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Expert

Terrence Collins

Exhibit G

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CONFIDENTIAL

REPORT

For

Korein Tillery, LLC
505 North 7th Street, Suite 3600
St. Louis, MO 63101

By

Dr. Terrence J. Collins

I Introduction

My name is Terry Collins. I am the Teresa Heinz Professor of Green Chemistry and the Director of the Institute for Green Science (IGS)[1] at Carnegie Mellon University in Pittsburgh, Pennsylvania. I teach the scientific, cultural and sustainability dimensions of unsustainable chemistry and the logic of how we can build a sustainable chemical enterprise.

My full CV can be found in Appendix 1.

My work on this case is pro bono.

I have been asked to address the role of paraquat (PQ) in the chemistry of oxidation-reduction (redox) reactions in cells and tissues and the relationships to Parkinson's Disease (PD). My explanation of redox chemistry will show why the adage "the dose makes the poison" does not apply to chronic, low dose exposure to PQ.

I will focus in this report on what I know about the fundamental chemical properties of PQ and I will integrate this knowledge into what is known about the chemical mechanistic relationships between PQ exposures and PD.

II Relationship my Research Program and Oxidative Stress

II.1 Reactive Oxygen Species (ROS) are central to both the causation of Parkinson's Disease and to my four-decade long research program

I will now highlight connections between my research focus and the underlying chemistry of PD. For four decades, I have been working to understand and gain design control over oxidation catalysis which, for the purposes of this case, can be restated as a pilgrimage to gain control through catalyst design over the chemical reactivity of notoriously uncontrollable Reactive Oxygen Species (ROS; ROS can be singular or plural). ROS are produced as essential components of aerobic life. In cells, oxidative stress results from an imbalance between ROS production and antioxidant defense mechanisms that quench surplus ROS that cells do not need for healthy functioning. Some antioxidant therapies show promise in the treatment of PD [1]. The lives of some members of this Court are likely be threatened at some stage or other by these imbalances by which ROS produces a multitude of pathologies related to oxidative stress such as vascular remodeling, where atherosclerosis is one form [2].

In the large multidisciplinary[2] IGS research program I direct, we focus on the design of small-molecule catalysts [3] to *safely* [4] [5] deploy the natural oxidants, oxygen and hydrogen peroxide, in processes that are both valuable and promoting of sustainability [6] [7] [8]. The technologies we have developed are based on what I have called TAML® activators (the terms "activator" and

[1] https://www.cmu.edu/igs/

[2] https://www.cmu.edu/igs/people/index.html

"catalyst" are interchangeable) which are the most effective hydrogen peroxide activating catalysts known. TAML® is a registered trademark to Carnegie Mellon University in the US and EU. I am the principal inventor of TAML® catalysts that were designed to control and avoid the formation of various ROS by confining the mechanism of hydrogen peroxide oxidations to the efficient catalytic cycle of the peroxidase enzymes [9]. Many of the ROS species we have learned how to avoid in peroxide oxidations are the same chemical species that produce the oxidative stress that PQ amplifies in the SNpc to accelerate the death of dopaminergic neurons [10]. The ability of TAML® catalysts to efficiently control [11] and direct the chemistry of ROS is unprecedented across both chemistry and biology [4]. Intellectual property based on TAML® catalysts is the basis of a new CMU-spinoff company Sudoc, LLC.[3]

Thus, I have spent much of my waking time over decades thinking about oxidation processes and in particular about the chemistry of ROS that I will report upon herein as to its bearing in this case.

II.2 The importance of iron in my design program and in Parkinson's Disease

TAML® catalysts are peroxidase mimics—they activate hydrogen peroxide via a related mechanism to that of the peroxidase enzymes. Iron is the key element in both the peroxidase enzymes and our miniaturized TAML® replicas—it is the atom in both the catalyst molecules and the enzymes where the hydrogen peroxide binds to become activated and where all the principal direct catalytic events take place [6][12]. Iron is widely recognized as facilitating oxidative injuries to dopaminergic neurons in the SNpc where catalytic activation of local hydrogen peroxide plays a role among other processes. [13] [14]

But there are major differences between iron atoms in TAML® activators and the simpler iron compounds that are abundant in food, water and the environment to provide sources for iron in the SNpc. The macrocyclic ligands of TAML® activators place iron atoms in highly electron-donating molecular straightjackets to accomplish stable confinement of the iron in an electronic environment that effectively directs the activation of hydrogen peroxide toward efficient peroxidase-like catalytic cycles where the reactive intermediates have oxygen atoms bonded to iron.

In more simple iron compounds, the molecular groups attached to the iron fall on and off easily and the compounds typically activate hydrogen peroxide via Fenton processes.[4] These iron-catalyzed processes produce the familiar ROS reactive intermediates of oxidative stress that, unlike the TAML® reactive intermediates, are not attached to iron. In the PQ-PD literature, in cases where the evidence suggests that iron is involved in ROS generating processes the processes are typically referred to as "Fenton" chemistry. By using my design protocol [3], we have learned how to escape the highly reactive but inefficient Fenton pathways that typically dominate the interactions

[3] www.sudoc.com

[4] Wikipedia gives an adequately informative introduction to Fenton chemistry https://en.wikipedia.org/wiki/Fenton%27s_reagent

between iron and hydrogen peroxide to direct the catalysis to proceed around a peroxidase enzyme-like highly efficient catalytic cycle instead. [12]

Have you ever used hydrogen peroxide to disinfect a wound? If you have, you will remember that you see bubbles while it is working and white skin and flesh when you rinse it off. If you leave the peroxide on for too long, it will start really hurting. The bubbles are oxygen produced via multiple ROS pathways including iron accelerated processes. The bubbles are a signal of Fenton Chemistry's inefficiency—hydrogen peroxide is made from oxygen and hydrogen and processes that send it back to oxygen are inherently inefficient. The white skin and flesh results from ROS attack at organic pigments bleaching the treated areas. The pain results from direct ROS and/or indirect ROS-induced chemical attack at nerves. When you apply peroxide to a wound, what you experience results from a marked increase over the background ROS flux in your tissues. PQ accelerates this type of chemistry such that a tiny amount over a prolonged period has the ability to increase the ROS flux causing damage in the SNpc over what would be present without it.

In the mechanistic Section V, I will focus not only on iron-mediated ROS lethality to dopaminergic neurons, but also on a process known as free radical autoxidation [15] that does not require an iron catalyst, although it may be augmented by such. This distinctive feature of ROS chemical reactivity also amplifies the hazardous nature of PQ toward the SNpc and deserves further explanation.

II.3 ROS connects the thinking in my catalyst design program to the literature of oxidative stress in Parkinson's disease

Our achievements have all been built upon a foundation of insight, acquired over decades, into all the various reactivities available to hydrogen peroxide and other oxidants, including in the ROS class, that are also major chemical actors in the PD-induced degradation of dopaminergic neurons in the SNpc. Lost integrity of SNpc dopaminergic neurons is the clearest pathological evidence of much of the rigidity and bradykinesia that provide significant morbidity in PD [16]. Today, the success my team has achieved in marshalling by catalyst design hydrogen peroxide catalysis away from typical ROS means that many existing chemical oxidation technologies that are practiced in ways that are foreign to natural chemistry (e.g. chlorine for water disinfection) may soon be replaceable with alternatives that are safer, in major part because they can effectively and efficiently deploy the same reagents that Nature uses for the targeted purposes, although often via a different mechanism (e.g. hydrogen peroxide for disinfection via a peroxidase-like mechanism) [17]. In contrast, immune cells employ ROS mechanisms in phagocytic processes [18]. Implicit in this achievement is the fact that our iron catalysts activate hydrogen peroxide in ways that avoid the radicals in the ROS class that are especially damaging to cells, superoxide ion (O_2^-) and the hydroperoxyl ($HO_2\cdot$, protonated superoxide) as intermediate species to extremely aggressive hydroxyl ($HO\cdot$) and alkoxyl ($RO\cdot$)[5] radicals.

PD is known to be difficult to study from an epidemiological perspective. Important exposures may occur many years before diagnosis and many individuals with early or mild disease may die from other causes before motor symptoms manifest, obscuring risk factor associations [10]. Nevertheless, PD typically develops in older humans with age being by far the strongest risk factor

[5] Where R is a carbon group.

[19]. In a recent review applying a multifactorial analysis to other risk factors [20], it was assessed that chronic low-dose exposure to PQ-inducing oxidative stress and consequent metabolic processes of excitotoxicity, α-synuclein aggregate formation, autophagy, alteration of dopamine catabolism, and inactivation of tyrosine hydroxylase conspire to cause the loss of dopaminergic cells that characterizes PD. This was presented as being consistent with (i) observations that repeated PQ exposures of different animal models can induce loss of dopaminergic neurons in the nigrostriatal dopamine system, (ii) epidemiologically revealed risk factors including living in areas where PQ exposure is likely to occur, and (iii) exposure to PQ during normal application procedures. It is noteworthy that various of the plaintiffs in this case have acknowledged repeatedly over years de-clogging with their bare hands the PQ herbicide delivery nozzles of the boom dispensers they were using to distribute large amounts of PQ in the fields of their farms. In my evaluation, this multifactorial paradigm is also entirely consistent with very low-level PQ exposures of the SNpc leading to PD because of the nature of the chemistry of PQ.

As I will explain in Section V, in an appropriate medium such as the lipid component of the cellular membrane, ROS radical fluxes augmented by PQ need no participation of iron to produce long chain reactions. This equates with one PQ molecule doing an immense amount of damage to the membrane each time it undergoes a single redox cycle. Again, this does not mean that iron cannot promote the ROS flux, simply that in suitable cellular media, iron is unnecessary. The terminology used by many authors in the PD literature that captures this specific type of chemical reactivity, among other things, is "lipid peroxidation" [1][13][14][20][21][22][23][24][25]. This component of what PQ-PD researchers call lipid peroxidation is called 'free radical autoxidation' by chemists and I believe it to be destructive of the phospholipid bilayers of cell membranes and vesicles in PQ-induced oxidative stress of SNpc cells. For example, as I will explain in Section V, dopamine rich synaptic vesicles in dopaminergic neurons show high susceptibility to oxidative stress and should be expected to be especially vulnerable to free radical autoxidative damage; aberrant tracking of dopamine rich synaptic vesicles has been speculated to be a factor in PD neurodegeneration [26].

III Discussion of "the dose makes the poison."

As is by now well-known in this case, in 1538, Paracelsus expressed the idea that "All things are poison, and nothing is without poison; Just the dose makes sure that a thing is not poison."[6] This is generally condensed to 'the dose makes the poison' which Defense experts have alleged is "the fundamental principle in toxicology for assessment of safety for all substances." My explanation of the chemical properties and redox chemistry of PQ (Sections IV, V) will show why "the dose makes the poison" and the corollary that it is possible to identify a safe dose for all substances defies common sense in the case of PQ.

[6] Google translation of, Paracelsus, Die dritte Defension wegen des Schreibens der neuen Rezepte, http://www.zeno.org/Philosophie/M/Paracelsus/Septem+Defensiones/Die+dritte+Defension+wegen+des+Schreibens+der+neuen+Rezepte

In the context of the acute or chronic toxicity of PQ as an etiology of PD, I do not challenge the alleged fundamental principle of toxicology that 'the dose makes the poison.' Based on its mechanism of action, more PQ in the SNpc creates an obviously worse situation than less.

I do challenge the concept that there is a knowable nontoxic dose of PQ in humans for chronic toxicity determinable by risk assessment because the inherent chemical properties of PQ mean that every PQ molecule can produce a large and continuous flux of ROS over a very long time. Among the mammalian species studied in the PQ-PD literature, including, inter alia, mice, rats, dogs, monkeys, humans, humans live the longest and therefore following exposure are subject to the longest onslaught of the fire-like ROS fluxes produce by PQ molecules (fire is oxidation chemistry supported by ROS plasmas). This is another way of asserting that, based on the fundamental chemistry of PQ, the lowest PQ chronic dose in the SNpc that is capable of killing dopaminergic neurons is immeasurably low and not assessable. Each PQ molecule will create a large flux of ROS on its arrival in the SNpc, and that flux will continue for as long as the PQ molecule remains in the SNpc—the residence time factor is discussed in Section IV.2. Moreover, the severity of ROS related toxicity will obviously depend on the sensitivity of the cells being impacted where it has become widely accepted that dopaminergic neurons in the SNpc are particularly vulnerable. [19]

This brings me to introduce a critical theme going forward that I will reflect on in more detail in Section VI. From the scientifically known chemical characteristics of PQ in the 1960s, I believe the defendants knew or should have known of the potential for health calamities with PQ such that its introduction into the market should never have happened. Moreover, as time went by, Syngenta became more and more aware of the powers of PQ to destroy dopaminergic neurons.

IV The Chemical Properties of PQ

IV.1 The long half-life of PQ

One important chemical characteristic of PQ is that it is persistent [27]. This means that PQ is to a significant degree resistant to all forms of destructive metabolism, including in the brain, and that this resistance conveys a long half-life to a PQ molecule located within a living organism. The half-life ($t_{1/2}$) is one common metric for measuring the duration of a chemical process, such as the disappearance of PQ from the SNpc starting from an initial measured concentration. The $t_{1/2}$ in this context is the time taken for the PQ concentration to fall to one half from its initial value when the time course started to be measured. Each half-life starts afresh at the end of the prior half-life time-

Table 1. Mathematical manifestation of the half-life ($t_{1/2}$)

Number of half-lives elapsed	Fraction remaining	Percentage remaining
0	$1/1$	100
1	$1/2$	50
2	$1/4$	25
3	$1/8$	12.5
4	$1/16$	6.25
5	$1/32$	3.125
6	$1/64$	1.5625
7	$1/128$	0.78125
...	...	...
n	$1/2^n$	$100/2^n$

block at the concentration present when the new half-life time block begins. The $t_{1/2}$ is a constant; it neither lengthens nor shortens as the value of the initial concentration changes—see Table 1.[7]

Table 1 shows how the theory of $t_{1/2}$ plays out in the real-life scenario of PQ in the SNpc. To illustrate how it works, the Table informs us that after five half-lives, 3.125% (I will round to 3%) of the starting amount of PQ will still remain in the SNpc. For an environmental contaminant with a relatively short $t_{1/2}$, say one day, 3% of the initial burden will still be present after five days. For an environmental contaminant with a moderate $t_{1/2}$, say one month, 3% of the initial burden will still be present after five months. And for an environmental contaminant with a long $t_{1/2}$, say 1 year, 3% of the initial burden will still be present after five years. If you can measure a concentration of PQ in any tissue, such as the SNpc, and you know to a reasonable degree of certainty the value of the $t_{1/2}$ in that tissue, then you also know how much PQ is still present at any time going forward from the measurement, even if the concentration has fallen below the limit of detection of whatever technique you used to detect the initial concentration of PQ.

The concept of the half-life ($t_{1/2}$) is crucial to this case, because whenever a chemical substance is both persistent and toxic, as with PQ, the $t_{1/2}$ value is always there to remind everyone of how slowly PQ may leave the brain. So quite naturally, this brings us to ask the question of what are the experimentally determined half-lives of PQ in the midbrain? There are no measurements of PQ half-life in the human brain, but we can look at data from rodents and non-human primates (NHP). In black mice administered a single 10 mg/kg dose of paraquat, Prasad and colleagues found a PQ half-life of about 28 days in the ventral midbrain [27]. This means that 0.3 mg (3%) was still present in the brains of the black mouse subjects after 140 days (0.38 years). An internal Syngenta study measured PQ in the brains of squirrel monkeys.[8] The monkeys had been treated with PQ (2.5 mg/kg weekly for 6 weeks) and then sacrificed at 2, 4, and 6 weeks after dosing. The levels of PQ found in the frontal cortex did not decrease even at 8 weeks after treatment. That means the $t_{1/2}$ in NHP brains is more than 8 weeks. In turn, this means that after 40 weeks (0.77 years), more, almost certainly much more, than 3% of the initial quantity measured in the frontal cortex was still present. Because NHP are closely related to humans genetically, we can surmise that PQ's half-life in humans is similar.

While the actual amount present at the end of each half-life depends on the initial quantity, a residual of 3% after five half-lives might convey the impression of being insignificant, regardless of the starting concentration. Here's why one should always be concerned about knowing that any amount of PQ is present in the SNpc. For a compound with PQ's dramatic oxidative chemical reactivity (Section V), after five half-lives even 3% of any initial

Inset 1

Impaired autophagy and lysosomal dysfunction play important roles in PD pathogenesis. PD causal mutations, including LRRK2 G2019S, impair autophagy *(the natural, regulated mechanism of the cell that removes unnecessary or dysfunctional components)*, and dysfunction is observed in PD brain and in genetic and toxicant animal models. Synuclein aggregates are cleared by chaperone-mediated autophagy and lysosomal degradation, and high levels of aggregates and damaged mitochondria can overwhelm the degradatory machinery, leading to a self-perpetuating cycle. (Goldman, 2014; italicized clause added.)

[7] https://en.wikipedia.org/wiki/Half-life

[8] Ray WJ. Analysis of brain samples from paraquat-exposed squirrel monkeys for residues of paraquat: final report. 2011 SYNG-PQ-00044965

concentration that was enough to be initially detected will always matter with respect to ongoing injuries to dopaminergic neurons. In the latter stages of PQ-stimulated ROS destruction of the dopaminergic neurons of the SNpc, these later half-life quantities could make all the difference in wiping out enough dopaminergic neurons to throw the midbrain irreversibly toward PD. Multiple related processes are implicated in PD pathogenesis, including mitochondrial dysfunction, oxidative and nitrative stress, microglial activation and inflammation, proteasomal impairment, aggregation of α-synuclein protein, and impaired autophagy [10]. ROS is an instigator of each of these pathologies. In serial exposures over decades, as in the cases of the plaintiffs, earlier exposures to PQ-induced ROS fluxes can be reasonably predicted to have increased mitochondrial dysfunction, amplified oxidative and nitrative stress, stimulated microglial activation and inflammation, impaired proteasomes, increased α-synuclein aggregation, crippled dopaminergic neuronal cleanup mechanisms (autophagy) and reduced the numbers of functioning dopaminergic neurons in the SNpc, leaving fewer neurons to absorb the amplified catalytic redox activity (explained below) and these many consequences at the beginning of the next PQ exposure (see Inset 1).

The plaintiffs were typically exposed to PQ multiple times over multiple years. This half-life process plays out within each of the multiyear exposure periods and also from one multiyear block of time to the next. Therefore, PQ from a previous exposure was very likely to still be present in the brains of the plaintiffs at the point of re-exposure.

When the functioning dopaminergic neuron count drops toward PD onset and another PQ exposure occurs, the much larger quantities of PQ present in the early half-lives of that final dose can be logically predicted to impact the vitality and count of remaining dopaminergic neurons, with the lower amounts of PQ still present in the later $t_{1/2}$ periods enacting ongoing damage. In this repeated cycle of exposure, years and even decades of PQ-augmented ROS injuries sooner or later can be reasonably expected to produce enough cell damage to hasten the onset of the hallmark characteristics of PD.

PQ possesses the ability to set off a complex suite of interacting chemical processes that are destructive to organic matter. As noted above, the complexity of this multi-functional reactivity of PQ is very well-documented in the literature of PD, which in itself represents a treasure trove of insight into this very reactivity. Each molecule features a dramatic ability to inflict localized oxidative stress on living cells. This oxidative stress is especially hazardous to any oxidatively sensitive tissue or aggregate of similar cells that form a definite kind of structural material with a specific function, such as the aggregate of primarily dopaminergic neurons, other cells and the intercellular substance of the SNpc tissue. Moreover, PQ's chemical reactivity is vigorously destructive of oxidatively sensitive cell products such as dopamine.

To bring better clarity to the especially hazardous nature of PQ, I will now group toxic substances into three separate classes.

IV.2 Stoichiometric vs. Catalytic vs. Amplified Catalytic Toxicants

The most common kind of toxicant is a stoichiometric toxicant where, as I am using the term here, "stoichiometric" means that a single molecule engages in a small number of toxic reactions,

usually a single reaction, and the term "toxicant" means a toxic chemical substance. An example of a toxicant behaving in a stoichiometric fashion is the inactivation by lead of the enzyme δ-aminolevulinic acid dehydratase in which lead displaces zinc in the active site to cripple this key enzyme in the heme synthetic pathway [28] while leading to a buildup of its native substrate δ-aminolevulinic acid, a neurotoxin. A stoichiometric toxicant produces an effect at a set ratio such that one molecule of the toxicant can produce a discrete number of toxic molecular events and that's it. Typically, a toxicant produces a 1 to 1 ratio for molecules of toxicant and toxic events produced.

But PQ is not a stoichiometric toxicant; *PQ is a catalytic toxicant.* In contrast to a stoichiometric toxicant, a single catalytic toxicant molecule can serially produce numerous injurious chemical events. For example, ricin is a catalytic toxicant. An amount as small as a grain of table salt can be sufficient to kill an adult if it is ingested, inhaled, or injected. Once in the target cell, a single ricin molecule can inactivate more than 1500 ribosomes per minute ultimately resulting in cell death.[9] Catalytic toxicants have the potential to be far more deadly than stoichiometric toxicants.

But PQ is not only a catalytic toxicant; *PQ is an amplified catalytic toxicant.* If a stoichiometric toxicant is like a single shot rifle, a catalytic toxicant is like a machine gun that can fire many rounds per minute, and an amplified catalytic toxicant is like a machine gun using bullets which are explosive rounds. PQ is an amplified catalytic toxicant because each catalytic redox cycle produces two superoxide ions and some percentage of these can set off the chain reaction processes of free radical autoxidation in an appropriate cellular medium.

In their review, Zhang et al. (2016) highlight work of the USEPA (Inset 2) reporting PQ administration and the following of its trajectory along with metabolites, that accumulated in the tissues of farm animals (cows, goats, pigs, and poultry) and rats. Although a large percentage of PQ is excreted through the feces and some metabolism occurs, PQ residues were found to accumulate in the animal tissues [20]. Because PQ is known to cross the blood-brain barrier [29], it is reasonable to conclude that PQ in the blood cannot be safe as it would lead to PQ in the brain and therefore to PQ in the SNpc.

Inset 2

The United States Environmental Protection Agency reported on a number of experiments on administering PQ and following the trajectory along with secondary compounds, which accumulated in the tissues of farm animals (cows, goats, pigs, and poultry) and rats. Although a large percentage of PQ is excreted through the feces, PQ residues accumulate in the animal tissues. [Locke KK. Toxicological significance of the following metabolites of paraquat: monoquat, QINA, monopyridone, dipyridone, and methylamine. United States Environmental Protection Agency: 1988 https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/061601/061601-1988-09-02a.pdf]

V Mechanisms of PQ Induced Injuries to Dopaminergic Neurons

I have relied on multiple sources and reviews that touch on mechanistic aspects of the different injurious processes that can lead to PD. A sampling of these highlight that oxidative stress is a unifying theme linking all such processes [1][10][13][14][19][20][21][22][23][24][25]. Since

[9] https://natoxag.ku.dk/toxin-of-the-week/ricin/

most PQ oxidative stress processes are well understood, I will focus in my mechanistic considerations on Free Radical Autoxidation (FRA) which seems unduly underrepresented in the mechanistic literature of the connections between PQ and PD. Based on the chemical composition of the lipid bilayer of cellular membranes and organelles and on what is known in synthetic and reaction chemistry about FRA, I believe that FRA is a major mechanistic component of lipid peroxidation induced by PQ creating a major oxidative stressor of dopaminergic neurons. I would expect that Syngenta, as a chemical company, should have deep knowledge of FRA.

V.1 Free radical autoxidation originating from superoxide generated by PQ catalytic redox cycling is expected to amplify the destruction of dopaminergic neurons in the SNpc

Autoxidation refers to oxidations brought about by oxygen at normal temperatures without the intervention of a flame or electric spark. The radical mechanisms of autoxidation have been extensively studied from the 1940s on, especially by Cheves Walling (1916–2007) and should be known to any scientist of ordinary skill in the art of ROS.

I will employ the common practice in chemistry of symbolizing a carbon–hydrogen (C–H) bond in any organic molecule as R–H, where R is a group attached through carbon to a hydrogen atom (H), usually an alkyl carbon group, in discussing cellular compounds relevant to the PQ-PD connection. I begin this section by noting that free radical autoxidation (FRA) (Figure 1) is a useful synthetic procedure for the conversion of liquid or gaseous hydrocarbons to alkylhydroperoxides (ROOH)[15]. In a typical synthetic procedure, one adds an organic initiator to a liquid or gaseous hydrocarbon R–H in the presence of oxygen, and then causes the initiator molecule to split into radicals, for example by heating modestly. As represented in Figure 1, typical FRA initiators decompose easily near room temperature to produce radicals that either directly or indirectly lead to abstraction of a hydrogen atom (H·) from R–H with formation of a carbon-centered radical (R·). Typically, the resulting R· radicals combine with molecular oxygen rapidly, sometimes at rates approaching diffusion control, to produce alkylperoxyl radicals (RO_2·). RO_2· radicals tend to be relatively stable members of the ROS family because the O–H bond of ROO–H that would result from their abstracting a hydrogen atom from R–H is typically weaker than the R–H bonds of the hydrocarbons that serve as the principal sources of hydrogen atoms.

However, when RO_2· meets another of its kind, or any of a variety of other radicals formed in the ongoing process, unstable nonradical species can be produced that upon degradation produce exceptionally reactive ROS species where the O–H bond that follows upon further abstraction is stronger than the R–H bonds that the synthetic chemist is targeting for oxidative transformation. For example (Figure 1), when two RO_2· radicals meet, the tetraoxide ROOOOR can form. RO_4R once formed fleetingly decomposes to release oxygen (O_2) and produce two alkoxyl radicals (RO·). Typically, these two RO· radicals may (i) combine to form a stable dialkylperoxide compound (ROOR), in effect terminating chains launched by two initiator radicals, (ii) react with each other to undergo decomposition forming nonradical products and therefore also terminating

the two chains or, (iii) escape each other's vicinity and, because alcohol RO–H bonds are typically stronger than alkane R–H bonds, react with more R–H to propagate the chain.

In a typical clean FRA synthesis, such as the conversion of the substrate (the compound on which the chemist will perform a reaction) cumene to cumene hydroperoxide or the substrate 2-methylpropane to tertiary-butyl hydroperoxide (see Figure 2), the reaction is carried out to low conversion, because in each case, one of the R–H bonds (highlighted in red in Figure 2) is considerably weaker than the others and the selectivity for its transformation to give the desired alkyl hydroperoxide is high. However, as the reaction proceeds, these bonds are removed from the medium and the frequency with which reactive ROS radicals encounter the targeted bonds is reduced. ROS then start attacking other R–H bonds in the substrate molecule leading to a mixture of products. Hence, obtaining high purity products requires that the processes be set up to stop at low conversions.

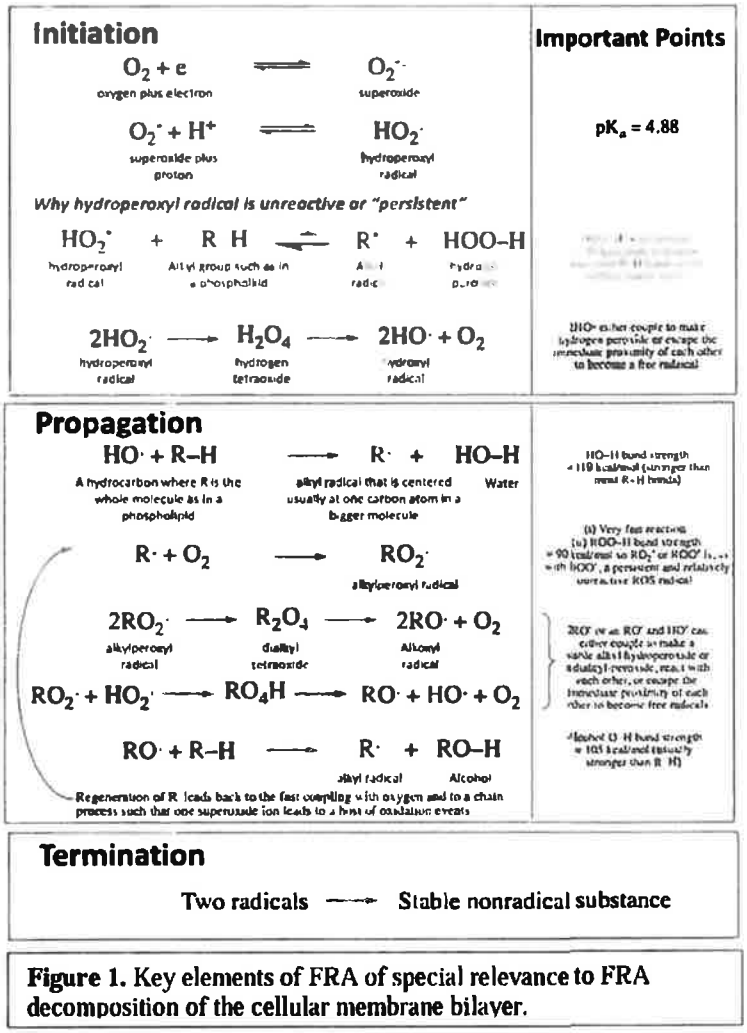


Figure 1. Key elements of FRA of special relevance to FRA decomposition of the cellular membrane bilayer.

This type of FRA reactivity is of particular significance to the PQ induction of PD via amplification of PQ's already catalytic induction of oxidative stress. Free radical autoxidation is fast chemistry typically proceeding with facility near room temperature, but it is hard to control unless ideal circumstances can be set by a synthetic chemist such as in the two syntheses I have described. In both these cases, all the ROS radicals "see" upon their formation is a sea of R–H bonds where their reactions with the targeted bond are much faster than their reactions with other R–H bonds. As noted, when they don't see enough of the targeted bond, they attack other R–H bonds.

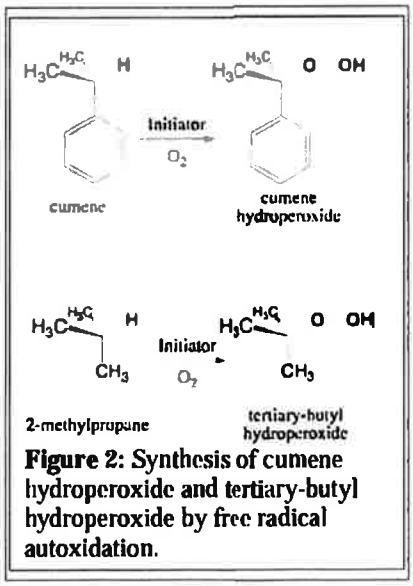


Figure 2: Synthesis of cumene hydroperoxide and tertiary-butyl hydroperoxide by free radical autoxidation.

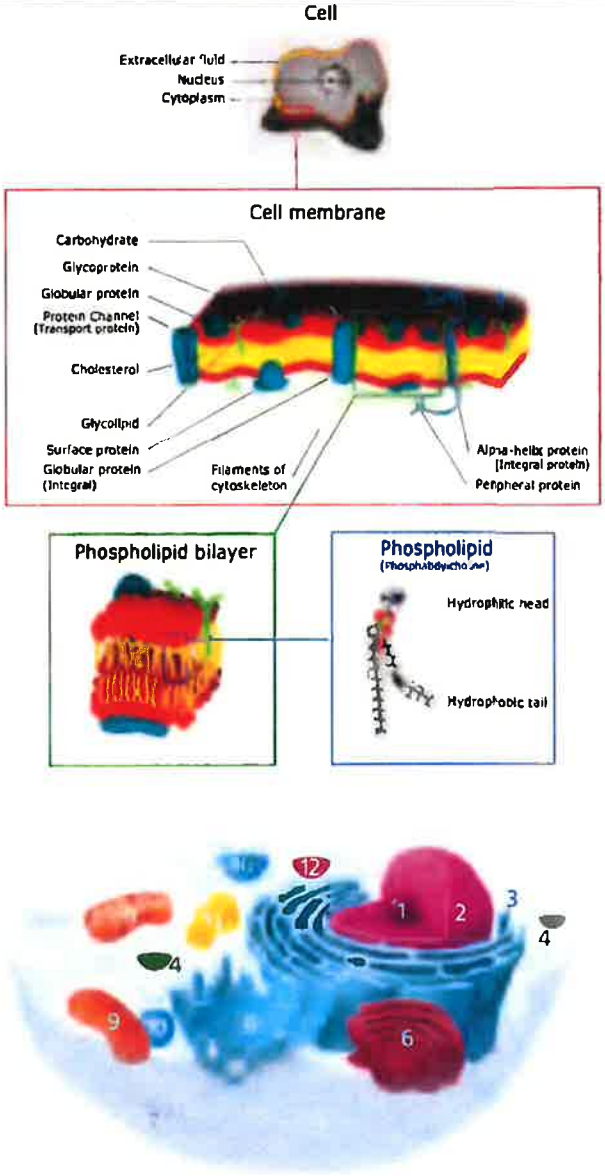
I have described these rather simple FRA syntheses to connect with compartments of dopaminergic neurons of the SNpc that approximate a liquid hydrocarbon, namely, the lipid bilayers of the cell membrane and endomembrane organelles such as the nucleolus, nucleus, ribosomes, vesicles, endoplasmic reticulum, Golgi apparatus, mitochondria, vacuoles, lysosomes, and centrosomes (Figure 3, bottom).

Figure 3 (top) presents readily available diagrams showing basic components of the cell membrane. The yellow sheet, the lipid bilayer, is the liquid hydrocarbon-like environment. The hydrophobic ("water hating") tails of the lipids are hydrocarbon chains. In the bilayer, the long chains adhere to each other through copious weak interactions known as Van der Waals forces. The phospholipid head groups pack tightly on the outer and inner surfaces of the lipid bilayer to present a hydrophilic (water loving) structure to the media of the external environment and the cytosol that are mostly comprised of water (see Inset 3). The lipid bilayer membrane is asymmetric in nature, i.e. its outer leaf (exterior facing) and inner leaf (interior facing) have different compositions of lipids, fatty acids and proteins.

Inset 3

Most of the cytosol is water, which makes up about 70% of the total volume of a typical cell. The pH of the intracellular fluid is 7.4, while human cytosolic pH ranges between 7.0–7.4, and is usually higher if a cell is growing.

https://en.wikipedia.org/wiki/Cytosol



1. Nucleolus
2. Nucleus
3. Ribosome
4. Vesicle
5. Rough endoplasmic reticulum
6. Golgi Apparatus
7. Cytoskeleton
8. Smooth endoplasmic reticulum
9. Mitochondrion
10. Vacuole
11. Cytosol
12. Lysosome
13. Centrosome
14. Cell membrane

Figure 3. Diagrams explaining basic features of cell membranes (top) and a typical animal cell components (bottom)
https://upload.wikimedia.org/wikipedia/commons/3/3a/Cell_membrane_detailed_diagram_4.svg
https://upload.wikimedia.org/wikipedia/commons/1/11/Animal_Cell.svg

Significantly for the PQ connection with PD, the inner membrane leaf is more negatively charged than the outer leaf because of a higher density of negatively charged phosphatidyl serine where the

outer leaf is comprised primarily of neutral phosphatidylcholines resulting in a surface potential that attracts and binds positively charged ions, proteins, and peptide motifs [30]. Thus, it can be confidently deduced that the double positive charge of the small resting PQ ion will have a very high affinity for sticking to the surface of the membrane inner leaflet. Various organelles have distinctive lipid compositions, but in all cases the oily inner spaces of the membranes represent media that is conducive to supporting the chain processes of FRA.

Inset 4

Oxygen enters cells by passing through the cell membrane in a process called diffusion, which is a transport process that does not require energy.

https://www.reference.com/science/oxygen-enter-cells-4b3275db9f63cf8f

This affinity of PQ for inner membrane surfaces means that it will produce superoxide via catalytic redox cycling in close proximity to the oily lipid space of the membrane interior. While negatively charged superoxide will have little attraction for the lipid space, its protonated form, the hydroperoxyl radical $HO_2\cdot$, is neutral and will have a much higher affinity for this medium. Hydrogen superoxide (the hydroperoxl radical) is expected to be of very low concentration in the cytosol because the acid dissociation constant (pK_a) of $HO_2\cdot$ is 4.88 and the human cytosolic pH varies from 7.0-7.4. However, the rate at which O_2^- can acquire a proton is extremely fast such that as the occasional $HO_2\cdot$ penetrates into the inner membrane space to be removed from its equilibrium with O_2^- in the cytosol, the equilibrium will adjust almost instantaneously to reproduce the lost $HO_2\cdot$. Once sufficient $HO_2\cdot$ is in the hydrocarbon inner space, FRA will begin in concert with homeostatic oxygen present in this space (see Inset 4).

As noted above, the occurrence of compartments of the dopaminergic neurons that are especially vulnerable to FRA is great, and all can be expected to experience this degrading chemistry to some degree when PQ increases the O_2^- concentration beyond the coping mechanisms of the cell. It is reasonable to assume that the PQ localization mechanism I just described and believe to be inescapable is actually a way in which the O_2^- produced by PQ can hide from the cellular defense mechanisms.

V.2 Dopamine containing synaptic vesicles are especially vulnerable to FRA

However, not all organelles of dopaminergic neurons maintain near neutral pH conditions. Synaptic vesicles for dopamine have a homeostatic pH of 5.6, where the low pH is presumed to provide protection from oxidative attack for dopamine [31]. The compartmentalization of dopamine has been suggested to be a protective mechanism that isolates dopamine from oxidation and other processes in the extravesicular media of the synapse [16]. However, at this pH, significant concentrations of $HO_2\cdot$ are continuously present inside the vesicles suggesting that these organelles should have extreme sensitivity to PQ's catalytic induction of oxidative stress that is then turbocharged by FRA in the acidic environment. Abeyawardhane and Lucas (2019) have reviewed iron redox chemistry and its implications in the PD brain and have detailed the oxidative transformation chemistry of tyrosine via L-dopa to dopamine and beyond, attributing the processes to augmentation by iron catalysis [14]. As shown in Figure 4, dopamine metabolism in the synapse leads to cascades producing toxic compounds tied to the development of pathologic indicators of PD. As noted, both the intravesicular medium and membranes of low pH dopamine rich vesicles are highly likely to be singularly subject to aggressive FRA initiated catalytically by PQ.

Thus PQ, a catalyst for turning oxygen into superoxide, can be toxic to dopaminergic neurons over time even if only infinitesimal concentrations are present in the SNpc. Through catalysis, each paraquat molecule produces untold numbers of superoxide ions. Then, each pair of protonated superoxide ions can initiate free radical chain processes as depicted in Figure 1. and cause untold numbers of sequential oxidative events to create oxidative stress that may overcome cellular repair mechanisms and lead to cellular death. With this picture in mind, it is easy to see that the defense experts' opinions that a tiny amount of PQ in the SNpc is harmless is a fantasy. PD symptoms typically present in later life [32], a fact that is entirely consistent with exposed humans suffering chronic damage to the dopaminergic nervous system over time from even infinitesimal contamination in the SNpc by PQ. And for these reasons, traditional dose-response analyses are inappropriate methods for assessing the toxicity of paraquat. Thus, catalytic redox cycling amplified by FRA and impacted by other mechanistic pathways such as Fenton processes together produce a continuously evolving stream of highly reactive ROS that attack and destroy the molecules of dopaminergic neurons.

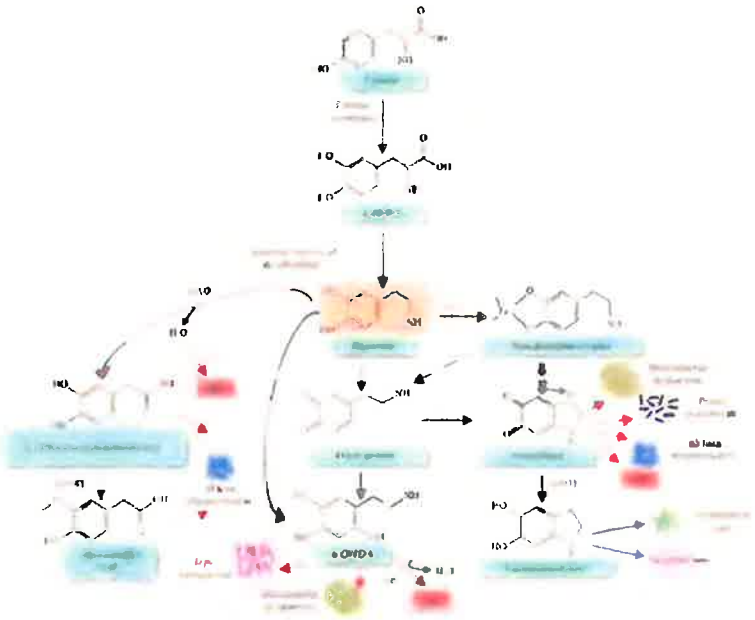


Figure 4. Schematic representation of dopamine metabolism in the synapse and toxic cascades associated with iron—from Abeyawardhane and Lucas 2019

V.3 The Knowable Known Case against Employing Dose-Response Testing to Identify the Connections between PQ and PD in Humans

What does the behavior of a chemical company look like when, (i) there is clear evidence that PD, a condition inevitably associated with a serious decline in the quality of life and premature death in the afflicted, is mostly caused by environmental and not genetic factors [10] and, (ii) there is strong evidence from the known chemical characteristics of PQ that it is fully capable of causing neurotoxicity in those who use it? I will conclude with an evaluation of what Defendants either knew or certainly should have known about the very likely probability that PQ would induce neurotoxicity (PD) in exposed humans. As discussed above, once inside tissues, PQ stays around for a very long time. And it is resistant to the destructive and removal biochemistry of the tissues where Nature's most potent weapons for destroying toxic compounds are typically found in cytochrome P450 and peroxidase oxidation enzymes such as we have mimicked. PQ is known to

engage in "redox cycling" (a form of catalysis) within tissues in the presence of resident oxygen and electrons supplied from the biological medium (e.g. by NADPH) to produce a continuous stream of superoxide ions. Once superoxide is produced. this rather unreactive molecule may then engage in various processes to induce tissue damage, significantly by acting as a source of the extremely reactive hydroxyl radical.

The redox properties of PQ have been known and discussed since the 1930s [33] and were certainly known to Syngenta and its predecessors when they first patented paraquat as an herbicide. In a remarkable streak of irony in this case, many of the cell damaging reactions initiated by PQ were written about in 1982 [34] and 1984 [21] by the Defense witness, James S. Bus, where a catalytic route, with PQ as the catalyst, to the hydroxyl radical is clearly postulated (Figure 5). Thus, the Defendants' expert knew enough by 1982 to understand that PQ is not a stoichiometric poison where one molecule does its damage and ceases to act. Rather, PQ is a catalytic poison whereby one molecule can keep spinning out a continuous stream of superoxide ions. This is exactly the point of Bus's 1984 paper; "Although the mechanism of paraquat toxicity is not precisely understood, it remains clear that redox cycling is a critical event leading to cell injury" [21]. Obviously, it is just a tiny leap of insight for the Defendants to imagine that what is going on in the lung could also be going on in the SNpc.

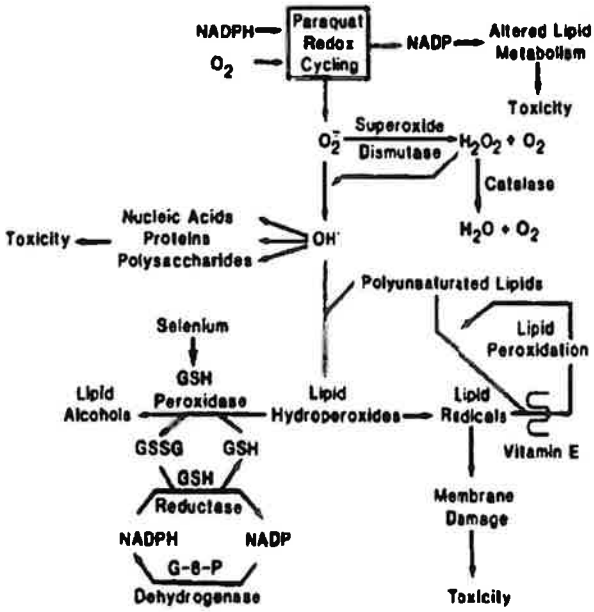


FIGURE 2. Potential mechanisms of paraquat toxicity.

Figure 5. From James S. Bus and James E. Gibson Paraquat: Model for Oxidant-Initiated Toxicity *Environmental Health Perspectives* **1984**, *55*, pp.37-46.

Moreover, if a chemical company knew that superoxide ion is produced catalytically in human tissues by one of its products, baseline competency would require that company to consider that there would be a high likelihood that the superoxide would transition to tissue damaging radicals not only via the mechanistic pathways shown in Figure 5, but also via FRA pathways that unleash chains of deadly reactive oxygen species or ROS—especially hydroxyl and alkoxyl radicals—that result in tissue injuries and even cellular death. These considerations make it nothing less than absurd to default to dose-response models for the toxicity of PQ. The fact that PQ induced catalytic redox cycling should have been more than enough information for the Defendant to remove PQ from the marketplace following the Bus paper. A simple dose-response argument is also inappropriate because a no-observed-adverse-effect level (NOAEL) or other Point of Departure (POD) for neuron loss in the SNpc of animal models has not been established.

I should note that individual genetic susceptibility also plays a role with regard to anti-oxidant defenses. An homozygous deletion of one of the glutathione genes (GSST1) resulted in an 11-fold increase in PD risk in a group of PQ applicators recruited from the Agricultural Health Study [35].

VI Conclusion

Pesticides are by the nature of their desired properties selected for commercialization because they kill targeted pests. PQ is a nonselective herbicide which means it kills all plants. The thought that PQ's mechanisms of toxicity might also adversely impact animals is inescapable as is the notion that it is obviously important to consider and react to the most sensitive animal endpoints in making marketing decisions.

In my judgment, the widely known idiom—a chain is only as strong as its weakest link—applies to the relationship between PQ and public health and therefore is relevant to this case. If among the human cell types that are essential to good health and a long life, one type is known to be exquisitely vulnerable to injury and death by PQ, then in my judgment as a professor of sustainable chemistry, the PQ purveyor has a duty to the public to prioritize the welfare of that weak-link cell type in all its marketing decisions. If the hazard is known beforehand, this duty applies at the time of consideration of introduction of the toxic chemical into commerce. If the hazard becomes known after commercial development, then the duty applies to decisions about continued commercial use. In this case, the most obvious weak-link cells are the dopaminergic neurons of the substantia nigra pars compacta, SNpc. One can identify various points in PQ commercial history where the purveyor chose to continue selling PQ in the US over clear warnings of a connection with PD. One such clear warning played out in the 1980s as explained in an extract from Samuel Goldman's review (see Inset 5) [10]. The refusals by 70 countries to allow PQ to be sold in their jurisdictions represent others.[10]

Inset 5

"In 1982, intravenous drug users in San Jose, California, began presenting to local emergency rooms with acute parkinsonism. They manifested the classic signs and symptoms of advanced PD and dramatically improved after treatment with L-dopa, the standard PD therapeutic medication. Langston and colleagues (18) identified the toxic agent as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Figures 1 and 2), which had formed during synthesis of a meperidine analog. Intravenous administration of MPTP to squirrel monkeys recapitulated the clinical observations seen in humans and caused specific destruction of nigral dopaminergic neurons (19). This observation reinvigorated environmental etiologic hypotheses of PD and launched a search for agents with similar toxicological profiles."

PQ possesses a rare and remarkable set of reactivity properties that allows one to conclude that any amount is capable initiating FRA and other mechanisms toward injuries to dopaminergic neurons of the SNpc. Because of PQ's amplified catalytic redox cycling, it should be treated like a carcinogen for purposes of risk assessment[11] or like the neuro-toxicant lead in children[12], i.e., there is no safe dose.

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[10] Syngenta has acknowledged that it is not legally allowed to sell paraquat in 70 countries around the world, including all member nations of the European Union and China. Syngenta's Response to Request to Admit Facts; *see also* Botham: 483-85, 1210-11.

[11] https://www.cdc.gov/tobacco/data_statistics/fact_sheets/tobacco_industry/low_yield_cigarettes/index.htm

[12] https://www.cdc.gov/nceh/lead/prevention/blood-lead-levels.htm

If the "dose makes the poison" were to apply to chronic low-dose PQ, it would mean that there is a dose below which PQ is not a poison, defined as a substance that causes injury, illness, or death by chemical means. The problem with the assertion that the principle holds for PQ is that the chemistry of PQ remains the chemistry of PQ regardless of the dose and the implication that there can be an identifiable safe dose defies common sense based on an accessible understanding of this chemistry.

January 27, 2021

Dr. Terrence J. Collins

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