, mutagenicity, carcinogenicity and hormonal actions.

The law, decree and current health authority rules on the hazard-based cut-off criteria make no comment on the relevance, to humans, of modes of action of adverse effects (cancer, birth defects/malformations, hormonal actions) in the test species. The new regulation proposed by ANVISA states that a pesticide is to be considered as carcinogenic if there is *"..evidence (of carcinogenicity) in at least one experimental animal species, with a mode of action relevant to humans, or a mode of action not elucidated"*[10]. Regarding teratogenicity, mutagenicity and hormonal actions, however, there is no word about the relevance, to humans, of the mode of action in the test species[10]. The proposed new regulation would greatly benefit if a phrase such as "*unless the mode of action in the laboratory animal species under consideration is demonstrated not to be relevant to humans*" were introduced for all cases in which regulatory decisions are based on experimental data.

Unsuitability of using teratogenicity as a cut-off criterion

Another weakness of the Pesticide Law[4] is that *teratogenicity* and not *developmental toxicity* was the outcome selected for application of a cut-off criterion. Although there have been inconsistencies in the use of the term, strictly speaking, "teratogenicity" refers to the ability to induce structural abnormalities (malformations) or dysmorphologies, also referred to as birth defects[15]. Teratogens, therefore, are biological (e.g., viruses, parasites, bacteria), chemical (e.g., drugs, environmental pollutants) or physical agents (e.g., radiation, hyperthermia) that cause malformations in developing embryos or fetuses. An increased occurrence of structural anomalies, however, is only one of several possible manifestations of adverse effects on prenatal development. In addition to a higher incidence of malformations, embryo and or fetus deaths, prenatal growth retardation (giving rise to "small for gestation age babies") and functional disorders (some of which appear and are detected only after birth) may also occur as a result of mothers' exposure to chemical, biological and physical agents during pregnancy. In summary, teratogenicity is a focus that is narrower than developmental toxicity and excludes a number of possible outcomes of chemical-induced harm to embryos and/or fetuses.

Furthermore, in rodent and rabbit fetuses at term, it is often difficult to distinguish malformations from (structure) deviations from the normal other than malformations (e.g., variations, retardations). The foregoing problem was addressed in a series of international workshops on harmonization of terminology and classificatory terms in developmental toxicology that were held in Berlin, Germany between 1995 and 2011[16,17]. During the second Berlin workshop, a consensus was achieved to put forward a scheme of classification for fetal abnormalities that consists of only two categories: "malformation and variation", which were then defined as follows: 1) *Malformation*: *"a permanent structural change that is likely to adversely affect the survival or health of the species under investigation*". 2) *Variation: "a change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health. This change might include a delay in growth or morphogenesis that has otherwise followed a normal pattern of development"*[16]. In the subsequent five workshops, discussions among experts from academia, regulatory agencies and industries focused on a number of fetal (structural) observations that did not fit readily into one of the two categories ("grey zone anomalies"),

and for which there has been no consensus whether they are to be classified as malformations or variations. The lack of agreement on the classification of "grey zone anomalies" has been ascribed mainly to insufficient knowledge of their consequences for health and survival after birth[17].

Since it has been agreed upon that teratogenicity is the ability to increase the incidence of malformations (but not the enhancement of variations only), occurrence of grey zone anomalies often leads to conflicting conclusions as to whether the substance is teratogenic (to the species under investigation) or not.

Another difficulty in consistently applying a cut-off criterion to teratogenicity is the still standing controversy on the interpretation of fetal anomalies found only at maternally toxic dose levels. Some researchers think that increases in the occurrence of fetal anomalies, noted only at doses which are overtly toxic to the dams, are likely to be effects secondary to changes of maternal homeostasis and thus should not be taken as evidence of "developmental toxicity" or "teratogenicity"[18,19]. Other experts, including the author of this article, think that developmental toxicity is developmental toxicity irrespective of being maternally mediated or not[18]. At any rate, it is questionable to classify (label) a chemical as a teratogen (and developmental toxicant) if it increases the incidence of malformations (and or variations) only at unrealistically high doses (at which severe maternal toxicity is also noted).

Along this line, to avoid misinterpretations of results from developmental toxicity studies on environmental chemicals, Erminio Giavini and Elena Menegola have recently suggested that the upper limit of tested dose range should be *"the maximum dose unable to produce maternal toxic effects extrapolated by previous short term toxicity studies"*[19]. Guidelines for developmental toxicity studies generally require that the highest dose(s) should induce some signs of maternal toxicity. The OECD guideline 414 (Prenatal Developmental Toxicity Study), for instance, states that: "*the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering*"[20]. Although Brazilian regulation (SVS No. 3, 1992) requires testing "*...a dose high enough to produce maternal toxicity...*", it makes no recommendation on the upper limit of tested dose range based on severity of maternal toxic effects[6].

Finally, it should be stressed that both controversial matters (i.e., to distinguish malformations from variations and the role of maternal toxicity) are less critical issues if a risk assessment rather than a hazard-based approach is adopted for decision-making on pesticide registration.

EU hazard-based criteria for labeling and registration of pesticides

As aforementioned, the Brazilian hazard-based cut-off criteria for pesticide registration are unique in the world. In the US, the Environmental Protection Agency (EPA) adopts a risk assessment approach to regulatory decision-making on pesticides. A hazard-based cut-off approach is employed for pesticide labeling and placing on the market in the EU[21]. There are, however, marked differences between the cut-off criteria that have been adopted in Brazil since 1989, and those introduced in the European Union in 2009. The differences between Brazilian and European Union cut-off criteria are summarized in Table 1.

The EU Directive 91/414, concerning the placing of plant protection products (pesticides) on the market, which entered into force on July 15th, 1991, stipulated that active substances contained in pesticide products must be assessed regarding possible risks for humans and animals[22]. More recently, the Plant Protection Products Regulation (EC) No. 1107/2009 introduced a hazard-based approach to regulatory decisions[21]. According to this regulation, "*An active substance, safener or synergist shall only be approved if … … it is not or has not to be classified as …mutagen category 1A or 1B,..carcinogen category 1A or 1B …*, toxic for reproduction category 1A or 1B …*, ..is not considered to have endocrine disrupting properties that may cause adverse effects in humans …*;. …* unless ... exposure of humans is negligible*"[21].

The EU, therefore, has recently moved from using risk assessment to adopting hazard-based cut-off criteria to place pesticides on the market. Nonetheless, contrasting to Brazil, where cut-off criteria are based purely on health hazards, cut-off criteria adopted by the EU also take into account whether or not anticipated human exposures are negligible (*unless ... exposure of humans is negligible*). By a negligible exposure it is meant (Regulation (EC) 1107/2009) that "*..the product would be used in closed systems or in other conditions excluding contact with humans and that residues of the active substance, safener or synergist concerned in food and feed do not exceed the default value set in accordance with point (b) of Article 18(1) of Regulation (EC) No 396/2005 (European Council 2005), which is 0.01 mg/kg food*"[21]. More pragmatic and better scientifically-based definitions of "negligible exposure" have also been suggested, such as using the concepts of margin of exposure (MOE) or threshold of toxicological concern (TTC)[23,24,25].

It is of note that EU cut-off criteria take advantage of hazard categories described in the "*Guidance on Classification, Labelling and Packing (CLP) of substances and mixtures*" (Regulation (EC) No 1272/2008),[26] which are essentially the same of the United Nations' "*Globally Harmonised System of Classification and Labeling of Chemicals*" (GHS)[27]. CLP/GHS classification scheme categories 1A (known) and 1B (presumed) are for known or presumed human mutagens, carcinogens, and reproductive toxicants[26,27]. Classification into category 1A is largely based on evidence obtained directly from humans, while allocation to category 1B is based on data from animal studies. In both cases the strength of evidence is taken into account for classificatory purposes. Chemicals for which there is "some" evidence are put into Category 2 (suspected human toxicant) while those chemicals that epidemiological studies

Table 1. Comparison of Brazilian current rules, and changes proposed by ANVISA (draft pproposal), with European Union rules for triggering hazard based cut-off criteria for placing pesticides on the market.

	Brazilian rules		European (EU) rules
	Current rules (1992)	**Draft Proposal rules (2011)**	
Level of Exposure	No mention to exposure	No mention to exposure	Not applicable if exposure of humans is negligible
Mode of action (MOA)	(Ir)relevance of MOA to humans not considered	(Ir)relevance of MOA to humans not considered §	Not applicable if MOA is shown not to be relevant to humans
Mutagenicity	Positive in at least two tests, gene mutation in vitro and in vivo clastogenicity	Positive in at least one in vivo test for induction of chromosome aberrations	CLP/GHS categories 1A (known) or 1B (presumed) human mutagen
Carcinogenicity	Evidence in humans and or positive in at least two species, dose-effect relationship strenghtens the evidence	Evidence in humans and or in at least 2 species, and or in at least one species with a MOA either relevant to humans or not elucidated yet. §	CLP/GHS categories 1A (known) or 1B (presumed) human carcinogen
Teratogenicity	Evidence in humans or positive in at least two species. The highest dose tested should be maternally toxic. No comment on the interpretation of fetal malformations found only in the presence of maternal toxicity.	Evidence in humans or positive in at least one species	No specific cut-off criterion
Toxicity to reproduction	No cut-off criterion	No cut-off criterion	CLP/GHS categories 1A (known) or 1B (presumed) human reproductive toxicant
Hormonal actions or endocrine disrupting effects	Applicable only to irreversible hormonal changes (all tested doses) and if NOAEL remains undetermined	Applicable also to reversible hormonal changes; what is to be considered an adverse effect is unclear	Applicable if pesticide has endocrine disrupting properties that may cause adverse effects in humans

§ Only for carcinogenicity draft proposal rules make a remark on the relevance of the MOA to humans. The rule, however, is not entirely clear. As written (in Portuguese) the reader may misinterpret that a positive result in two animal species would trigger the cut-off for carcinogenicity even if, for both species, the MOA is shown not to be relevant to humans. The text should be rephrased to make it clearer that cut-off shall not be triggered if MOA is demonstrated not to be relevant to humans.

have proved to be not hazardous to humans, and those that have not been studied yet remain unclassified[26,27]. It is of note that allocation to hazard category 2 does not prevent a pesticide from being placed on the market according to EU cut-off criteria[21]. The CLP/GHS classification scheme also takes into consideration other facts depending on the particular hazard category. For instance, presumed human reproductive toxicants (category 1B) should exhibit "*... clear evidence of an adverse effect....in the absence of other toxic effects,... or (that) adverse effect is considered not to be a secondary non-specific consequence...*"[26,27]. Moreover, relevance of mode of action to humans is also addressed (if "*mechanistic information raises doubt about relevance for humans, classification in Category 2 may be more appropriate.*")[26,27].

Although still being basically a hazard-based cut-off approach, the EU decision-making process on pesticides incorporates elements of risk assessment, i.e., the idea that if exposure is very low ("negligible exposure") the substance will pose no health risk. What is to be considered a "negligible exposure", without previously assessing the risk of the substance under consideration, however, is a debatable topic and thus a weakness of the EU approach compared to the risk assessment process adopted by the US EPA.

At any rate, compared to the EU approach based on CLP/GHS classification (hazard categories 1A and 1B), the hazard-based cut-off approach currently adopted in Brazil has plenty of deficiencies and is a worse option if the goal is to put into effect regulatory decisions on pesticides aimed at protecting public health.

Concluding remarks

Hazard-based cut-off criteria do not take into account the "level of exposure". Since the likelihood of being harmed by a chemical substance depends on exposure, approaches based only on hazard do not provide a rational basis for regulatory decisions. For guiding regulatory decisions on chemicals, health risk (probability to harm) should always take precedence over hazard (potential to harm).

Brazil adopts hazard-based cut-off criteria (mutagenicity, carcinogenicity, teratogenicity, hormonal actions) for pesticide registration[4]. Contrasting to the EU cut-off criteria for plant protection products that exempt "negligible" exposures[21], the Brazilian cut-off criteria are based purely on hazard, i.e., they do not take exposure into account. Additionally, a number of shortcomings (e.g. no clear reference to the strength of evidence and to the relevance of the Mode of

Action to humans) of the Pesticide Law and health authority regulations make the Brazilian cut-off criteria difficult to be consistently implemented. A draft proposal of a new regulation on pesticides has been recently published by ANVISA and is on the table to receive criticisms and comments[10]. As a rule, the proposed changes are not based on sound toxicological science principles and make implementation of cut-off criteria far more restrictive. This particularly holds true for proposed new rules to put into effect cut-off criteria for teratogenicity and hormonal actions. According to the draft proposal, "*evidence of teratogenesis in at least one laboratory animal*" would trigger the cut-off criterion[10]. No remark is made, however, on exemption if the mode of action is shown not to be relevant to humans. The proposed rules for triggering a cut-off criterion for hormonal actions are even more drastic. ANVISA proposes that any hormonal disturbance, irrespective of being reversible or irreversible upon treatment discontinuation, would trigger the cut-off criterion for hormonal action[10]. Again no remark is made on relevance to humans. It also remains unclear what is to be considered an adverse effect resulting from a reversible hormonal disturbance.

Brazil has become the world's largest consumer of agricultural pesticides and there has been growing concern about the adverse health consequences of uncontrolled and careless use of such products in some rural areas. The time has come to move from cut-off criteria based purely on hazard to risk assessment, which is a more rational, reliable, flawless, and effective approach to health risk management purposes. Any amendment to a Federal law (Pesticide Law), however, has to be discussed and approved by the parliament before being enforced. A cut-off approach based primarily on hazard but also incorporating "negligible exposure" as an exemption - as adopted by EU - is an alternative that does not require changing the Pesticide Law. It can be put into effect by a lower ranking regulation issued by the health authority (ANVISA). Along this line, Brazil would also greatly benefit from taking advantage of GHS hazard categories 1A and 1B[27] as cut-off criteria for mutagenicity and carcinogenicity. The potential to induce malformations ("teratogenicity") is part of a more comprehensive GHS category ("toxicity for reproduction") and thus it is needed to redefine 1A and 1B categories for this particular hazard-based cut-off criterion. As far as hormonal actions are concerned, however, the current rules (SNVS regulation No. 3, January 16th, 1992)[6] make more sense than those proposed by ANVISA in January 2011[10]. According to the current rules, a cut-off for hormonal actions is triggered if "no observed adverse effect levels remains undetermined" and "the effect is not reversible" upon discontinuation of exposure, whereas the proposed new rules do not make an exception to the existence of an experimentally determined NOAEL (no observed adverse effect level) and triggers the cut-off even if hormonal changes are reversible. Reversible hormonal alterations are not necessarily harmful and experimentally derived NOAELs are reliable tools that can be used by regulators to manage health risks. As reminds us one of the most fundamental concepts of the modern science of toxicology, foreseen by Paracelsus nearly 500 years ago, the dose (exposure) "*makes a substance not a poison*".

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