National Health Surveillance Agency
Superintendence of Toxicology
General Toxicology Office
Coordination of New and Low Risk Products

TECHNICAL REASSESSMENT OPINION NO. 01 of 2015/GGTOX/Anvisa

Analysis of the technical reassessment note of the active ingredient paraquat elaborated by Fiocruz.

I – REPORT

This opinion aims to analyze the technical note of the reassessment of the active ingredient paraquat elaborated by specialists of the Oswaldo Cruz Foundation (Fiocruz) referring to process number 25351.056773/2013-21. The objective is to evaluate the content and legal basis presented in the technical note and the conclusion regarding the toxicological effects of paraquat. The assessment of the arguments of the technical note will enable the conclusion of the aspects that are relevant from the regulatory point of view to suport the public consultationand technical regulation proposition for this active ingredient, according to the legal determinations of the reassessment of pesticides.

1. Legal aspects of the Reassessment of Pesticides

The registration of pesticides in Brazil is regulated by Law no. 7.802 dated July 11, 1989. Paragraph 6 of Article 3 of this Law establishes the prohibition of the registration of pesticides, their components and similar products:

> *a) for which Brazil does not have methods to deactivate their components, in order to prevent their remaining residues from causing risk to the environment and public health;*
> *b) for which there is no antidote or efficient treatment in Brazil;*
> *c) that show teratogenic, carcinogenic or mutagenic characteristics, according to the updated results of experiences of the scientific community;*
> *d) which causes hormonal disorders, damages to the reproductive system, according to the updated results of experiences of the scientific community;*
> *e) which are more dangerous to man than laboratory tests with animals have been able to demonstrate, according to the updated technical and scientific criteria;*
> *f) whose characteristics cause damages to the environment*

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The reassessment of pesticides is established in Article 13 of Decree no. 4.074, dated January 4, 2002, which establishes:

> *Pesticides, their components and related products that indicate reduction of their agronomic efficiency, change of risks to human health or to the environment may be reassessed at any time and have their registrations maintained, changed, suspended or cancelled.*

The Ministry of Health Ordinance no. 03 of January, 1992 regulates how the toxicological assessments of pesticide products should be conducted in the country, thus it is alsoimportant for toxicological reassessment. It establishes that the toxicological assessment should detect possible severe effects to health that can impede the registration and use of certain pesticide . Therefore, after the analysis of all data and studies, the reassessment process of a pesticide should include the evaluation of the weight of the evidence obtained, the relevance to humans and if the effects falls into the legal prohibitive characteristic of registration.

According to Article 13 of Decree 4.074, of 2002, and item 1.3 of Ordinance no. 3 of 1992, prohibitive characteristics of registration for pesticides, their components and similar products are:

there is no methods for deactivation of the components in order to prevent the remaining residues from causing risk to public health (Item I of Article 31 of Decree no. 4.074, of 2002).

there is no antidote or efficient treatment (Item II f Article 31 of Decree no. 4.074, of 2002).

Scientific evidences, based on validated data, of teratogenesis in human species or in studies with at least two laboratory animal species, which should include a dose sufficiently high to produce maternal toxicity and one low dose without effect to the adult or to the offsprings (Item III of Article 31 of Decree no. 4.074, of 2002, and item 1.3.4 of Ordinance no. 3 of 1992).

Scientific evidence of carcinogenicity in human based on validated epidemiological studies, carried out with the scientific precision of WHO, in Regional bodies and their specialized Centers; or scientific evidences of carcinogenicity based on validated data in at

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least two laboratory animal species with high incidence of malignant tumors: a) on a certain part of the body or organ, with tumors of the same type; b) in various tests, preferably with different administration routes and with different doses; or c) at abnormal level with reference to the incidence, site, type of tumor or onset age, with the evidence reinforced when there is direct relationship between the number of animals positive to tumors and the increase of the doses (Item IV of Article 31 of Decree no. 4.074, of 2002, and item 1.3.2 of Ordinance no. 3 of 1992).

Scientific evidence of mutagenicity based on validated data, with induction of mutations in at least two tests, one to detect gene mutations (conducted with and without metabolic activation and with one positive control) and the other to detect chromosome mutations (Item V of Article 31 of Decree no. 4.074, of 2002, and item 1.3.3 of Ordinance no. 3 of 1992).

Scientific evidence of endocrine disruption, when there is no relevant dose with no adverse effects in experiments with laboratory animals or humans. The changes should occur in all the tested relevant doses and the effect should not be reversible with the interruption of administration of the substance (Item VI of Article 31 of Decree no. 4.074, of 2002, and item 1.3.5 of Ordinance no. 3 of 1992).

evidence of relevant damages to the reproductive system (Item VI of Article 31 of Decree no. 4.074, of 2002).

evidence
Evidence that the active ingredient is more dangerous to humans than that shown in laboratory tests with animals (Item VII of Article 31 of Decree no. 4.074, of 2002).

Even if the active ingredient does not falls in the prohibitive characteristics, after the analyses, the need for changes in the registration may be concluded, such as exclusion of a type of application, review of the Acceptable Daily Intake (ADI), exclusion of crops and change of safety interval, among other measures.

Therefore, as established by Article 19 of Decree no. 4.074 of 2002, and based on the assessment of all the relevant toxicological aspects in the reassessment, the conclusion may be to maintain the registration of the active ingredient without changes; change of formulation, dose or application method; restriction of the production, import, trade or use; prohibition or suspension of the production, import or use; or cancelation of the registration.

2. Reason for Reassessment of Paraquat

The reassessment of the active ingredient Paraquat was determined by Resolution – RDC No. 10 dated February 22, 2008, due to the existence of studies showing the high

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acute and chronic toxicity of this active ingredient. On December 31, 2007, an agreement was signed between the National Health Surveillance Agency (Anvisa) and Fiocruz, in which Fiocruz became responsible for the elaboration of the technical note addressing all the relevant toxicological aspects of the active ingredient paraquat based on bibliographic survey and analyses of studies and technical-scientific documents of the technical products. The technical note of Fiocruz was submitted on October 23, 2009, as reported on page 9 of the reassessment process of paraquat no. 25351.056773/2013-21.

Article 9 of Resolution – RDC no. 48 of July 2008, which regulates the toxicological reassessment procedures, establishes that the analysis of the toxicological aspects should be conducted by Anvisa together with a technically and scientifically recognized institution in the field of toxicology and that has no conflict of interest with pesticides. Therefore, on September 1, 2014, Anvisa started the review of the technical note of the reassessment of paraquat elaborated by Fiocruz. It is worth highlighting that for this review, all the relevant toxicological aspects addressed by Fiocruz are verified for possible inconsistencies, discussed, contested, confirmed and compared with the legislation to minimize the risk of decision making based on aspects that may be addressed incorrectly or that have no solid weight of evidence.

Like in the technical note of Fiocruz, the relevant regulatory aspects regarding the international status of the active ingredient paraquat were mentioned superficially and were based on old information and, also, the national scenario of this active ingredient was not addressed by the technical note, an updated survey on the national and international status of paraquat was conducted.

3. International Status

It is observed that the use of paraquat is no longer authorized in various countries or that its use is restricted in countries where the use is still allowed, as shown in Table 1. The restrictions imposed on the use of paraquat include the concentration limit of the active ingredient, exclusive terrestrial application or application by accredited persons. Some countries that restrict the use are still reassessing this active ingredient, such as the case of Malaysia (a country where the use of paraquat is intensive) and Australia.

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Table 1. International status of paraquat.

Country	Regulatory status
United States	Restricted use. The application should be monitored by a certified applicator. Application close to schools and homes, in parks, golf courses and playgrounds is prohibited. Re-entry interval of 12 to 24 hours.
Canada	Restricted use. Reassessment started December 2013. There are no registered products with over 37% of active ingredient (AI) and the products containing paraquat cannot be sold to anyone.
Australia	Restricted use. Only terrestrial application.
New Zealand	Restricted use. Only terrestrial application.
European Union	Prohibited since 2009, but the import of products with this herbicide is allowed.
Switzerland	The registration was never authorized.
Norway	Prohibited. Voluntary cancellation of the products.
Bosnia-Herzegovina	Prohibited.
Indonesia	Use for certain crops only by professionals with special permission.
Kuwait	Prohibited since 1985.
Malaysia	Prohibited since 2002 but was permitted in 2006 for palm oil (permanent decision awaiting the assessment of alternatives).
Cambodia	Prohibited since 2003.
Laos	Prohibited since 2010.
Arab Emirates	Prohibited since 2005.
Syria	Prohibited since 2005.
South Korea	Prohibited since 2012.
Philippines	Restricted use.
China	Prohibited. Cancelation of the registrations and production in 2014. Production allowed only for export. Use permitted until 2016.
Sri Lanka	Prohibited.
Columbia	Aerial application is prohibited.
Uruguay	Restricted use. Only products with concentration below 28%, packages of 1 to 30 liters and with dye are allowed.
Belize	Restricted use. Only terrestrial application.
Chile	Restricted use. Aerial application is prohibited.
Costa Rica	Restricted use. Aerial application is prohibited.
Jamaica	Restricted sale.
Ivory Coast	Prohibited since 2004.
Cape Verde	Prohibited since 2011.
Senegal	Prohibited since 2011.
Nigeria	Prohibited since 2011.
Mauritania	Prohibited since 2011.
Mali	Prohibited since 2011.
Republic of Chad	Prohibited since 2011.
Burkina Faso	Prohibited since 2007.
El Salvador	Prohibited since 2013, with period of one year to substitute this active ingredient with others.
Guinea Bissau	Prohibited since 2011.
Gambia	Prohibited since 2011.

Sources: http://curia.europa.eu/juris/liste.jsf?language=en&num=T-229/04#; synergies.pops.in; http://www.cirs-

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http://en.centralamericadata.com/en/article/home/El_Salvador_Use_of_53_Chemicals_Banned;
http://eatlocalgrown.com/article/12038-el-salvador-bans-monsanto-s-roundup.html;
http://paraquat.com/safety/regulation;
http://www.cloc-viacampesina.net/campanas/campana-contra-los-agrotoxicos-y-por-la-vida/1564-el-salvador-asamblea-legislativa-prohibe-el-endosulfan-paraquat-glifosato-y-clorpirifos-entre-una-lista-de-53-plaguicidas-y-compuestos-toxicos;https://www.google.com/fusiontables/DataSource?docid=11jsB_C9mlOMrlOavDYlb517SfecepHQqOt3qGFc#rows:id=1; http://www.epa.gov/; http://www.epa.gov/;
http://www.pic.int/

The American regulatory agency, United States Environmental Protection Agency (USEPA), classifies paraquat as having no evidence of carcinogenicity (Class E). However, this active ingredient showed positive results in lymphoma test of mice and in sister chromatid exchange tests with lung fibroblasts of chinese hamsters. Furthermore, there was induction of the DNA synthesis of human embryonic epithelial cells and positive results in DNA repair error tests and in mutation tests with *Salmonella typhimurium* and *Saccharomyces cerivisiae* (USEPA, 1997).

The California Environmental Protection Agency considers paraquat to be neurotoxic and capable of affecting the development of the brain functions in children. The World Health Organization (WHO) classifies paraquat in class II (moderately hazardous) while the Pesticide Action Network (PAN) classifies it in class I (WHO, 2009). PAN criticizes the position of WHO, suggesting that paraquat should be classified in class Ia (extremely hazardous) or Ib (highly hazardous) because besides the high acute toxicity, there are delayed acute toxic effects such as pulmonary fibrosis, and there is no antidote for this substance (PAN, 2011).

Paraquat was prohibited in the entire European Union after a court order that contested the decision of the reassessment by the European Commission to maintain the use of this active ingredient (The Court of First Instance of European Communities, 2007). The reasons that lead the Court to remove paraquat from the list of authorized products were:

Existence of studies in scientific literature relating paraquat to Parkinson which was not considered in the reassessment;
The reassessment concluded that there was no neurotoxicity but it does not satisfy all the necessary procedural requirements;
There was no assessment of a French study on occupational exposure considered relevant;
In a Guatemalan study, the exposure of one of the operators was 118% above the permitted level, although he was using the product in the appropriate conditions.
The French study used as basis to allow the use of paraquat warns in its conclusions regarding the use of backpack sprayers in the application of products, reinforcing the doubts regarding the conclusion of safe use of paraquat-based products.

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3.1. Rotterdam Convention

Besides the prohibitions and restrictions imposed on paraquat in various countries, the Rotterdam Convention placed on the agenda and debated the use of products with paraquat. In 2013, at the sixth Conference of Parties (COP) held in Geneva, over 120 countries supported the decision to include the liquid formulations containing a concentration of paraquat dichloride equal or greater than 276 g/L in the list of annex III of the Rotterdam Convention. This proposal was made by Burkina Faso that observed the serious exposure of workers to this pesticide (UNEP/FAO/COP.6/11, 2013). The Chemical Assessment Committee of the Convention, created to assess the risk of the exposure to paraquat, had recommended the inclusion of this active ingredient in the list of annex III. With this decision, paraquat is classified as seriously hazardous; however, Guatemala and India vetoed the inclusion of paraquat in this list (UNEP/FAO/RC/CRC.7/11Add.4, 2013). The representatives against the inclusion argued that paraquat continued to be used in their countries on crops important to agriculture. The representatives in favor argued that paraquat is dangerous to human health and to the environment and that the inclusion in the list will not imply in restrictions to the substance because it will only make the prior grant procedure of Rotterdam Convention necessary. The COP presented a document deciding on the inclusion of paraquat, but due to the impasse between the countries, it decided on the non-inclusion and the matter will be placed on the agenda again at COP7 in 2015 (UNEP/FAO/RC/CRC.8/9/Ver.1, 2013). On March 3, 2015 there was confirmation of the recommendation for inclusion of paraquat formulation in the list of annex III of the Rotterdam Convention at COP7 in 2015 (PIC, 2015a).

COP 7 was held between May 4 and 15, 2015 in Geneva, but till present the official documents have not been disclosed; however, in a statement available on the website after the event, it clarifies that only the methamidophos was included in the list of annex III of the Rotterdam Convention. For paraquat, there was apparently no consensus regarding its inclusion in the list, but more information regarding the future actions and discussions were not mentioned. In general, it was decided the chemicals that had not consensus regarding the inclusion in the list would have their discussions postponed or indicated as topics of special inter-sectoral groups (PIC, 2015b).

The documents of the 2013 Rotterdam Convention have the reasons for inclusion of paraquat in the list of hazardous substances of annex III and especially include the concerns resulting from the Burkina Faso reports. In Burkina Faso a total of 296 cases of intoxication among 650 workers were reported between 1996 and 2010. Fifty three of these cases were due to the backpack application of the paraquat-based Gramoxone® Super formulation. A study in Burkina Faso showed that the use of personal protective equipments (PPPEPPEs) by farmers is very low in the country. The adverse effects in the 53 registered cases appeared immediately or several hours after application of the pesticide. The symptoms reported were headache, excessive sweating, itching, tingling, burning skin, eruptions on the skin and injuries, complete destruction of contaminated site, fever, dizziness, bone pain,

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fainting, breathing difficulty, cough, vision problems, eye pain, ringing in the ears, abdominal pain, nausea, vomit and locked jaw. Treatment is unknown in 16 cases, but patients were treated in 26 cases and hospitalization was required in 11 cases. The treatment for this type of intoxication is symptomatic and there is no antidote. After these observations, Burkina Faso decided to cancel all the registered products (UNEP/FAO/RC/CRC.8/9, 2013). The documents presented at the Convention by the member countries showed the existence of alternative methods that involve chemical and non-chemical strategies. For example, glyphosate based herbicides are pointed out as an alternative but recently the International Agency for Research on Cancer (IARC) classified glyphosate as likely carcinogenic to humans (Guyton et al., 2015).

The criteria adopted by the Committee to include paraquat in annex III was the occurrence of intoxication during the agricultural use in the field conditions of users in Burkina Faso The misuse was not used as a criteria for this proposition despite the numerous cases of suicide and accidental exposure. Therefore, the Committee concluded that there is sufficient evidence that the incidents reported by Burkina Faso are relevant for other countries with similar climatic conditions, citing Brazil as one of the countries where the PPPEs are also not used correctly and where the backpack applicators used are mostly defective (UNEP/FAO/RC/CRC.8/9, 2013).

Besides the documents that report the entire discussion process about the inclusion of paraquat in the list of annex III, there are a number of documents in the Rotterdam Convention files on the position of the countries regarding the use of paraquat (Rotterdam Convention, 2013).

a) Chile:

Considers that paraquat does not have an antidote, the medical treatment alternatives does not reverse the damage caused by the pulmonary fibrosis and, therefore, the intoxication generally results in death. Different conditions of use of this pesticide in Chile resulted in intoxication and adverse effects in humans, with the main risk being to the manipulators (those who applied, mixed and transported it). It was observed that paraquat can be hazardous to birds, mammals and plants. Based on these problems, the aerial application was prohibited and it was established that the application of products with paraquat should be executed according to the specific recommendations. The packaging should contain information on: protective measures to avoid contact with the pesticide during and after application; danger warnings during application, with indication of period and time of treatment; besides other information. The mandatory production, import, distribution and sale of paraquat with dye and emetic agent or odorizing agent were also established.

b) African Economic and Monetary Community – Pesticides Committee of Central Africa:

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Reported that the oral DL_{50} in humans is 35 mg/kg and in rats it is 100 to 150 mg/kg. Furthermore, it concluded that paraquat causes degenerative diseases, brain injuries and Parkinson's disease. It also reported indications of resistance to the pesticide and its use has been reducing, and it is no longer the mostly used pesticide in the member countries.

c) Argentina:

Paraquat was replaced by other herbicides. There are no statistics or intoxication study for paraquat in Argentina.

d) Australia:

Paraquat is not allowed for domestic use. The use of dye, emetic agent and repulsive odorizing agent is mandatory. Australia also reported that the product Gramaxone at 200 g/L is not registered in the country and, therefore, there is no information of intoxication for this formulation, but there is registration of 61 paraquat-based formulations. Also, this active ingredient is under analysis by the regulatory body of the Federal Australian Government, the Australian Pesticides and Veterinary Medicines Authority (APVMA), due to concerns regarding the potential risk to occupational health and regarding environmental safety. If the review establishes that the current strict controls on the use of this chemical product in Australia does not satisfy the human and environmental safety requirements of APVMA, then stricter measures may be adopted. The review is still ongoing and other toxicological studies are being conducted to determine if there is any concern regarding the subtle effects after prolonged exposure to low doses. The acute toxicity of paraquat is well characterized and therefore it is an herbicide with greater utilization restriction and control in Australia.

e) United Kingdom:

The absence of genotoxicity was observed *in vitro* and *in vivo*, with two negative *in vitro* results and four *in vivo* results. Positive results were observed *in vitro* in a sister chromatid exchange test, in a cytogenetic test in human lymphocytes and in a gene mutation test in the lymphoma cells of mice. The positive *in vitro* results indicate clastogenic and mutagenic potential in mammal cells. There are no positive *in vivo* results and therefore paraquat was not considered mutagenic. The cited studies are the same used by the reassessment committee of the European Food Safety Authority (EFSA). Some interesting findings but not considered relevant were the testicular injuries, the degeneration of the peripheral nerve, the pulmonary adenomas and carcinomas (not confirmed after review of the slides), the consistent reduction in the number of leukocytes in the highest dose in males (7.2 mg/kg b.w./day), the changes in the ossification and the discrete abnormalities in the highest dose. The short term Acceptable Operator Exposure Level

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(AOEL) was 0.0005 mg of paraquat / kg b.w./day; the long term AOEL was 0.004 mg/kg b.w./day and the limit in water of 13 µg/L.

f) Hungary:

Banned since 1990.

g) Norway:

The assessment of the registration renewal in 1993 resulted in the cancelation of the registration of the products with the active ingredient paraquat; however, on appeal, this decision was revoked and the products were sold until 1996. No paraquat-based product can be sold in the country since 1996. In genotoxicity studies, paraquat was positive in the arabinose-resistance gene mutation test in *Salmonella typhimurium* and in *Aspergillus nidulans* and clearly induced unscheduled DNA synthesis in human epithelial cells. Therefore, paraquat appears to have a weak mutagenic potential. Significant increase of chromosome damage in vitro and increasing trend in lower doses were also observed. Therefore, paraquat showed clastogenic effects. In an in vivo test (wing spot test in drosophila), the result was positive, with paraquat presenting non-dose-dependent genotoxic effect. Norway also concluded that paraquat is not teratogenic and is slightly embryotoxic.

4. National Status

In Brazil, according to the Phytosanitary Pesticides System (Agrofit), paraquat is registered for pest control in avocado, pineapple, cotton, rice, asparagus, banana, potato, beet, cocoa, coffee, sugarcane, tea, citrus, coconut, kale, beans, apple, corn, pasture, pear, peach, rubber tree, soybean and sorghum crops. It is also authorized as a drying agent for cotton, rice, potato, sugarcane, corn, soybean and sorghum. Fifteen paraquat-based technical products and eight formulated products are registered as observed in tables 2 and 3.

There is a large volume of sales for paraquat-based products (table 4), but there are also other products registered for the same pests, on the same crops and therefore, with the potential use as substitutes of paraquat, according to the data provided by Agrofit. However, a deeper analysis of the possibility of these pesticides substitutes paraquat should be done by Ministry of Agriculture, -Livestock and Supply (MAPA).

The reports of various agencies and international bodies report the importance of paraquat as a broad-spectrum pesticide, of which the main substitute is glyphosate. However, the use of glyphosate is being restricted because many plants show resistance to it; furthermore, with the new classification of glyphosate as likely carcinogenic adopted by

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IARC, its use should be reviewed in various countries. It is worth highlighting that there are indications of resistance even to paraquat (Rotterdam Convention, 2013; PAN, 2008; Neumeister and Isenring, 2011; Watts, 2011; Lancet, 2015).

Table 2. List of paraquat-based technical products in Brazil according to Agrofit.

Trademark	Registration Owner	Registration
Alamos Paraquat Technical	Alamos do Brasil	8314
CHN Paraquat Technical	ALLIERBRASIL AGRO LTDA.	11312
Helm Paraquat Technical	HELM DO BRASIL MERCANTIL LTDA	3808
Rainbow Paraquat Technical	RAINBOW DEFENSIVOS AGRÍCOLAS LTDA.	2513
Sinon Paraquat Technical	SINON DO BRASIL LTDA.	07805
Zeneca Paraquat Technical	SYNGENTA PROTEÇÃO DE CULTIVOS LTDA.	0678498
Paraquat Technical 500	STOCKTON - AGRIMOR DO BRASIL LTDA.	2108
Paraquat Technical ZY	ALLIERBRASIL AGRO LTDA.	11712
China Paraquat Technical	ALLIERBRASIL AGRO LTDA.	11212
Syngenta Paraquat Technical	SYNGENTA PROTEÇÃO DE CULTIVOS LTDA.	014507
Paraquat Technical Yn	ALLIERBRASIL AGRO LTDA.	11112
Ouro Fino Technical DCP	OUROFINO QUÍMICA LTDA	9814
Alta Paraquat Dichloride Technical	ALTA – América Latina Tecnologia Agrícola Ltda.	12414
Nufarm Paraquat Technical	NUFARM Indústria Química e Farmacêutica S.A.	8914
Atanor Paraquat Technical	ATANOR DO BRASIL LTDA.	9214

Table 3. List of paraquat-based formulated products in Brazil according to Agrofit.

Trademark	Registration Owner	Registration
Laredo	HELM DO BRASIL MERCANTIL LTDA	13309
Orbit	SINON DO BRASIL LTDA.	2010
Tocha	STOCKTON - AGRIMOR DO BRASIL LTDA.	13208
Gramocil	SYNGENTA PROTEÇÃO DE CULTIVOS LTDA.	1248498
Gramoxone 200	SYNGENTA PROTEÇÃO DE CULTIVOS LTDA.	1518498
Helmoxone	HELM DO BRASIL MERCANTIL LTDA	14908
Paradox	SINON DO BRASIL LTDA.	5006
Pramato	AGROLI INDÚSTRIA QUÍMICA LTDA.	396

Table 4. 2014 Trade data of technical products (TP) and formulated products (FP) registered in Brazil.

Product	Quantity Imported	Quantity Exported	Domestic Production	Domestic Sale		
				Customer/ Quantity	Industry/ Quantity	Resale/ Quantity
Total TP (Kg)	3,908,011.77	0.00	0.00	1,918,101,00	0.00	0.00
Total FP (Kg)	1,981,422.41	313,363.99	4,119,343.36	2,289,476.29	1,533,650.04	1,957,968.67

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Some toxicological aspects reported in the technical note of Fiocruz needed to be analyzed closely because in general the technical note was vague: for having used a scarce number of bibliographic references on certain aspect: for concluding incoherently about some cited studies, for not having completely addressedthe studies of the dossiers of products registered at Anvisa or for not havingaddressed the essential regulatory aspects on the toxicity of paraquat. Below is the analysis of the relevant toxicological aspects of the technical note, with some additions considered important which had not been addressed.

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			progressive dyspnea. The patient arrived at the hospital with 3% of the body surface burnt. There was no liver or spleen impairment but there was renal damage and pulmonary fibrosis. Blood and urine tests were also normal. Case 2: 55-year-old woman presented 2% body surface with burns on the lower limbs. Worked with the man in case 1 from 9 am to 12 noon. The woman did not protect herself and did not wash the skin immediately after contact with paraquat. In the afternoon there was formation of erythema and blisters on the skin. The patient was treated for five days by a community doctor. The condition of the patient worsened and she was taken to the hospital. The organs and biochemical tests were normal.	
LIN et al., 2003.	Taiwan	Accidental / Intentional Dermal	24-year-old man, smoker and with a history of amphetamine abuse, washed his face and body with paraquat when drunk. Sixty hours later he was hospitalized with painful lesions on the skin. The patient was washed with water and soap. The patient had multiple burns on over 10% of the body surface and corneal and lip ulcers. The patient also had significant leukocytosis, renal failure, hepatic failure and pulmonary fibrosis.	The patient was treated with cyclophosphamide, dexamethasone and therapy with steroids, and patient is recovering after three months.
PAPIRIS et al., 1995.	Greece	Occupational Dermal	57-year-old male smoker exposed to paraquat through the use of a defective backpack applicator. The paraquat spilled on this back and testicles. After five hours the man noticed burning and blisters on the testicle. Initially the doctor suggested only washing the site. Five days later the man experienced short of breath, cough and fever. The testicular lesions were cured but there was interstitial infiltrates in the lung. The man developed acute respiratory failure with infiltrates in the lung a few days after dermal intoxication, which rapidly evolved to fibrosis. Treatment with anti-inflammatories and antioxidants.	In a rare event of intoxication by paraquat, the patient survived after treatment despite the residual pulmonary fibrosis. The article calls attention to sub-lethal intoxication which may explain workers with pulmonary fibrosis with no known cause.
WONG et al., 2011.	Hong Kong	Occupational Dermal	22-year-old man presented lesions on the skin of the lips and testicles five days after paraquat application without using protective equipments. His hands, lips and testicles were contaminated with paraquat. There were no symptoms until the second day when pain lesions appeared on the skin. There was impairment of the hepatic function, which was gradually re-established.	The lesions were completely cured. The hospital discharge occurred before complete recovery of the liver but the patient did not show up to the follow-up consultation.

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ATHANASELIS et al., 1983.	Greece	Occupational Dermal	64-year-old man applied paraquat solution at 0.5% using an old backpack applicator, which caused leakage of the liquid on his back for 3.5 hours (concentration of 5 g/L). The man did not wash until night time. On the next day he felt burning sensation on his back, with skin irritation. Two days later he looked for a doctor who confirmed apparent necrosis of the lesions. The man also complained of weakness. The doctor recommended hospitalization but the man did not accept and went back home. Three days later the clinical condition deteriorated. Serum level of paraquat: 0.08µg/mL.	Twelve hours after hospitalization the man died of renal and respiratory failure.
WOHLFAHRT, 1982.	Papua New Guinea	Occupational / Accidental Dermal	Five men exposed to paraquat through the skin. Three cases resulting from occupational exposure, one accidental in which the paraquat was used to kill lice and one to treat scabies. All the men had blisters and abrasions on the skin.	The five men died from respiratory failure.
BINNS, 1976.	Papua New Guinea	Accidental Dermal	Patient thought that paraquat could kill lice and applied the solution at 20% on his head and beard, causing painful lesions on the skin.	Patient died after the sixth day, with hemorrhage in the lungs.
GARNIER et al., 1994.	-	Accidental Dermal	Case 1: 36-year-old man applied paraquat solution at 20% on his entire body to treat scabies. He developed erythema on the skin and blisters. He was hospitalized two days later. Presented renal failure and dyspnea. Case 2: Man applied diluted paraquat to hair and beard to treat lice. Developed extensive dermatitis.	Case 1. The condition of the patient deteriorated resulting in death, which occurred 26 days after exposure. Case 2. Moderate and temporary changes of the renal and respiratory functions.
CHESTER et al., 1993.	Sri Lanka	Occupational Dermal	Collected the urine of twelve workers involved in the preparation of the liquid and in spraying paraquat. These workers already used paraquat for at least five years. The workers used their own normal clothes (short sleeve shirt and shorts, and they did not use socks or closed shoes). The urine was collected every 24 hours, starting one day before the first day of application and ending seven days after the last application, for a total of 13 days. After the thirteen days of urine collection, the potential dermal exposure was measured. To measure the absorption, the workers used hooded overalls, gloves and socks to apply the paraquat. The quantities of paraquat used and their concentration in the spray solution were below the normal values used in the backpack applications.	Paraquat detection limit in urine of 0.03 µg/mL. The quantity of paraquat recovered from from the clothes of the workers was 66 mg (mixed) and 74 mg (sprayed). For the mixed, the hands were the most affected while for the sprayed the percentage absorption of the hands, legs and feet were similar. The dose absorbed based on skin absorption was 0.0004-0.009 mg/kg/day, exceeding 18 times the AOEL. The dose absorbed based on blood and urine analysis was 0.001-0.004 mg/kg/day, exceeding 2 to 8 times the AOEL (calculated assuming urinary excretion equal to the analytical limit, which is high in this

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				study). The dose absorbed is likely lower. Furthermore, the worker did not correctly use the PPEPPEs PPEs (SCP/PARAQ/002, 2001).
LEE et al., 2009.	Costa Rica (2001)	Occupational Dermal / Inhalation	Urine was collected from 119 paraquat operators and from 59 non- operators that also worked on the crops. The use of paraquat occurred through backpack applicators. The normal clothes of the workers consisted of long pants, long sleeve shirts or overalls and boots. In banana and palm kernel crops, the workers also used rubber gloves but did not use these in coffee crops. In banana and palm kernel crops all the workers used overalls, gloves, mask and apron. In coffee crop only 50% used overalls and apron, but all used gloves.	Paraquat detection limit in urine: 2 ng/mL. The inhalation was also measured in the group of workers. For manipulators, 83%, 47% and 64% of the urine samples were below the detection limit before, during and after spraying, respectively. The crop in which the workers presented the highest level of paraquat in the urine samples was banana, followed by coffee and lastly palm kernel. Regarding the inhalation exposure, paraquat was detected in the urine of 60% of the workers, but the aerial exposure level (23.8 $\mu g/m^3$) was well below the value established by the American Conference of Governmental Industrial Hygienists (500 $\mu g/m^3$). The detection of paraquat in urine can result from dermal exposure.
LEVIN et al., 1979.	South Africa	Occupational Dermal	29-year-old man received care at the hospital for dyspnea. He was well two weeks before spraying the vineyards with paraquat (2-8%). The patient spilled paraquat on the shoulder when carrying the sprayer tank, which caused skin burn (high concentration solution of 28 g/L). One week after exposure the man started developing breathing difficulty and was hospitalized to treat tuberculosis. When this hypothesis was discarded, which occurred three weeks after intoxication, the man was transferred to another hospital. Ulcer on the shoulder, tachypnea, cyanosis, lung opacification in the radiography and altered levels of various blood parameters were observed.	The patient became hypotensive in three days, requiring nasotracheal intubation and ventilation, but he died three days later. The autopsy showed cell infiltration and fibrosis.
FITZGERALD et al., 1978.	Ireland (1968-1977)	Occupational Oral or Dermal	Thirteen cases of exposure to diluted Gramoxone. The oral exposures generally occur during an attempt to unblock the equipment with the mouth. There was only one case of suicide. Contact with the diluted sprayed solution but with previous dermal lesion.	In eight of the cases there was impairment of the kidneys, lungs and/or liver. Six cases were fatal, including the suicide case. Two fatal cases resulted from dermal exposure, four cases resulted from oral exposure through incorrect handling and one

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				case was suicide.
NEWHOUSE et al., 1978.	Canada	Occupational Dermal	39-year-old woman sought medical care with lesions on the skin that had lasted for four weeks. The woman regularly came in contact with diluted paraquat solution. In some occasions, even with certain injuries on the arm, the woman did not use protective clothing when handling the paraquat solution.	Death due to respiratory failure eight weeks after the skin contact with paraquat.
OKONEK et al., 1983.	Germany	Accidental Dermal	Four-year-old child spilled paraquat on the pants but it was not possible to determine if the exposure was only dermal or if the child put the hand in the mouth, but the standard symptoms indicated dermal intoxication. The child's cloth was not changed for a long period of time. At night the skin presented redness and the following day the skin was sensitive to touch. One day later the skin had a blister and necrosis and the boy was taken to the hospital. Serum level of paraquat: 5.53 µg/kg	The health condition of the child and the treatment were not described, but the child recovered.
WOJECK et al., 1983.	USA	Occupational Dermal	Exposure of workers during application of paraquat on tomato and citrus crops through tractors. The workers used the paraquat in the usual manner and their clothing consisted of long sleeve or short sleeve shirts, long pants, socks, closed shoes or boots. Many workers used hats. Only one worker used gloves.	The exposure to paraquat was highest in open tractor. The use of closed cabin or high clearance tractor reduced the exposure by about 85%. Paraquat was not observed in the urine (detection limit: 0.012-0.041 ppm). Open tractor: 168.59 ± 81.85 mg/h Closed cabin tractor: 26.91 ± 25.63 mg/h High clearance tractor: 18.38 ± 13.58 mg/h
STAIFF et al., 1975.	USA	Occupational Dermal	Workers operating spray tractors in orchards and voluntary workers spraying paraquat through backpack application in gardens. The potential dermal contamination was established through absorbent paper adhesives attached to the body. The dermal exposure on the hands was measured through the collection of the water used to wash the hands after application of paraquat. The inhalation was measured by specific filters in the masks. The clothing consisted of short sleeve shirt, no gloves and no hat.	The dermal and respiratory exposure was considered low. The highest exposure was the hands. The dermal exposure varied from 0.01 to 3.4 mg/h. Paraquat was not observed in the urine samples (detection limit of 0.02 ppm).
EUROPEAN UNION, 2007.	-	Occupational Dermal	Man spilled paraquat on his clothes while transferring the container and waited 48 hours before cleaning.	Ten days after exposure, the lungs stopped functioning and he died on the fifteenth day.

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6. Neurotoxicity

For some time, the neurotoxicity of paraquat remained uncertain. In the past, the JMPR (Marrs and Adjei, 2003) stated that paraquat was not neurotoxic, but currently there is evidence that it is able to trigger or accelerate the development of Parkinson's disease (Dinis-Oliveira et al., 2006; Ren et al., 2009; Ntzani et al., 2013). Parkinson is a progressive neurodegenerative disease of multifactorial etiology and may be influenced by age, genetic makeup and environmental factors (Franco et al., 2010). The technical note of Fiocruz addresses a number of scientific articles that contribute to the characterization of paraquat as an important environmental factor in the development of Parkinson's disease in people exposed to this pesticide. In addition to these articles, there are still a number of other studies that corroborate this relationship between exposure to paraquat and Parkinson's disease, including epidemiological studies (Prasad et al., 2007;. Tanner et al., 2011) and a careful analysis performed by the EFSA on epidemiological studies ever published on the subject (Ntzani et al., 2013).

The paraquat has structural similarity with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is known to induce Parkinson's disease (Richardson et al 2005; Jackson-Lewis and Przedborski, 2007) . Despite this similarity, there were doubts as to paraquat's ability to reach the central nervous system, because it was believed that he would not cross biological membranes with the same efficiency of MPTP. However, there is already evidence that paraquat is also able to cross the blood brain barrier and promote neurotoxic (Pardridge, 2012). The paraquat can be transported across the blood-brain barrier by the action of neutral amino acid transporters, as the carrier system L (LAT-1), and the maximum levels in the brain are achieved after 24 hours of exposure (Brooks et al., 1999; McCormack et al., 2002; Richardson, 2005; Dinis-Oliveira et al., 2006; Kuter et al., 2007; Kuter et al., 2010).

Studies have shown that MPTP and paraquat have distinct mechanisms of action, but both are able to cause the death of dopaminergic neurons, and consequently triggering Parkinson's disease. Unlike MPTP neurotoxic action of paraquat did not depend on the dopamine transporter. Several studies describe the modes of action of paraquat by tests *in vivo* and *in vitro*, showing that this herbicide is capable of triggering the production of ROS, α-synuclein aggregation and selective

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damage to the nigrostriatal pathway neurons. It is noteworthy that the paraquat administered parenterally is used as Parkinson's disease inductor in animal models for mechanism studies and treatments of disease (Brooks et al., 1999; McCormack et al., 2002; Richardson, 2005; Dinis-Oliveira et al., 2006; Kuter et al., 2007; Kuter et al., 2010).

Parkinson's disease is characterized by the selective degeneration of dopaminergic neurons in the black substance *pars compacta* attributed to aggregation of proteins (eg, α- synuclein), oxidative stress, the proteasome dysfunction, mitochondrial dysfunction and apoptosis induction. Several studies relate paraquat to the development of Parkinson for influencing such outcomes (Ding et al., 2001; Manning-Bog et al., 2002; Goers et al. 2003; Yang et al., 2007;. Fei et al., 2008 ; Moran et al., 2008; Yang et al., 2009; Chorfa et al., 2013)..

The destruction of dopaminergic neurons by paraquat has been demonstrated in non-mammals (Menzies et al., 2005; Chauduri et al., 2007;. Wash-Culebras et al., 2007) and also in mammals. In these, there are several studies describing neuronal degeneration with neurobehavioral effects, neuropathological and reduction of neurotransmitters compatible with the changes that are observed in Parkinson's disease (Calo et al., 1990; Bagetta, 1992; Liou et al., 1996; Brooks et al ., 1999; McCormack et al., 2002; Chanyachukul et al., 2004; Li et al., 2005; Miranda-Contreras et al., 2005; Kuter et al., 2007; Ren et al., 2009; Kuter et al., 2010; Mehdi and Qamar, 2013).

Studies of neurotoxicity submitted before June 26, 2015 by the company holding the registration of a technical product (PAraquat Tecnico Syngenta = Syngenta technical paraquat) have some important limitations and indications of toxic paraquat action to the nervous system, as can be seen in Table VI. The two studies initially submitted, which used similar protocols contains elements that may had affected the attainment of the results. Assays were performed in a shorter period of time (only three weeks) and conducted with maximum three applications of paraquat. That is, the frequency of exposure was low because study of the scientific literature demonstrate that the perception of significant changes related to Parkinson's disease may be more than four weeks due to compensatory mechanisms (McCormack et al., 2002; Ossowska et al. 2005a; 2005b Ossowaska; Ossowaska et al., 2006; Kuter et al., 2007; Kuter et al., 2010).. Therefore, the biochemical changes (reduction of dopamine and its metabolites and increased I dopamine) from exposure to paraquat may differ from

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The promoted by MTTP and therefore, the protocols used in the studies of the technical product dossier may not have been possible to give the clear perception of these changes. Therefore, although MPTP is recognized as a positive control for studies of Parkinson's, this molecule does not have the same mechanism of action on the nervous system as paraquat, as explained above. Thus, while the administration of MPTP has resulted in changes able to trigger Parkinson's in the protocol used, it is not expected that paraquat has exactly the same behavior outlined in the experiment.

Chronic exposure to multiple low doses of paraquat likely causes the activation of degenerative and compensatory mechanisms. After intraperitoneal administration of paraquat, there intracellular ROS production in the dopaminergic neurons and subsequent cell death (Dinis-Oliveira et al., 2006; Franco et al., 2010; Kuter et al .; 2010; and Mehdi Qamar, 2013) . The pattern of change of dopaminergic neurotransmission after exposure to paraquat is peculiar: first is increased release of dopamine, and subsequently reducing the basal level in striatum. Thus, the application of paraquat at low doses (10 mg/kg) shows delayed effect (neurodegeneration), probably due to the activation of protection mechanisms (antioxidant system) which would be initially saturated with maintaining the generation of reactive species. Studies have shown that repeated exposure (over

24 doses) leads to degeneration of the nervous system, a reduction of 45% of dopaminergic neurons even in a low intraperitoneal dose. Thus, the protocols used in the assessment of technical products are not able to detect these changes (Richardson et al., 2005; Ossowaska et al., 2005a; Ossowaska et al., 2005b; Ossowaska et al., 2006; Kutter et al., 2007; Kuter et al., 2010).

Despite the limitations, in one study (n° 639092) there is evidence of neuronal degeneration, because there was a reduction of dopaminergic neurons, and this feature is one of the key elements for recognition of Parkinson's disease. It is observed in the study file a standard of reduction of dopaminergic neurons similar to the positive control with MPTP. There was not a statistically significant decrease for most of the evaluated assays, but even in the positive control there were situations where such reduction was also not significant. In the same study, there was no reduction of dopamine, but it is noteworthy that there were few exhibitions and the time to access the central nervous system effects after the last

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application was too long, seven days. That is, this time may have been sufficient for the compensatory mechanisms were triggered, since three days after paraquat administration already start compensatory mechanisms to restore dopaminergic transmission. The surviving neurons are activated to functionally compensate for the loss of neuronal. The degeneration of neurons is very slow and effective reduction of dopamine would be perceptible only after a long period of administration of paraquat. At 24 weeks, for example, one notices a significant decrease in dopamine about 30% (Richardson et al., 2005; Ossowaska et al., 2005a; Ossowaska et al., 2005b; Ossowaska et al., 2006; Kutter et al., 2007; Kuter et al., 2010)..

The studies of Breckenridge and colleagues (2013) and Minnema and colleagues (2014) were sent by Syngenta in addition to complementing the response to the demand Official Letter No. 0042/2015/GGTOX. These studies were submitted on the grounds to verify the absence of relationship between exposure to paraquat and the development of Parkinson's disease.

Breckenridge et al (2013) evaluated weekly intraperitoneal administration of paraquat in doses of 10, 15 or 25 mg/kg/week, for one, two or three weeks in mice. The dose of 35 mg/kg/week was also tested, but 3 of 5 animals died or were euthanized because of high toxicity. According to the authors, there was inconsistency in the evidence of loss of dopaminergic neurons, neuronal degeneration there was no hint of the striatum or cell death by apoptosis and also there was no activation of astrocytes and microglia in the conducted study. Breckenridge et al (2013) challenge the literature studies have shown reduction of dopaminergic neurons because argue that the detection by immunoreactivity to tyrosine hydroxylase, an enzyme involved in dopamine synthesis does not necessarily reveal cell death. However, it is noteworthy that this technique is widespread and well standardized (Jackson-Lewis and Przedborski, 2007). Moreover, the authors point out that in some articles it was observed entails the reduction of total neurons.

Breckenridge and colleagues' assays (2013) were conducted with paraquat obtained by Syngenta (99.9%) and Sigma-Aldrich (100%) in 9 to 10 weeks old mice. That is, exposure to paraquat was at most up to 12 to 13 weeks (less than 24 weeks used in the literature studies). The neurotransmitter level was assessed after 7 days of administration of the last dose.

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Although the authors have concluded that there was no significant reduction in signs of dopaminergic neurons, one can observe a significant reduction of this parameter in the animals treated for three weeks with 15 mg/kg of paraquat. This reduction was even greater than that of the positive control with MPTP. In both cases, there was also a significant decrease in total neurons count (immunomarked or not to TH) and although not statistically significant, was also observed reduction in total neurons stained with violet cresyl. The authors disregarded this reduction because it is not significant, however, it was not significant even in the positive control, and even then, it is true the action of MPTP. Therefore, we conclude that there is no relevance of these findings, especially since there is convergence between the result of exposure to the highest dose tested and the result of the positive control. Furthermore, for all other doses (3 times/week to 10 mg/kg, 1 time/week to 15mg/kg, 2 times/week to 15 mg/kg), there was a reduction of dopaminergic neurons and even without statistical difference, there is a clear trend of reduction of dopaminergic neurons after exposure to paraquat.

In another assay, Breckenridge et al (2013) compared the amount of TH positive neurons by two techniques (chromogenic staining and fluorescence labeling). It is observed that in chromogenic staining no significant reduction in the number of TH+ neurons at all doses of paraquat (3 times/week of 10 mg/kg, 3 times/week of 15 mg/kg and 1 time/week of 25 mg/kg) and MPTP, in the number of total neurons in the higher dose of MPTP and paraquat and significant reduction in the average volume of the black substance *pars compacta* for MPTP and the two higher doses of paraquat. For the lower dose of paraquat, was also reduced, but not significantly.

In the fluorescence analysis, the same pattern of reduction was observed in all cases and at all dose levels, but without significant difference. It is noteworthy that there was only a significant reduction in the number of TH+ neurons of the positive control. Apparently, these decreases observed are dose-dependent. Thus, these results do not allow conclusions about the total absence of paraquat action in the death of dopaminergic neurons; on the contrary, they provide important evidence that paraquat can act in these neurons and because it has not been shown to reduce dopamine in the striatum, it may be due to the fact that exposure duration was short and therefore compensatory mechanisms are working.

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The authors explain that in another study of 13 weeks also did not show any decrease of dopamine, even with a longer exposure time (Minnema et al., 2014). These results contradict those reported by Prassad and collaborators (2009), which carried out *in vivo* studies in mice with intraperitoneal administration of 10 mg/kg paraquat two or three times a week and observed a significant reduction of dopamine and its metabolites from 18th dose associated with significant reduction of TH protein in the 6th and 12th doses, with even smaller values than the control values, but growing from the 18th dose. The neurotransmitter level and the number of TH were evaluated 7 days after the last dose. Prasad et al (2009) argue that, in opposite of what has been observed, several published studies found reductions of dopaminergic neurons with no correlation to reduction of dopamine, but it can occur due to low exposure dose does not lead to the plateau contourline in the brain or because paraquat did not act in the brain for the appropriate time period.

In the study Minnema et al (2014) carried out for 13 weeks (91-94 days), the paraquat was administered orally at doses of 1.7 (males); 2.7 (females); 10.2 (males) and 15.6 (females) mg/kg/day in animals of 10 weeks of age, reaching brain concentrations compatible with those affected after intraperitoneal administration. Therefore, animals were treated until they are 22 weeks (4.5 months).

It is noteworthy that the positive control with MPTP followed different methodology, being administered intraperitoneally and not orally, because this is the standard methodology it is possible to observe changes in the striatum. The use of two different methods can damage the comparison and makes it difficult to access the efficiency of proposed methodology for paraquat, especially as the maximum concentration of paraquat in the brain was reached in 90 days (time that it reached the plateau) and was well below the observed following intraperitoneal administration of paraquat (Breckenridge et al., 2013). However, the accumulation was similar, namely, the half life of paraquat is about 20-22 days for both oral as for intraperitoneal administration. Thus, we conclude that at the intraperitoneal administration a greater dose is maintained in the brain. Therefore, the time required to verify the reduction in dopamine by oral administration would be even greater than what was proposed in articles that used intraperitoneal administration, since the reduction of dopamine and even neural degeneration would be slower.

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Therefore, the development of Parkinson after exposure to paraquat cannot be ruled out, and you can only conclude that, if it occurs, should be much slower than a usual subchronic study could access. In view of these findings, for oral administration, perhaps a chronic study is more appropriate to assess the depletion of dopamine, its metabolites and striatal neurons. So, despite a subchronic study reveal a similar exposure to exposure of the general population, it does not adequately reflect the actual occupational exposure in which both subchronic exposure as recurrent acute intoxication are relevant for farm workers who use paraquat and This complex combination of exposures may be related to increased incidence of Parkinson's disease observed in epidemiological studies.

In the study of Minnema and colleagues (2014), despite the long exposure time, there was no reduction in the levels of dopamine, except for one of its metabolites in females at the lowest dose. These authors consider that there was no reduction of dopaminergic neurons in the black substance, however, there is decrease of dopaminergic neurons in males at the highest dose (not statistically significant). This result alone would not really be very relevant, but adding it to all literature and other company's studies raises doubts as to the action of paraquat on dopaminergic neurons.

On June 29, 2015, the company filed the study reports that supported the publication of the above-described items (Breckenridge et al., 2013; Minema et al 2014.). The analysis of these studies was added to the sixteenth frame. Below are some important considerations about them:

a) Study No. 639 058:

There is a clear significant reduction of dopaminergic neurons in the black substance *pars compacta* compared to the negative control, both for the positive control (mice treated with MPTP), and to the group treated with the higher dose of paraquat (3 doses of 15 mg/kg/dose once a week for three weeks). The stereological analysis of brain injury immunomarked cuts for tyrosine hydroxylase revealed reduction of dopaminergic neurons, however there was no evidence of neuronal degeneration in the neuropathological analysis.

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In the statistically significant decrease in dopaminergic neurons and total neurons in the group that received the highest dose in the other groups treated with paraquat also occurred reduction of these parameters. Adding to this, the Nissl staining revealed the reduction of total neurons and although this reduction was not statistically significant, it corroborates the findings of stereological analysis of reduction of dopaminergic neurons.

Thus, the author of the study should not have considered the evidence of the highest dose promote reductions of dopaminergic neurons in the black substance is limited. He justified this position by the need to better understand the discrepancy between neuropathological and stereological findings, to confirm the reproducibility of the study and to evaluate more times between dosing and euthanasia, to rule out premature death hypothesis. Surely it is important to answer these questions raised by the author, however the results of this study show the potential of the paraquat to cause the death of dopaminergic neurons in the black substance.

The author explains the non-occurrence of statistical difference in image analysis for the high variability between animals, but this high deviation may also be due to the lower sensitivity of this technique in relation to stereological analysis. Furthermore, Nissl staining seems to underestimate the results, since the boundary area of the black substance *pars compacta* is smaller as well as the number of sampling sites and the thickness of the sections. Still, the study report itself, the counting of stained neurons is generally less accurate than the immunomarked, for the determination of the borders of the region tends to be less accurate. Thus, stained image analysis help but are not ideal for scanning death of dopaminergic neurons, with an immunostaining more accurate and, therefore, commonly used (Jackson-Lewis and Przedborski, 2007). Ascoli (2002) reports that Nissl staining showed no significant reduction of neurons in the black substance after exposure to 3,4,6 trihidroxiphenylalanine, but showed with the TH immunostaining and also concludes on the superiority of immunostaining to detect dopaminergic neurons.

Despite the statistical analysis of the results from imaging analysis have shown no significant difference, there was dopaminergic neurons reduction trend in the brain

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of animals treated with paraquat, confirming the positive result of stereological analysis and proving that the loss of neurons is real and not a limitation for the loss of immunoreactivity of the assay. In front of the above, the reduction of dopaminergic neurons in the treatments of paraquat appears to be consistent, as was also accompanied by a reduction of total neurons.

b) Study 639092:

Despite not having been statistically significant difference in most groups treated with paraquat compared to the negative control, we can see a pattern of reduction of dopaminergic neurons immunostained for TH in fluorescence analysis and chromogenic, including a trend towards significance in the group treated with three doses of 25 mg/kg of paraquat (10% reduction in dopaminergic neurons, p=0.0732). The same fact happened with regard to reducing the total neurons and the total contour volume after exposure to MPTP, and the study report itself concluded that the *p* value was very close to significance.

Apparently, the dose of 10 mg/kg was not sufficient to observe the action of paraquat on the black substance, therefore the observed decrease was very low. However, in the groups at three doses of 15 or 25 mg/kg, the action of paraquat was more evident; so that the evaluation of the total contour volume, there is reduction of neurons with a statistically significant difference compared to the negative control for these two groups and the positive control. According to the study report, it is not clear the relevance of these significant results of reduction in the total volume of contour in the groups exposed to paraquat; however, considering that there was a pattern of decrease of dopaminergic neurons in all parameters and groups analyzed, it is clear the relevance of these results.

While it is possible to observe a pattern of reduction of dopaminergic neurons in this study, the results were unclear probably by the low sensitivity of detection of the reduction of the evaluated parameters, even for the MPTP. This low sensitivity was observed in all studies of the dossier, including the validation testing techniques. Nevertheless, there are indications of changes in the nervous system, with possible reduction of dopaminergic neurons as observed pattern of reduction of positive neurons to TH. The positive control

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showed a similar pattern, preventing any conclusion that paraquat is not related to Parkinson's disease. Even if much of the data from this study shows no significant differences, they reveal a standard reduction compared to the negative control in all evaluated parameters in both chromogenic analysis, as in fluorescence, except for the total number of neurons in chromogenic analysis. Another pattern observed in the trial is the reduction of neurons between the groups with exposure to paraquat, which appears to be dose-dependent, even if there is no statistical difference. These reductions of dopaminergic neurons, while not statistically significant, are important, as the result of that test shall be added to the results of other company's own studies and literature in the evaluation of the evidence.

c) Study 639158:

Initially, it is worth to point out that this study carried out through oral exposure does not necessarily reflect the best route of exposure to paraquat, since the main concern is the workers who directly handle the pesticide, since there is evidence that the actual exposure of workers may exceed the AOEL. On the other hand, so far, the oral exposure of food consumption is not of great importance, because it is not expected to find residues of paraquat above the established in food. It is important to emphasize this aspect because it is not expected that oral administration is adequate to predict the long-term exposure of workers under current conditions of this active ingredient pesticide based uses. In stereologic analyzes, the results were not satisfactory even for positive control with MPTP; therefore, although there was a significant reduction in the number of neurons immunostained for TH, and the total contour of the black substance in animals exposed to MPTP, no significant reduction in the total number of neurons or all of these parameters in females. Thus, the subtle reduction observed in the positive control does not allows that the results of the treatments with paraquat in concentration of 50 ppm being disregarded. At this concentration there was slight no significant reduction in all parameters evaluated for males, and for the total volume contour, there was a reduction with a trend towards significance

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(p = 0.0615). For females, it was not observed reduction or total dopaminergic neurons, probably due to the lower sensitivity of this sex, it has been demonstrated in other studies in which the treatment was carried out with intraperitoneally route (Miller et al., 1998).

Although Miller and colleagues (1998) have observed reduction in the response of females, the reduction of dopaminergic neurons was well tipycal (45% reduction), not affecting the analyzes. However, in the dossier's study, there was not so prominent reduction even in males (10% maximum reduction). Therefore, the dose for females in the dossier's study was not enough to reach the MPTP and paraquat's neurotoxicity.

After detailed analysis of these studies submitted by the company it's possible to reaffirm that the test results with paraquat do not allow conclusions about the lack of action of these herbicides as trigger of dopaminergic neurons death; rather, they provide important evidence that paraquat acts on these neurons, triggering similar changes to those of Parkinson's disease. Yet in these studies is not clear paraquat action on dopamine and its metabolites. It is important to say that the sensitivity of the technique chosen for this analysis (HPLC-EC) was not the one with the best result in the validation tests. The EC-MS / MS technique was more sensitive in detecting the differences between the groups in the validation assay. In addition, as discussed above, the dose and the interval used to access the paraquat action in the evaluation period may be insufficient to realize the dopaminergic reduction.

Evidence of neurotoxicity of paraquat were also observed in chronic studies submitted to Anvisa (Table XVII): increase of cysts in the spinal cord, greater degeneration of sciatic nerve and cases of hydrocephalus in groups treated with paraquat. The direct correlation of these findings with Parkinson's disease may not be as well evident; however, as stated by the director of one of the studies, these effects may result from exposure to paraquat, showing, once again, the oxidative damage of this active ingredient.

Given all what has been said about the toxicity resulting from the release of ROS on the depletion of dopaminergic neurons and dopamine levels, it is clear that paraquat really acts in important brain areas and related to the development of Parkinson's disease, however, is not possible doubtless affirm that this pesticide can be an etiological agent in cases of Parkinson's disease in humans.

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This extrapolation would require best estimate of doses, use of more precise and resolute experimental designs and more know how on its mechanism of action. The doses in which effect was observed were high (250 times higher than the likely exposure of workers), even considering that this actual exposure exceeds 100 times the AOEL. On the other hand, knowledge about Parkinson is still scarce and cannot determine that a 100 safety factor could be used, as with other toxicological endpoints, especially because there are studies showing paraquat to remain in the encephalom of animals for one month after the end of treatment and there epidemiological studies showing that exposure doses may be responsible for increased Parkinson in field workers exposed mainly to paraquat (Prasad et al., 2007).

Epidemiological studies have associated exposure to paraquat to the development of Parkinson's disease, although some studies have limitations (confounding factors and evaluation methodology), much of it was well conducted and has consistent findings, so much so that a recent publication of the EFSA indicates paraquat association with the development of Parkinson (Ntzani et al., 2013). In this publication, Ntzani and colleagues (2013) conducted a systematic and extensive review of literature of epidemiological studies to analyze the association between exposure to pesticides and some diseases. Thirty studies were analyzed to assess the association between exposure to pesticides and Parkinson's diseaseusing meta-analysis. The association between occurrence of Parkinson's and exposure to paraquat was moderate and statistically significant heterogeneity. Therefore, the result of meta-analysis corroborate the countless studies with experimental animal models already published which demonstrated the effects of paraquat in the development of Parkinson's disease.

Epidemiological studies allow determining the real consequences of pesticides in the population and allow us to observe important effects that cannot be fully characterized in laboratory animal studies. Thus, the analyzes made by Ntzani et al (2013) corroborate the association between exposure to paraquat and the development of Parkinson's disease.

One must consider that the consequences of exposure to paraquat in humans are more important than animals, because Parkinson's is a serious, progressive disease, whose treatment only delays the symptoms but cannot reverse the situation, ie there is no cure.

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With the development of the disease, the person becomes disabled and with severe respiratory problems and motors until the occurrence of death. In face of the recognized severity of this disease to humans, it can be concluded that paraquat may prove more dangerous to humans than the tests with laboratory animals can demonstrate, and therefore the risk of developing Parkinson's disease unacceptable.

Given the above regarding the neurotoxicity of paraquat, the use of paraquat in Brazil should not be allowed because it can endanger the lives of pesticide handlers. This conclusion can be based on section II of Article 31 of Decree 4074 of January 4, 2002, which prohibits the registration of pesticides without effective treatment for their toxic effects, since there is no cure for Parkinson's, and clause VI Article 31, which prohibits the registration of pesticides which are most dangerous to humans than assays on laboratory animals can demonstrate.

Chart VI. Analysis of the neurotoxicity studies of the technical product dossiers of the active ingredient paraquat.

NEUROTOXICITY					
Assay	**Purity**	**Via**	**Exposure time and doses**	**Toxic effects**	**Assay limitations**
Subchronic Neurotoxicide in rats. R1322 – 2006	33,4%	VO	Time of exposure: 90 days Doses: 15, 50 and 150 ppm *This study was carried out in Rats Alpk:APfSD.*	In general, the landing foot-splay, tail removal and limb force measurement tests apparently did not present important variances correlated to ingestion of paraquat. Landing foot-splay test: there was a significant increase of the limb extension in the 14th week, at 50 ppm (at 150 ppm there was a slight increase). Tail removal test: there was a significant increase for males and females in the 2nd week, at dose of 50 ppm. Limb force test: significant increase in the 14th week at 15 ppm for females. Apparently, there was an increase at all doses in all weeks, in general. In the motor activity test, non-significant and non-dose-depend and increase in all locomotion of the rats exposed to paraquat is perceived (1st, 2nd and 9th weeks). In the 14th week, there is a reduction of the total number of movements for males and females. Degeneration of the tibial distal nerve, the proximal sciatic nerve and the tibial proximal nerve is observed at 150 ppm; however, it was not different from the control, which had similar number of cases. However, these alterations were not present in the groups, which received treatment at 15 ppm and 50 ppm. **NOAEL: 10.2 mg/kg/day.**	-
A dose Tolerability Study of Paraquat in Mice. Study n° WIL-639063 – 2010	39,9% (72.4% Paraquat Cation)	IP	Time of exposure: 3 weeks. Doses: 20, 25, 30 and 35 mg/kg/week. 5 animals/group	**35 mg/kg/week:** high mortality, severe clinical signs (curved posture, hypoactivity, defecation reduction, difficult breathing, cold body, among others), histological pulmonary (edema, inflammatory infiltration, endothelial hypertrophy), kidneys (tubular degeneration) and adrenal (vacuolization of the fasciculate zone) alterations considered related to the treatment. **Maximum tolerated dose:** 30 mg/kg	-

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Multi-time and multi-dose using paraquat in mice. Report n° WIL-639093 – 2012	99% or 72.4% Paraquat cation	IP	**Group 1 – negative control:** vehicle administered 1 time/ week, for 3 weeks. **Group 2:** 1 single dose 10 mg/kg paraquat. **Group 3:** 1 single dose 15 mg/kg paraquat. **Group 4:** 1 single dose 25 mg/kg paraquat. **Group 5:** 2 doses 10 mg/kg/dose paraquat, 1 time/ week, for 2 weeks. **Group 6:** 2 doses 15 mg/kg/dose paraquat, 1 time/ week, for 2 weeks. **Group 7:** 2 doses 25 mg/kg/dose paraquat, 1 time/ week, for 2 weeks. **Group 8:** 3 doses 10 mg/kg/dose paraquat, 1 time/ week, for 3 weeks. **Group 9:** 3 doses 15 mg/kg/dose paraquat, 1 time/ week, for 3 weeks. **Group 10:** 3 doses 25 mg/kg/dose paraquat, 1 time/ week, for 3 weeks. **Group 11 – positive control :** 4 doses 10 mg/kg/dose MPTP in a single day, at 2 h interval. 5 animals from each group were euthanized in the following times after the application of the last dose: 4, 8, 16, 24, 49, 96 and 168 hours. Thus, the maximum interval between the application of the last dose and the euthanasia was 7 days.	Two males were euthanized *in extremis* at the highest dose and at the positive control (cold body, curved posture and hypoactivity, with no alterations in the necropsy). Lack of clinical signs in the other treated animals. There was a reduction in the body weight of the animals after the treatment, but then the weight was reestablished. In the positive control group, hypoactivity was observed in one animal, reeling walk was observed in three animals and semi-opened eyelids in two. No alteration in the brain appearance in the treatments and in the positive control. The pathological analysis did not reveal brain damage in the animals treated with paraquat. In the positive control, damage was observed in the black substance and in the striatum (neuronal necrosis, glial reaction, reduction of tyrosine stain, hydroxylase and disintegration of the synaptic terminals).	Low frequency of exposure and in short period of time.
	99.9% Syngenta	IP	- Negative control :vehicle	**Clinical signs:**	According to the study director,

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Validation Study on MPTP or Paraquat in Mice. Study nº WIL-639028			administered once a week (for 3 weeks). - Treatment: 10 mg/kg/week paraquat (for 3 weeks) - Positive control: 10 mg/kg MPTP intraperitoneal (4 doses in one day = 40 mg/kg/day) - 20 animals/group	Weight reduction immediately after the dose administration, but there were no significant alterations in the total treatment. There was no clinical alteration in the animals treated with paraquat, however the animals treated with MPTP presented light to severe tremors, curved posture, flattened posterior limbs and *Straub* tail. **Neurochemical analyses by the LLC laboratory (LC-MS/MS):** - Positive control: reduction of dopamine, DOPAC, HVA and serotonin and increase in the dopamine *turnover*. - Treatment: slight, not significant reduction in the dopamine, DOPAC, HVA and serotonin concentration; slight increase in the dopamine *turnover* in mice exposed to paraquat. **Neurochemical analyses by the RTI laboratory (HPLC-EC):** - Positive control: significant reduction of dopamine, DOPAC, HVA and serotonin and significant increase in the *turnover*. - Treatment: the variance was less perceptible than that of the LLC laboratory; there was no reduction of dopamine and HVA and there was no increase of the *turnover*, but the increase of serotonin was significant. **Stereological analyses:** - Positive control: significant reduction by 41% of positive neurons for TH and 29% of total neurons. - Paraquat: no effect. **Image analyses:** - Significant reduction by 57% of positive neurons for TH in the animals exposed to MPTP. The study was able to validate the assays for the determination of MPTP and paraquat by HPLC/UV in aqueous solutions, considering that the formulations are stable and homogeneous for 7 days. The tested substances were not found in the vehicle. There was validation of the analyses of the samples from mice striatum regarding the concentration of DOPAC neurotransmitters and metabolites and the stereological and image analyses.	

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A Two-Week Sequential Necrosis/Pathology Study on Paraquat Administered by Intraperitoneal Injection in Mice – Study n° WIL-639046 – 2011	99.9% Syngenta	IP	**Group 1 – negative control:** received the vehicle on days 0 and 7. **Group 2:** received 10 mg/kg paraquat on days 0 and 7. **Group 3:** received 10 mg/kg paraquat on day 7. **Group 4 – Positive control:** received 4 doses of 10 mg/kg MPTP on day 7 (total 40 mg/kg). 25 animals/group were used, but each group was divided in subgroups of 5 animals: **Subgroup A:** euthanized on day 8 for pathological analysis. **Subgroup B:** euthanized on day 9 for pathological analysis. **Subgroup C:** euthanized on day 11 for pathological analysis. **Subgroup D:** euthanized on day 14 for pathological analysis. **Subgroup E:** euthanized on day 14 for serotonin, GABA, DOPA, DOPAC and HVA dosage in the left and the right *striatum* separately.	**Signs clinical:** - MPTP: tremors, hypoactivity, defecation reduction, Straub tail, hypothermia, walking on the tow tips, significant reduction of the body weight (12.7%) and the food consumption. - Groups 2 and 3: absence of clinical signs **Pathological analyses:** - MPTP: absence of alteration in the weight of the brain and in the brain macroscopic characteristics. Damage to the *substantia nigra* (neural necrosis, glial reactions, reduction of TH+ neurons) and to the *striatum* (glial reactions, disintegration of the synaptic terminals and reduction of TH+ neurons). The histological alterations described for MPTP were not so prominent, because a big part was classified as light (incidence lower than 1%), minimum (incidence lower than 5%) or soft (incidence from 5 to 14%). Moderate (incidence from 15 to 40%) or severe (incidence higher than 40%) classifications occurred mainly in the *substantia nigra* and in the *striatum*, but these were not always prevalent. - Groups 2 and 3: absence of histological alterations in the *striatum and* in the*substantia nigra*. **Neurochemical analyses:** RTI Laboratory (HPLC-EC): - MPTP: significant reduction in the concentration of dopamine (90%), DOPAC (80%) and HVA (69%). Increase of the DOPA *turnover* (280%). - Paraquat: there was no modification in the concentration of the neurotransmitters and the metabolites in relation to the control. Reduction of DOPAC (15% in group 2 and 17% in group 3). LLC Laboratory (LC-MS/MS): - MPTP: reduction significant of dopamine (98%), DOPAC (73%) and HVA (64%). Increase of the DOPA *turnover* (778%). - Paraquat: there was a reduction in the concentration of HVA, dopamine and serotonin in the striatum of the animals from group 3, with significant difference for the latter two. The reduction was not as prominent as the one observed for MPTP. In group 2, there was also reduction, but not significant. There was increase in the dopamine *turnover*, considered important in group 3 (81%) and slight increase (6%) in group 2, both not significant.	

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Three-Week Sequential Necrosis/Pathology Study of Paraquat Dichloride and MPTP in Mice – Study n° WIL-639058 – 2013	99.9% Syngenta	**Group 1 – Positive control:** received the vehicle once a week, for 3 weeks (30 animals). **Group 2:** 3 doses of 10 mg/kg/dose de paraquat, once a week (30 animals). **Group 3:** 3 doses of 15 mg/kg/dose, once a week (20 animals). **Group 4:** 2 doses of 15 mg/kg/dose de paraquat, once a week (20 animals). **Group 5:** 1 dose of 15 mg/kg/dose paraquat (20 animals). **Group 6:** 3 doses of 10 mg/kg/dose paraquat (Sigma- Aldrich), once a week (30 animals). IP **Group 7 – Positive control:** 4 doses of 10 mg/kg/dose MPTP on a single day, with 2h interval (total dose of 40 mg/kg, 20 animals). The groups were divided in subgroups of 5 (A, B, C and D) or 10 animals (E): **Subgroup A:** euthanized on day 15 for neuropathological analysis (groups 1, 2 and 6). **Subgroup B:** euthanized on day 16 for pathological analysis (all groups). **Subgroup C:** euthanized on day 18 for pathological analysis (groups 1, 2 and 6). **Subgroup D:** euthanized on day 21 for pathological analysis (all groups).	There was an increase in the concentration of paraquat in the brain of the animals with dosage increase, confirming the exposure of this tissue to the test substance. **Signs clinical:** - MPTP: after the second, third and fourth doses there were behavior alterations, tremors, Straub tail, hypoactivity, walking on toe tips, light material around the mouth and the urogenital area. - Paraquat: no relevant alterations. **Pathological analyses:** - MPTP: damage to the *substantia nigra* (neuronal necrosis, glial reaction, tyrosine reduction, reduction of the marking for tyrosine hydroxylase in the *substantia nigra* and *striatum*). The cellular death was not due to apoptosis, as evidence by the absence of marking for caspase 5 and TUNEL. The silver marking (indication of necrosis) was more consistent in subgroup B (16 days), although it was also present in the other euthanasia periods. The reduction of the marking with TH occurred only in the group treated with MPTP, especially in *pars compacta* of the *substantia nigra* and in the *striatum*. There was increase of the marking with GFAP and IBA-1. Although there was marking for the used stains, in general, it was not very prominent, considering that the marking was predominant in the *substantia nigra* and in the *striatum*. *In the* midbrain, the marking was considered minimum, compatible to that of the negative control. Subgroup B had more relevant marking in the *striatum* or in the *substantia nigra*, but it was not so prominent: - Increase of AmCuAg: marking classified as mild, minimum or moderate; - Reduction of TH+: marking in the *substantia nigra* classified as light or mild (4 animals out of 5); - Increase of GFAP: marking in the *substantia nigra* classified as minimum (2 animals out of 5) and in the *striatum* classified as moderate or severe (all 5 animals); - Increase of IBA-1: marking in the *substantia nigra* classified as minimum or mild (all 5 animals). In subgroup D, there was also certain marking for some parameters, but also less prominent: - Reduction of TH+: marking classified as moderate in the *substantia nigra* (4 animals out of 5); - GFAP: marking classified as minimum, mild or moderate (2/5) - IBA-1: marking classified as minimum or moderate (3/5).	

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Subgroup E: euthanized on day 21 for stereological analysis (all groups).				I the other subgroups, the findings were not relevant to indicate the effect from MPTP. - Paraquat: no neuropathological alteration was considered relevant, with marking not consistent and non-specified considered and not related to the treatment. The TH marking observed in *pars compacta* of the *substantia nigra* and the *striatum* was considered very different from MPTP and at levels similar to the negative control.	

Image and stereological analyses:

1) Immunomarking with TH:
- MPTP: reduction by 31% of TH+ neurons and 15% of total neurons ($p<0.05$).
- Paraquat: reduction of TH+ neurons (23%, $p<0.01$) and total neurons (15%, $p<0.05$) in group 3.

In group 4 there was also a slight by 7% of TH+ neurons and 2% total neurons, although not significant.
In group 6 there was also a reduction of TH+ neurons (14%) and total neurons (8%), although not significant.

2) CVO Staining:
- MPTP: there was no significant reduction in the total number of neurons stained with CVO. The reduction was 12%, i.e., similar to the reduction of TH+ neurons of the immunomarking (15%); however, due to the high deviation, there was no significant difference.
- Paraquat: there was no significant reduction in the total number of neurons stained with CVO, however, it was possible to observe a slight reduction in some groups:

Group 3: the reduction was very low, only 3%, and the deviation was low. Group 4: reduction by 8%.
Group 6: reduction by 6%.

3) Volume outline:
- MPTP: although without significant statistical difference, there was reduction by 32% of TH+ neurons.
- Paraquat: although without significant statistical difference, there was reduction by 15% of TH+ objects in group 3.

Remark: there were alterations in the optical nerve of some animals.

Statistical analysis carried out by consulting:
- In relation to the negative control, the only statistical differences were for reduction of TH+ and total neurons marking for groups 3 and 7.

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				Group 3: significant reduction of TH+ in relation to the negative control (p=0.0012). Group 6: TH+ reduction trend in relation to the control (p=0.0683). Group 7: reduction de TH+ significant in relation to the negative control (p=0.0174). Group 3: significant reduction of total neurons in relation to the negative control (p=0.0233). Group 6: non-significant reduction of total neurons in relation to the control, but which might be relevant (p=0.0972). Group 7: significant reduction of total neurons in relation to the negative control (p=0.0251). - For the statistical analyses of CVO staining, there was no significant difference for any group.	
Dose Ranging-Finding and method Comparison Stereology Study Using Paraquat Dichloride and MPTP in Mice – Study n° WIL 639092 –2012	99,9% Syngenta	IP	**Group 1 – Positive control:** received the vehicle once a week, for 3 weeks for stereological analysis (25 animals). **Group 2:** 3 doses of 10 mg/kg/dose paraquat, once a week for stereological analysis (20 animals). **Group 3:** 3 doses of 15 mg/kg/dose paraquat, once a week for stereological analysis (20 animals). **Group 4:** received 3 doses of 25 mg/kg/dose* paraquat, once a week for stereological analysis (20 animals). **Group 5 – Positive control:** 4 doses of 10 mg/kg/dose** of MPTP in a single day, with 2 h interval (total dose of 40 mg/kg, 20 animals). **Group 6 – negative control:** vehicle once a week, for 3 weeks for neurological analysis (10 animals).	**Signs clinical:** - MPTP: curved posture, hypoactivity, hypothermia, loss of body weight, reduction in the food consumption. - Paraquat: no relevant clinical signs were observed, only dose-dependent loss of weight and, consequently, less weight gain in the treatment as a whole (groups 3 and 4). The reduction in the food consumption occurred only in group 4. An animal that received 25 mg/kg/dose was found dead on the 7th day, with no signs of intoxication and its death was considered related to the treatment. **Neurochemical analyses:** - MPTP: significant reduction in the concentration of dopamine and dopaminergic metabolites, as well as increase in the dopamine *turnover*. - Paraquat: the concentration of dopamine, DOPAC, HVA and the dopamine *turnover* were not changed. There was a slight non-significant and probably non-representative reduction in the concentration of DOPAC. **Stereological analysis:** **Chromatogenic:** - MPTP: non-significant reduction of dopaminergic TH+ neurons in the substantia nigra, but there was a significant reduction of the total volume outline in relation to the negative control. - Paraquat: reduction of the TH+ neurons, however not significant. The reduction was by 5% (10 mg/kg/dose), 14% (15 mg/kg/dose) and 12% (25 mg/kg/dose).	The neurochemical analysis was carried out by HPLC-EC. Low frequency of exposure and in short period.

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<table>
<tr>
<td></td>
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<td></td>
<td>Group 7: 3 doses of 15 mg/kg/dose paraquat, once a week for neurochemical analysis (10 animals).
Group 8: 3 doses of 25 mg/kg/dose paraquat, once a week for neurochemical analysis (10 animals).
Group 9 – Positive control: 4 doses of 10 mg/kg/dose de MPTP on a single day, with 2h interval, for neurochemical analysis (total dose of 40 mg/kg, 10 animals).

* According to the author, the dose of 25 mg/kg paraquat would be the maximum tolerated dose.

** According to the author, this would be the maximum tolerated dose with no need of additional care for the animals.</td>
<td>It shall be pointed out that there was a significant reduction in the total volume outline in groups 3 and 4, when compared to the negative control. According to the study report, the relevance of this data is not clear.

Fluorescence:
- MPTP: significant reduction of TH+ dopaminergic neurons in the substantia nigra, but there was no significant reduction in the total volume outline.
There was non-significant reduction in the total neurons (14%) and in the total volume outline (11%), as well. Although non-significant, the reduction of the total neurons and the total volume outline for administration of MPTP, the proper study report concluded that the p valor was very close to the significance.
- Paraquat: reduction in the percentage of TH+, total neurons and the total

volume outline in all groups, however, this was not significant. Remark: there

were alterations in the optical nerve of some animals.
<table>
<tr><th></th><th colspan="3">CHROMATOGENY</th><th colspan="3">FLUORESCENCE</th></tr>
<tr><th></th><th>TH+</th><th>TOTAL</th><th>CVT</th><th>TH+</th><th>TOTAL</th><th>CVT</th></tr>
<tr><td>G1</td><td>↓14%
p=0.0621</td><td>↓7%
p=0.2342</td><td>↓17%
p=0.0047</td><td>↓18%
p=0.0457</td><td>↓14%
p=0.0781</td><td>↓11%
p=0.0766</td></tr>
<tr><td>G2</td><td>↓5%
p=0.2842</td><td>↑7%
p=0.8271</td><td>↓5%
p=0.1754</td><td>↓2%
p=0.4090</td><td>↓5%
p=0.2903</td><td>↓2%
p=0.3919</td></tr>
<tr><td>G3</td><td>↓14%
p=0.1089</td><td>↑7%
p=0.8050</td><td>↓10%
p=0.0435</td><td>↓12%
p=0.1216</td><td>↓9%
p=0.1578</td><td>↓6%
p=0.2613</td></tr>
<tr><td>G4</td><td>↓12%
p=0.0732</td><td>↓6%
p=0.2028</td><td>↓10%
p=0.0439</td><td>↓13%
p=0.1064</td><td>↓8%
p=0.1539</td><td>↓6%
p=0.2346</td></tr>
</table>
G1-4: groups 1 to 4.
TH+: neurons positive for TH+ (dopaminergic)
Total: total neurons
CVT: Total volume outline</td>
<td></td>
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<tr>
<td>Subchronic (91-day) Dietary Study to Assess the Effects of Paraquat Dichloride on Dopaminergic Neurons in</td>
<td>99,9%</td>
<td>VO</td>
<td>Group 1: negative control. Group 2: 10 ppm paraquat in the food, equivalent to the ingestion of 1.7 mg/kg/day for male and 2.7 mg/kg/day for female.
Group 3: 50 ppm paraquat in the food, equivalent to the ingestion of 10.2 mg/kg/day for male and 15.6 mg/kg/day for female.</td>
<td>Three males from group 2 and one male from group 3 died during the study, but these deaths were not considered related to the treatment. One female from group 3 was euthanized in extremis, presenting curved posture, defecation reduction and weight reduction, and these symptoms were not considered related to the paraquat.

Clinical signs:
- MPTP: curved posture, hypoactivity, tremors, piloerection and body weight loss.</td>
<td>The neurochemical analyses neurochemical were carried out with HPLC-EC.

The study report mentions the reduction of the sensitivity in the detection of alterations in the substantia nigra pars compacta. In the neuropathological analyses, it was difficult to conclude about the</td>
</tr>
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<table>
<tr>
<td>C57BL/6J mice – Study n° WIL 639158 –2013</td>
<td></td>
<td></td>
<td>Group 4: 4 doses of 10 mg/kg/dose (intraperitoneal) MPTP in a single day, with 2h interval.</td>
<td>- Paraquat: no relevant clinical signs were noted during the observation period. There was reduction of the weight gain only in the first week and in group 3.

Neurochemical analyses:
- MPTP: significant reduction of dopamine, DOPAC and HVA in males. In females, there was reduction of these parameters, but it was not significant. In males, there was also significant increase of the dopamine turnover, a parameter, which was not altered in females.
- Paraquat: non-significant reduction of DOPAC in males and females from group 3, considering that for the females, this reduction was compatible to that observed for MPTP. In addition, reduction significant (p<0.01) of HVA was observed in group 2. No increase in the dopamine turnover was observed.

Stereological analyses:

<table>
<tr><th></th><th colspan="3">Male</th><th colspan="3">Female</th></tr>
<tr><th></th><th>TH+</th><th>Total</th><th>CVT</th><th>TH+</th><th>Total</th><th>CVT</th></tr>
<tr><td>Group2</td><td>↓10%
P=0.0317</td><td>↓6%</td><td>↓9%</td><td>↓5%</td><td>↓3%</td><td>↓3%</td></tr>
<tr><td>Group 3</td><td>NR</td><td>NR</td><td>NR↓</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>Group 4</td><td>↓2%</td><td>↓3%</td><td>↓4%
P=0.0615</td><td>NR</td><td>NR</td><td>↓2%</td></tr>
<tr><td colspan="7">TH+: neurons positive for TH+ (dopaminergic)
Total: total neurons
CVT: Total volume outline
NR: Groups, in which there was no reduction in the evaluated parameter.</td></tr>
</table>
Neuropathological analyses:
-MPTP: reduction of TH+ neurons in the striatum (in males and females) and in the substantia nigra pars compacta (only in males), but the classification rate was mild to minimum. Increase in the GFAP and IBA-1 marking was also observed in the striatum, but not in the substantia nigra.
- Paraquat: no relevant alterations were observed, only some isolated cases.

Remark: there were alterations in the optical nerve of one animal.</td>
<td>reduction of TH+ neurons in the substantia nigra pars compacta, because certain marking agglomeration shall be observed for a more accurate interpretation.

According to the study director, the staining with AmCuAg was impaired due to the long period (7 days) between the exposure of the animals to MPTP and the euthanasia.</td>
</tr>
<tr>
<td colspan="6">All assays were carried out with mice C57BL/6J
TH: Tyrosine hydroxylase
CVO: Cresyl violet well accepted for this kind of analysis. IP: Intraperitoneal
VO: Oral administration</td>
</tr>
</table>

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Chart VII. Relevant toxicological aspects of the studies of the technical product dossiers of the active ingredient Paraquat.

Product	Remarks
PARAQUAT TECHNICAL SYNGENTA 25351.370669/2005-20	**Sub-acute toxicity sub-acute in dogs (1 years):** fibroses and respiratory failure were observed. **NOEL:** 15 ppm **Toxicity sub-acute in rats Fischer-CD:** alveolar epithelial hypertrophy and increase of staining in the spleen. **NOEL**: 6.57 mg/kg/day **Chronic Toxicity Study Result – 104 Week Dosing Study in Mouse - CTL/C/1871A:** **Doses** / **0 ppm** / **10 ppm (1.31 mg/kg/day) (3.82 mg/kg/day)** / **30 ppm** / **100 ppm (13.03 - mg/kg/day) (0.26 mg/kg/day)** Female / - / - / - / - / ↓ erythrocytes, hematocrit, hemoglobin, leukocytes and lymphocytes ↓ total protein 26 weeks Male / - / - / - / ↓ adrenal weight (total and relative) / ↓ erythrocytes, hematocrit and leukocytes. ↓ total protein ↓ adrenal weight and thyroid (total and relative) ↑ lung weight (total and relative) ↓ erythrocytes and hemoglobin Female / - / - / - / - / ↓ GOT activity and alkaline phosphatase ↑slight of the blood sugar , with no relation dose-response 52 weeks ↓ total protein Male / - / - / - / - / ↓ erythrocytes, hematocrit and leukocytes ↑ heart weight (total) ↓ weight of thyroid, liver and bladder (total) ↑ kidney weight (relative) ↓ erythrocytes and hemoglobin Female / - / - / - / - / ↓ total protein ↓ weight brain (total) ↑ light of blood sugar with dose-response relation 104 weeks ↓ erythrocytes, hematocrit and leukocytes Male / - / - / - / - / ↓ total protein ↑ slight of the blood sugar , with dose-response relation Several histopathological lesions in several organs. None attributed to the treatment. **Other information** Male showed high incidence of adenocarcinoma, leukemia and hepatic adenoma and female - leukemia and adenocarcinoma. With no significant difference in relation to the control and with no dose-response relation. **NOAEL:** 30 ppm (3.82 mg kg day)

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Chronic study in rats F344:

Doses		25 ppm	75 ppm	150 ppm
113/ 122 weeks	Female	-	Dilation of the fourth ventricle of the brain Proliferative lesions, probably non-oncogenic (difficult classification), of the alveolar epithelium Lenticular cataract	↓ liver weight Dilation do fourth ventricle do brain Proliferative lesions, probably non-oncogenic (difficult classification), of the alveolar epithelium Lenticular cataract
	Male	-	Proliferative lesions, probably non-oncogenic (difficult classification), of the alveolar epithelium Light degeneration in fibers of the sciatic nerve Lenticular cataract	↓ liver weight ↓ testicular weight Proliferative lesions, probably non-oncogenic (difficult classification), of the alveolar epithelium Light degeneration in fibers of the sciatic nerve Lenticular cataract
Other information	**NOAEL:** 1.25 mg/kg/day *Assuming that 1 ppm = 0.05 mg/kg/day (USEPA)			

Lifetime feeding study in mouse Report n° CTL/P/556 – 1981:

Doses		12.5 ppm	37.5 ppm	100/125 ppm
99 weeks	Female	-	-	Alterations in the kidneys (hydropic degeneration and increase of the eosinophilia) Pulmonary lesions
	Male	-	-	Alterations in the kidneys (hydropic degeneration and increase da eosinophilia) Pulmonary lesions
Other information	**NOAEL:** 1.87 mg/kg/day			

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Paraquat: Multigeneration reproduction study in rats – Two generations Study n° CTL/P/649:

	0 ppm	25 ppm (1.25 mg/kg/day)	75 ppm (3.75 mg/kg/day)	150 ppm (7.5 mg/kg/day)
Parental generation (F0 and F1) Females	-	↓ in the body weight gain in relation to the control, without statistical difference . ↑ slight of focal alveolar histiocytosis in relation to the control.	↑ dose-dependent of focal alveolar histiocytosis. ↓ in the body weight gain in relation to the control, without statistical difference. One death due to paraquat pursuant to severe pulmonary alterations and urinary inflammation.	↑ dose-dependent of focal alveolar histiocytosis. Severe pulmonary alterations pursuant to the administration of paraquat. Death of 5 animals F0 and 13/13 F1 during or after the birth of offspring A and B (majority). Thirteen deaths due to pulmonary alterations pursuant to the administration of paraquat before the final observation period, considering that 38% of these cases also presented increase of siderophages in the spleen (probably indicating recycling of erythrocytes as response to the alterations in the oxygen tension in the blood pursuant to the severe dyspnea). There was 26.7% deaths in generation F0 and 43.3% in generation F1. ↓ in the body weight gain in relation to the control, without statistical difference.
Parental generation (F0 and F1) Males	-	-	↑ dose-dependent of focal alveolar histiocytosis. ↓ in the body weight gain in relation to the control, without statistical difference.	↑ dose-dependent of focal alveolar histiocytosis. Two deaths during the treatment. Small ↑ of light prostatitis. One animal with hydrocephaly, hypoplasia and testicular hyposecretion.
Offspring (F1A, F1B, F2A, F2B)	-	↓ in the body weight gain in relation to the control, with and without statistical difference, depending on the group.	↓ in the body weight gain in relation to the control, with and without statistical difference, depending on the group.	Light perivascular inflammation in the lungs with possible toxicological relevance. ↓ in the body weight gain in relation to the control, with and without statistical difference, depending on the group.
Other information	**NOAEL general:** 1.25 mg/kg/day **NOAEL reproductive:** 7.5 mg/kg/day Other non-dose-dependent findings: 1) Urinary inflammation (one animal in each treatment). 2) Pelvic dilation in the offspring. 3) Few cases of the seminiferous tubules atrophy , only in the treated offspring (at all doses).			

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Paraquat three generation study in the rat CTL/P/719S:

	0 ppm	25 ppm (1.25 mg/kg/day)	75 ppm (3.75 mg/kg/day)	150 ppm (7.5 mg/kg/day)
Parental generation (F0, F1 and F2) Females	Renal, pulmonary and hepatic alterations, in general, mild.	Pulmonary histopathological alterations. Moderate renal histopathological alterations. Increase of siderophages in the red pulp of the spleen (1 animal).	Pulmonary histopathological alterations. Moderate to severe renal histopathological alterations. Increase of siderophages in the red pulp of the spleen (2 animals).	Severe pulmonary histopathological alterations, most characterized as pursuant to paraquat. Severe renal histopathological alterations. Increase of siderophages in the red pulp of the spleen (6 animals). Histopathological alterations in the liver. Increased death of animals, pursuant mainly to pulmonary alterations (27-43%) (USEPA).
Parental generation (F0, F1 and F2) Males	Renal, pulmonary and hepatic alterations, in general, mild.	Pulmonary histopathological alterations. Moderate renal histopathological alterations. Increase of siderophages in the red pulp of the spleen (1).	Pulmonary histopathological alterations. Moderate to severe renal histopathological alterations.	Severe pulmonary histopathological alterations, most characterized as pursuant to paraquat. Severe renal histopathological alterations. Histopathological alterations in the heart. Alterations in the cornea.
Offspring (F1A, F1B, F2A, F2B, F3A, F3B)	Renal, pulmonary and hepatic alterations, in general, mild.	Hydrocephaly (1 animal).	Moderate pulmonary histopathological alterations.	Moderate pulmonary histopathological alterations. Alterations in the cornea. Hydrocephaly (1 animal).
Other information	**NOAEL general:** 1.25 mg/kg/day **NOAEL reproductive:** 7.5 mg/kg/day *Assuming that 1 ppm = 0.05 mg/kg/day (USEPA) Other non-dose-dependent findings: 4) Histopathological alterations in the liver, with high incidence in the control, as well. 5) Alterations in the heart, such as, interstitial lymphocyte infiltration in the heart and myocarditis, with high incidence in the control, as well. 6) Few cases of testicular immaturity, mainly in the treated animals (75 ppm and 150 ppm). 7) Few cases of testicular atrophy, mainly in the treated animals (25 ppm, 75 ppm and, mainly, 150 ppm). 8) Few cases of testicular degeneration, but only in the treated animals (25 ppm and, mainly, 150 ppm). 9) Low incidence of alterations in the cornea at 25 ppm dose. 10) Low incidence of increase of hematopoiesis in the spleen and, although it has also been observed in the control animals, it was more prevailing in the treated animals (25 ppm, 75 ppm and 150 ppm). 11) Low incidence of prostatitis and, although it has also been observed in the control animals, it was more prevailing in the treated animals (25 ppm, 75 ppm and 150 ppm).			

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		12) Many cases of infertility, either in the control animals or in the treated ones. 13) Corpus luteal cyst observed in the ovaries of animals treated, but with low incidence (25, 75 and 150 ppm).		
	Combined Toxicity and Carcinogenicity Study in Rats. LSR Report n° 82/ILY217/328 – 1983:			
	Doses		**25 ppm (2.9 – 0.8 mg/kg/day)**	**75 ppm (8.5 – 2.4 mg/kg/day)**	**150 ppm (16.8 – 4.8 mg/kg/day)**
PARAQUAT TECHNICAL ICI/ZENECA 25000.012491/95-61	113/ 122 weeks	**Females**	↑ Lenticular lesions (significant as of 119 weeks) with progression to cataract. Histopathological alterations pursuant to the toxicity of paraquat in the liver and in the lung. ↑ Incidence of cysts in the spinal cord (the study report says that it might be related to the treatment). ↓ Reduction of the body weight ↓ ALT and AST	↑Lenticular lesions (significant as of 103 weeks) with progression to cataract. Secondary eye lesions: glaucoma, hemorrhage, uveitis. Compatible histopathological alterations pursuant to the toxicity of paraquat in the liver and in the lung. Significant pulmonary alterations. Hydrocephaly, dilation of the 4th ventricle (significant and the study report says that it might be related to the treatment). ↓ Reduction of the body weight ↑ Glucose up to the 40th week. ↑ light of the parafollicular and diffuse hyperplasia in the thyroid. ↓ ALT and AST	↑Lenticular lesions (significant as of 103 weeks) with progression to cataract. Secondary eye lesions: glaucoma, hemorrhage, uveitis. Histopathological alterations compatible pursuant to the toxicity of paraquat in the liver and in the lung. Significant pulmonary alterations. ↑ Glucose up to the 40th week. ↑ Relative weight of the brain ↑ Incidence of cysts in the spinal cord (the study report says that it might be related to the treatment) Hydrocephaly, dilation of the 4th ventricle (significant and the study report says that it might be related to the treatment) ↓ Reduction of the body weight ↓ Transitory in the erythrocytes parameters. ↓ Total count of leukocytes, with statistical difference, but which was considered normal for the species. ↑ Partially activated thromboplastin time (light) ↓ Platelet count (light). ↑ Incidence of ovarian cysts. Uterine distension area. ↑ Light of the parafollicular and diffuse hyperplasia in the thyroid. ↓ ALT and AST

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		Males	↑ Lenticular lesions (significant as of 110 weeks) with progression to cataract. Histopathological alterations pursuant to the toxicity of paraquat in the liver and in the lung. ↑ incidence of cysts in the spinal cord. ↓ Reduction of the body weight ↑ Cellular debris in the epididymis lumen (non-significant). ↑ light adrenal lipid vacuolization. ↑ light of the parafollicular and diffuse hyperplasia in the thyroid. ↑ incidence of fibroma on the skin (without difference significant) ↓ ALT and AST	↑Lenticular lesions (significant as of 103 weeks) with progression to cataract. Compatible histopathological alterations pursuant to the toxicity of paraquat in the liver and in the lung. ↓ Reduction of the body weight ↑ Degeneration of the sciatic nerve. ↑ Incidence of cysts in the spinal cord. ↑ Glucose up to the 40th week. ↑ Cellular debris in the epididymis lumen (non-significant). ↑ incidence of fibroma on the skin (without difference significant) ↓ ALT and AST	↑Lenticular lesions (significant as of 103 weeks) with progression to cataract. Glaucoma, hemorrhage, uveitis. Histopathological alterations compatible pursuant to the toxicity in the liver, lung and kidneys. Pulmonary alterations significant. ↑ Partially activated thromboplastin time (light) and ↓ Platelet count (light). Renal cysts. Big, prominent and congested cervical lymphonodes. Swollen spleens. Increased and hemorrhagic pituitaries. ↑ Relative weight of the brain ↑ Incidence of cysts in the spinal cord. ↑ Glucose up to the 40th week. ↓ of the epididymis lumen content (without statistical difference). ↑ Cellular debris in the epididymis lumen (significant) Reduced luminal secretion in the epididymis (at the end of the treatment). Thickening of the tunica albuginea. Atrophied testicles (5/33), which did not occur in any of the 60 control animals. ↑ Degeneration of the sciatic nerve ↑ of the testicles. ↑ Sub-capsular plates in the testicle. Reduced epididymis. ↑ incidence of fibroma on the skin (with difference significant – 7/35) ↑ incidence of fibroma on the skin (with difference significant – 4/26) ↓ ALT and AST ↓ Reduction of the body weight
	Other information	**NOAEL:** < 25 ppm (2.9 – 0.8 mg/kg/day)			

Paraquat Dichloride: Teratogenicity Study in the rat Report n° CTL/P/365 – 1978:

Doses		**1.0 mg/kg/day**	**5.0 mg/kg/day**	**10.0 mg/kg/day**
Exposure: From the 6th to the 15th day. Females euthanized on the 21st day of pregnancy	**Female**		Toxicity to lungs, kidneys and liver in some animals. Hyperplasia of myeloid elements in the spleen of one animal. Focal degeneration of the sciatic nerve in one female.	Toxicity to lungs, kidneys and liver in most animals.
Other information	**NOAEL:** 5 mg/kg/day			

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Thus, the development of methodology and analyses still falls back on these aspects, i.e., it shall be expected there to be few reliable studies of endocrine disruption to assess other parameters, as in the case with paraquat, whose weak evidence point out to a deregulation associated to metabolic disorders. Without a substantial weight of the evidence about the endocrine disruption, it is not yet possible to confirm or discard such effect. Thus, it would be necessary to carry out specific studies to classify paraquat in the registration-prohibition category pursuant to its endocrine deregulation potential or not. Without such studies, it is impossible to determine what the risk of paraquat to the exposed population is.

III – CONCLUSION

Considering the arguments presented in the technical note for reevaluation of paraquat by Fiocruz, the bibliographic review and the analysis of the dossiers reported by the companies holding registrations of paraquat-based products and considering the determinations in the Brazilian legislation, it was concluded that:

Considering the lack of substantial evidence and the gaps left by the studies already carried out with paraquat, it is not possible to affirm that this active ingredient has endocrine distruption, carcinogenesis, reproductive toxicity and teratogenesis potential. Thus, the weigth of evidence is not sufficient to prove these toxic effects according to the motivations for the paraquat prohibition determined by the Brazilian legislation.

Without a substantial weight of the evidence about these aspects, it is still not possible to confirm them or discard them, or to determine what the risk of exposure to paraquat for the population is. Thus, deeper information will be necessary and even specific studies to classify paraquat in the registration-prohibition category pursuant to its endocrine disruption, teratogenesis, carcinogenesis or reproductive toxicity potential.

Paraquat has high acute oral toxicity and important dermal toxicity; acute exposure to this pesticide leads to appearance of pulmonary fibrosis, which is irreversible and fatal in most cases. Although these findings are found in assays with animals, at the time of registration of technical products, the tests on animals were not able to reveal the actual hazard from the exposure to paraquat, i.e., high lethality after accidental and occupational exposure.

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The high acute toxicity is even more relevant for this pesticide because the use of personal protection equipment does not guarantee full protection against intoxication by paraquat and the real level of exposure of the workers has exceeded the established AOELs.

The absence of antidote or efficient treatment for pesticide with so high mortality rate as paraquat is worrying. There are reports of some isolated cases of full recovery after intoxication with this pesticide; however, there are not broadly accepted protocols for the intoxicated patients´ treatment. Consequently, patients with severe intoxication do not have any real changes of recovery and the treatment is only palliative, considering that there is no antidote or efficient treatment for acute exposure to paraquat, and this is the reason for the prohibition, as provided in Law No. 7.802, dated June 1989.

Added to the high acute toxicity of paraquat, there is sufficient evidence about the mutagenic potential of paraquat and although other international agencies do not classify paraquat as mutagenic, according to the Brazilian legislation, paraquat fits the concept of mutagenicity defined by Ordinance No. 03 dated 1992, because it has registration-impeding characteristics:

A pesticide is considered mutagenic when there is scientific evidence of induction of mutations based on validated data, observed in at least two tests: one of them to determine genic mutations and the other one to detect chromosomal mutations. These proofs shall be performed without or with metabolic activation when necessary and using a Positive control in parallel.

The evidences of development of the Parkinson´s disease by paraquat are quite consistent and reveal unacceptable risk to the human being. The use of paraquat might place the life of the workers who use it at risk. It is necessary to consider that the consequences from the exposure of human beings to paraquat are more relevant than in animals, because the Parkinson´s disease is a severe, progressive disease, whose treatment only delays the symptoms, but which is not able to revert the condition.

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With the development of the disease, the person becomes disabled and with severe breathing and mobility problems until death. Considering the recognized severity of this disease for human beings, it is possible to conclude that paraquat may reveal to be more hazardous to human beings than the demonstrated in the laboratory animal tests and that there is no antidote or efficient treatment for this disease; these two aspects are included in the registration -prohibition motivation.

Considering the high acute toxicity of paraquat, the fact that AOEL has been exceeded during the application, the evidence of development of Parkinson´s disease, the absence of antidote for acute intoxication and for the Parkinson´s disease and the evidence of mutagenicity, it is recognized that there is a legal support for the prohibition of the registration of paraquat-based products in Brazil and thus, the use of this active ingredient shall be interrupted in our Country.

Considering the exposed, I suggest canceling the registers of products based on the active ingredient paraquat.

This is the opinion for superior consideration.

Brasília, September 01, 2015.

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