Environmental fate and ecotoxicology of paraquat: a California perspective

Fabio Sartori and Edgar Vidrio

Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, California, USA
Environmental fate and ecotoxicology of paraquat: a California perspective

ABSTRACT
The herbicide paraquat belongs to the group of the bipyridylum salts. In California, it is used primarily for control of broad-leaved grasses in fruit orchards and plantations, as a cotton defoliant, and for inter-row control in many crop and non-crop areas. In plants, paraquat causes the formation of reactive radicals leading to cell membrane damage and ultimately rapid desiccation. Soil clay minerals have a greater influence on paraquat adsorption and inactivation compared with soil organic matter following an application. Degradation mechanisms include photolysis, chemical, and microbial degradation, but these processes are generally extremely slow. In California during 2000–2014, paraquat was used primarily for the cultivation of almonds, cotton, alfalfa, and grapes: median value for an application and annual mass applied statewide were 0.53 kg ion/ha and 280 Mg, respectively. Paraquat was undetected in groundwater as a non-point source pollutant. Detections in surface waters (0.42–3.6 μg/L) were <1 %. In earthworms and other invertebrates there is limited paraquat accumulation as toxic effects are mitigated via soil inactivation. Paraquat is among the most embryotoxic contaminants for bird eggs, but not to adult; it causes toxic and teratogenic effects in amphibians, and toxic effects in honeybees, fish, and other aquatic species.

KEYWORDS: Paraquat; California; environmental fate; toxicity; soil deactivation
## Contents

1. Introduction ...................................................................................................................... 4  
2. Physical and chemical properties ..................................................................................... 5  
3. Overview of paraquat use ................................................................................................. 6  
   3.1 Regulation .................................................................................................................. 7  
4. Use profile of paraquat in California ............................................................................... 8  
5. Plant resistance ................................................................................................................. 9  
6. Environmental fate ......................................................................................................... 11  
   6.1 Soil .......................................................................................................................... 11  
   6.2 Water ....................................................................................................................... 15  
   6.3 Air ........................................................................................................................... 17  
7. Environmental degradation ............................................................................................ 17  
   7.1 Microbial ................................................................................................................. 18  
   7.2 Photochemical ......................................................................................................... 19  
8. Ecotoxicology ................................................................................................................. 20  
   8.1 Mode of action: paraquat redox chemistry .................................................................. 21  
   8.2 Plants ....................................................................................................................... 22  
      8.2.1 General physiological effects ........................................................................... 22  
      8.2.2 Dose-response studies .................................................................................... 25  
   8.3 Soil fauna and flora ................................................................................................. 26  
      8.3.1 Toxic effects .................................................................................................... 26  
      8.3.2 Teratogenic effects ......................................................................................... 28  
      8.3.3 Soil bacteria ..................................................................................................... 29  
   8.4 Birds ........................................................................................................................ 29  
      8.4.1 Toxic and teratogenic effects ........................................................................... 29  
      8.4.2 Reproductive effects ......................................................................................... 31  
   8.5 Honeybees ............................................................................................................... 31  
   8.6 Amphibians .............................................................................................................. 32  
      8.6.1 Toxic, teratogenic, and reproductive effects .................................................... 33  
   8.7 Fish and other aquatic species .................................................................................. 35  
      8.7.1 Toxic effects .................................................................................................... 35  
      8.7.2 Oxidative stress indicators .............................................................................. 37  
      8.7.3 Behavioral analyses ......................................................................................... 38  
9. Summary and Conclusions ............................................................................................. 39
1 Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridinium) was first synthesized by Weidel and Russo (1882) and used as an herbicide in 1955 at the Jealott’s Hill Research Center (Brian et al. 1958; Calderbank and Slade 1976). The first commercial paraquat formulation for agricultural use became available in 1962 (USEPA 1987). Paraquat belongs to the “bipyridylium” (BP) or “viologen” salts, a group of compounds that has been known for their redox properties since 1933 (Michaelis and Hill 1933). The ability of BP salt compounds to accept or release an electron in biochemical systems has long been considered the first step responsible for paraquat toxicity in animals and plants (Homer et al. 1960). Paraquat is a fast-acting, non-selective contact herbicide, absorbed by foliage and is used in both agricultural and non-agricultural areas, as well as on both food and feed crops (Homer et al. 1960; USEPA 1997), in reforestation programs, pine plantation establishment (USEPA 1997), and in some countries for the control of aquatic weeds (Brooker and Edwards 1975; Peterson et al. 1994).

In California, it is used primarily for control of broad-leaved grasses in fruit orchards and plantations (Wilson and Orloff 2008; Moretti et al. 2015), as a cotton defoliant (Chester and Ward 1984; Scarborough et al. 1989), and for inter-row control in many crops (Kim and Hatzios 1993), such as alfalfa (*Medicago sativa* L.) stands (Wilson and Orloff 2008) and non-crop areas (CDPR 2016; Dennis et al. 2016). It remains among the top five most used herbicides in California, with statewide applications averaging an annual mass of about 280 Mg ion during 2000–2014 on over 150 agricultural commodities (CDPR 2016).

Paraquat has revolutionized farming in the 1960s, because it generally does not leave an active residue in soil or plants to affect future or established crops, does not leach into groundwater, and reduces or eliminate the need of ploughing the soil for vegetation control.
acting as a “chemical plough”. It is currently banned in 32 countries, including the European Union, based primarily on human health concerns (The Court of First Instance 2007; USEPA 2018); however, it is still registered and applied in over 90 countries (Fortenberry et al. 2016). Because of its toxicity to human health and potential impacts to the environment, paraquat remains one of the most controversial and studied herbicides of the last 50 years (Chester and Ward 1984; USEPA 2018). Several past and recent reviews and analyses have already assessed paraquat toxicology in mammals including humans (Autor and Schmitt 1977; Dinis-Oliveira et al. 2008; Lock and Wilks 2010; Xie et al. 2016) and occupational and non-occupational exposure (Hart 1987; Tsai 2013).

Because persistence and terminal residues play a major role in environmental regulation, the objectives of this review are to (1) summarize current knowledge of the physico-chemical properties of paraquat; (2) provide an historical overview of paraquat use focusing on California agriculture; and (3) critically review the most current and past knowledge on its environmental fate, degradation, and ecotoxicology.

2 Physical and chemical properties

This herbicide is suitable for many agricultural uses because of its physical and chemical properties (Figure 1 and Table 1) (Florêncio et al. 2004). Its association with the chloride or bromide anion to form a salt has no effect on the herbicidal properties of the paraquat cation, because cation and anion are dissociated when in aqueous solution (Calderbank and Slade 1976). (Throughout this document we will refer to the paraquat cation as “ion” or simply as “paraquat”, and its salt, paraquat dichloride, as “PD”.) Michaelis and Hill (1933) first discovered the redox properties of the BPs (γ,γ’-dipyridyl and dimethyl-dipyridylum chloride) in 1932. During a titration with paraquat in an alkaline solution, they used sodium hydrosulfite as a reducing agent
and measured an electrode potential $E = -0.446$ V, which remained relatively stable at pH ranging 8.4–13. Redox potential measurements of the BP compounds have been used to assess their herbicidal properties, considering that the more easily a BP compound is reduced, the more the toxic radicals that will be formed and vice versa (Homer et al. 1960). These findings by Homer and colleagues were instrumental and led to the commercialization of paraquat by the Imperial Chemical Industries Limited London, England, who registered paraquat in that country in 1962 and in the United States in 1964 (USEPA 1987). Reported Freundlich ($K_f$) soil-water distribution coefficients are extremely variable because of the variability in soil type: 68–50000 mL/g (USEPA 1997) and 28.7 (sandy loam)–1419 (muck) mL/g (Cheah et al. 1997). However, this and other coefficients, such as the organic carbon normalized partition coefficient ($K_{oc}$), may not be meaningful or applicable for certain soil types, due to paraquat strong adsorption onto clay minerals.

3 Overview of paraquat use

In California, all agricultural applicators are required to report information on the use of any registered pesticide to the California Department of Pesticide Regulation (CDPR). The submitted use information is recorded in the CDPR’s Pesticide Use Report (PUR) database (CDPR 2017). During 2000–2014, there were 11 products registered that contained PD as an active ingredient (a.i.). These six most common trade names of commercial paraquat herbicides are Gramoxone Inteon, Gramoxone Max, Gramoxone Extra Herbicide, Gramoxone SL 2.0, and Firestorm, representing more than 80% of reported paraquat uses (CDPR 2016). All these products are formulated as aqueous solution with the a.i. concentration equal to or greater than 30.1% (USEPA 2016).
Paraquat is typically used as a nonselective herbicide to rapidly desiccate vegetation. It is defined as a “contact” herbicide because it quickly disrupts the plant tissues that are contacted by the chemical due to its rapid desiccant action. It is used as a “post-emergence” herbicide alone or in combination with other herbicides (Moretti et al. 2015). In California, it is primarily used individually or in combination with other herbicides in almond orchards (Connell et al. 2001), cotton plantations to cause cotton bolls to open or to control regrowth of late-season cotton (Seiber and Woodrow 1981; Chester and Ward 1984; Wilhoit et al. 1999), alfalfa stands (Wilson and Orloff 2008), and grapes (UC IPM Program 2016). In most recent years, paraquat has become an important management tool to control glyphosate-resistant species (Moretti et al. 2016). Paraquat is one of the few products registered for aerial application in alfalfa and is used when fields are too wet to apply other herbicides by ground. In grapes it is preferred to simazine, because simazine is a well-known groundwater contaminant in some parts of the state, thereby having more regulatory restrictions associated with its use (Wilhoit et al. 1999).

3.1 Regulation

Paraquat was first registered in the United States as a contact herbicide and has been subject to periodic reviews by the USEPA and other authorities. Due to its acute toxicity to humans and animals, the USEPA classified paraquat as a restricted use material in 1978, and thereby ruled that it can only be sold to and used by certified applicators (USEPA 1997). Additionally, paraquat is listed as a Restricted Use Pesticide (RUP) under Title 3 of the California Code of Regulations (CCR), section 6400, in the production of an agricultural commodity. The use of both federally-restricted and California-restricted materials subjects paraquat to additional restrictions and use limits. Buying or using a California-restricted use pesticide requires a permit
Use profile of paraquat in California

There was an upward trend from 2001 to 2006 (the overall maximum use year) followed by a general decline, with the lowest use occurring in 2013 (Figure 2, CDPR 2016). These trends may be related primarily to the climatic conditions that affect the use of water-soluble herbicides. As found in a previous analysis on paraquat use from the PUR database by Wilhoit et al. (1999), paraquat use typically increases in several areas of the state in years of abundant rainfall, which promotes weed growth. Similarly, there was a weak (Spearman’s $\rho = 0.49$) but significant correlation (significance of both regression coefficients $[p < 0.05]; R^2 = 0.3$) between the annual, statewide precipitation data (2000–2014, series [NOAA 2017]) and one-year lagged annual use, supporting these previous findings (data not shown).

Many factors may affect paraquat use patterns: changes in the area treated, development of plant resistance, changes in the paraquat label requirements, and in agricultural management practices. Paraquat can also be applied during wet years when fields are too wet to apply diuron or hexazinone (Wilhoit et al. 1999). When resistance occurs combinations of other herbicides are typically used in alternative, for example, glufosinate and glyphosate (Moretti et al. 2015). All of these factors may ultimately affect the rate of application, which remained relatively constant during 2000–2014 as there were no major label changes (Figure 3[c]), although the median value by year did show an increasing trend from 0.42 kg ion/ha in 2001 to 0.56 kg/ha in 2011 and 0.7 kg/ha in 2012. The average median value for an application (computed as average over 2000–2014) was 0.53 kg ion/ha or 0.73 kg PD/ha (data not shown). In the years of greater paraquat use and annual precipitation (76.5 cm and 60.0 cm for 2005 and 2006, respectively) the area of and
mass applied per application were also greater (Figure 3[a,b]), and vice versa in the years of lower use and precipitation (20.1 and 50.5 cm for 2013 and 2014, respectively). (The six years of lowest precipitation in California were 2011, 2002, 2008, 2009, 2007, and 2013 with annual precipitation of 47.7, 47.4, 45.3, 43.3, 35.2, and 20.1, respectively [NOAA 2017]).

The overall highest uses (2000–2014) were in Kern, Fresno, Kings and Tulare counties. Other counties with lower uses over the same period were Merced, San Joaquin, and Madera. All these counties are located in the San Joaquin Valley that is part of the Central Valley of California (Figure 4), one of the most productive regions of agricultural crops in the world. It is historically an area of high pesticide use including the use of paraquat (Chester and Ward 1984; Weinbaum et al. 1995; Carmichael et al. 2014). Five commodities/agricultural crops had a corresponding 15-year cumulative paraquat use greater than 3300 Mg ion or, in terms of percentage, greater than 77 % of total use in California for that period (Figure 5). In descending order of paraquat mass used, they were almond (23.9 % of total statewide use), cotton (20.4 %), alfalfa (15.2 %), grape wine (9.3 %), and grapes (8.8 %). During 2000–2014, PD was mainly applied via ground application methods (80.1 % of applications on a mass basis), whereas most of the remaining mass (19.6 %) aerially (data not shown).

5 Plant resistance

Increased resistance is among the important factors affecting paraquat application rates and the development of new strategies for vegetation control (Wilhoit et al. 1999). Resistance to paraquat has been developing relatively slowly and is believed not to represent a serious economic threat to agricultural production (Hawkes 2014). Researchers have thus far documented 63 cases worldwide of species that have developed some sort of paraquat resistant. The reported cases in the United States were in eight separate locations: two in California (hairy
fleabane, *Coryza bonariensis* L. [Cronq.] and horseweed, *Coryza canadensis* L. [Cronq.]; Moretti et al. 2016), three in Florida (*Solanum americanum*, *Eleusine indica*, and *Landoltia punctate*), two in Mississippi (*Coryza canadensis*), one in Delaware involving horseweed (*Coryza canadensis*), *Coryza bonariensis*, and *Landoltia punctate* (Heap 2016).

Plant resistance to paraquat has generally developed following prolonged selection pressure from many applications of the herbicide (Preston et al. 1994). An herbicide must be present for prolonged times to foster the high selection pressure required to obtain resistant biotypes, particularly for paraquat that completely lacks active persistence. In recent years, hairy fleabane and horseweed have become well adapted in tree nut and vineyard plantations of the Central Valley of California, thereby becoming one of the most problematic species to control despite herbicidal control treatments. The combined glyphosate-paraquat treatment has been shown to be one of the least effective herbicide treatment combinations due to the development of herbicide resistant in *Coryza* sp. populations (Moretti et al. 2015; Moretti et al. 2016). Those authors were the first to confirm the presence of populations of hairy fleabane that are resistant to both glyphosate and paraquat in the California Central Valley. The hypothesis of independent mechanisms of resistance for the two herbicides is supported by the concurrent presence of a multiple resistant population as well as glyphosate-resistant—but paraquat-susceptible—populations of hairy fleabane in California (Moretti et al. 2013).

Most recently, such evolved resistance has prompted new research efforts in identifying environmental conditions and new herbicides, such as saflufenacil (Dennis et al. 2016) for the control of glyphosate-paraquat-resistant biotypes of *Coryza bonariensis* [L.] Conquist in the Central Valley of California, similarly to other researchers in other U.S. regions, for example, combined paraquat-metrobuzin combination (Eubank et al. 2012).
6 Environmental fate

Under current U.S. regulations, paraquat cannot be applied directly to aquatic environments (Dial and Bauer 1984; USEPA, 1997); therefore, the focus of this review is on paraquat use in terrestrial ecosystems. Its environmental fate in aquatic environments was previously reviewed by Calderbank and Slade (1976). Following a terrestrial application, the primary targets are biological materials and soils. Because of its low vapor pressure (Table 1), the main pathway of paraquat dissipation is sorption onto soil particles or plant residue materials. The research conducted in the 1960s and 1970s was the foundation to understand the fate and transport of paraquat in the environment (Calderbank and Slade 1976). That key work showed three possible pathways of paraquat degradation in soil: (1) photolysis under ultraviolet (UV) or solar radiation (Slade 1965), (2) chemical (Hance 1967), and (3) microbial (Funderburk and Bozarth 1967). However, other researchers further confirmed that if these degradation processes do occur they are typically extremely slow or non-existent, and cannot be considered a viable option for the degradation of polluted soils or waters, as paraquat does not hydrolyze under neutral or acidic conditions (Staiff et al. 1981; USEPA 1997).

6.1 Soil

The rapid sorption and consequent rapid deactivation of the BP cations in soil is among the important reasons the BP herbicides, diquat and paraquat, have been important crop management tools worldwide in the agriculture and forestry sector for over 40 years (Roberts et al. 2002). Together with glyphosate and glufosinate, paraquat is one of the three non-selective and soil-inactivated herbicides (Hawkes 2014). Such inactivation generally allows the replanting or sowing of new crops almost immediately in treated soil without the risk of phytotoxicity (Calderbank and Slade 1976; Bromilow 2004), although in sandy soils inactivation of paraquat
occurs slowly and occasionally some toxic effects to the new crops can occur following an application (Khan et al. 1975).

A large body of research has shown that the main mechanism in the adsorption of BP cations to soil particles is cation exchange due to electrostatic (coulombic) forces by the soil’s negatively charged sites at mineral and organic surfaces of the soil particles (Hayes et al. 1975), and it is speculated that, to a lesser degree, other forces are also responsible (Weber et al. 1965; Burns et al. 1973). These include charge transfer, hydrogen bonding, and van der Waals forces (Knight and Tomlinson 1967; Burns et al. 1973; Cheah et al. 1997; Gondar et al. 2012).

Paraquat sorption and desorption onto soils depend on the relative quantity and quality of the clay minerals and organic matter (Knight and Tomlinson 1967). Those authors showed that the main driving variable controlling paraquat adsorption to soil is the presence of clay minerals rather than soil organic matter (SOM), although, in certain soil types, SOM may also be relevant in controlling paraquat adsorption and transport (Khan et al. 1975; Senesi et al. 1995). Adsorption behavior onto the expandable 2:1 type clay minerals, such as montmorillonite, that allow for isomorphic substitutions in interlayer positions greatly differs from that of non-expandable 1:1 types, such as kaolinite (Knight and Tomlinson 1967; Kookana and Aylmore 1993), and is controlled by the soil pH-dependent charge (Khan 1974; Calderbank and Slade 1976). In the case of kaolinite, adsorption occurs on the edges or faces of the clay particles rather than in the interlayer positions (Weber et al. 1965). Other variables that may affect paraquat adsorption/desorption with a relatively weaker influence are oxide minerals, such as iron oxides (Weber et al. 1965; Amondham et al. 2006). Weber et al. (1965) also showed that temperature and time of exposure did not significantly affect paraquat sorption onto kaolinite or montmorillonite (Weber and Weed 1968).
In clay-rich soils, the presence of SOM may not necessarily increase paraquat adsorption. The work by Pateiro-Moure et al. (2009) showed that adsorption of BPs by agricultural soils clearly increased when SOM was removed by treating the soil with H₂O₂. Their interpretation was that in the presence of SOM-clay mineral complexes, SOM occludes some of the potential sites afforded by the clay minerals for binding paraquat cations. In contrast to paraquat adsorption onto clay particles, paraquat adsorption onto SOM-rich charcoal was found to be strongly temperature and time dependent relative to the adsorption of other herbicides (Weber et al. 1965).

The study of paraquat adsorption/desorption to/from soils has important implications for paraquat fate into ground and surface waters. Key investigations that elucidated the underpinning mechanisms of paraquat fate and transport in soils with different SOM and clay contents were conducted in the 1960s and confirmed further in the 1970s by the work of Burns et al. (1973) and Khan et al. (1975). Paraquat transport through a soil’s profile may occur when the herbicide is adsorbed onto colloidal clays or as paraquat-laden SOM (Burns et al. 1973; Khan et al. 1975; Senesi et al. 1995), although paraquat transport in soil is generally very limited (Vintén et al. 1983; Cheah et al. 1997). The soil column experiments conducted by Vintén et al. (1983) showed that a Li-montmorillonite suspension transported over 50% of the applied ¹⁴C-paraquat to a depth of 12 cm in sandy loam soils; and the experiments by Leonard et al. (1979) in the Georgia Piedmont demonstrated that surface erosion of paraquat-laden sediment was the only possible mechanism of paraquat transport to surface waters. These authors used paraquat as a tracer to study sediment runoff at a watershed level. Their work represents one of the few publications validating results from previous laboratory experiments, showing that paraquat transport in clay-rich soils occur mainly as runoff rather than as leaching through a soil’s profile.
The determination of the total paraquat concentration in a typical agricultural soil requires the “extraction” of paraquat through a soil digestion, that is, boiling the soil samples in 12 N sulfuric acid for five h, because paraquat is immobilized/protected in soils. The ion can withstand such treatment because of its stability in acid solutions (Burns and Audus 1970; Hance et al. 1980).

Researchers have also investigated paraquat release overtime from soils—mainly as leaching—due to long-term safety concerns about its displacement by exchangeable cations applied through agricultural management practices (e.g. fertilization). While Roberts et al. (2002) concluded that this is an unlikely occurrence based on their review of the literature, other authors believe that certain agronomic practices, such as liming, gypsum application, and intense fertilization, could generate such high soil cation saturation, and foster the release of soil-bound paraquat particularly in coarse soils with low SOM or highly weathered soils rich in kaolinite (i.e. with low CEC) (Kookana and Aylmore 1993). Similarly in their work about sorption onto and desorption from clay minerals of the BP cations, Weber and Weed (1968) found that, using the 1M BaCl₂ extraction method, only 5 % of the total adsorbed cation mass could be extracted from montmorillonite, as opposed to 80 % from kaolinite due to its weaker sorption capacity.

In the presence of organic-rich soils/materials—rather than clay particles—the desorption of paraquat from soils can be expected to behave as the reverse process when ion exchange is the dominant process controlling adsorption. However, the analysis of adsorption and desorption isotherms by Burns et al. (1973), using aqueous paraquat and five types of organic sorbent (soil, humic acid, humin, and two ion exchange resins), showed that paraquat desorption is not a purely reversible phenomenon, because the same mass action type of ion exchange isotherms should have applied to both processes. Those authors could extract only a small fraction of the
total paraquat adsorbed onto the organic materials in suspensions (through mixing and centrifugation of the samples at varying HCl concentrations), indicating that other cooperative mechanisms in addition to simple cation exchange were involved in paraquat adsorption.

Several authors believe that when paraquat is applied following the typical “good agricultural practices”, as specified by a product’s label, it becomes strongly bound to soil particles (>99 % of total mass), thereby extremely low paraquat concentrations are generally present in the soil solution phase (Roberts et al. 2002). Their proposed conceptual model was that most paraquat (>99 %) in soil is strongly bound to soil particles and in equilibrium with soil-solution paraquat undergoing slow microbial degradation/mineralization (Funderburk and Bozarth 1967; Lee et al. 1995; Ricketts 1999) (Table 2).

It is common belief that no microbial degradation generally occurs once immobilized/protected by clay minerals, because it becomes unavailable to microbes (Burns and Audus 1970; Fryer et al. 1975; Kookana and Aylmore 1993; USEPA 1997). The work of Hance (1967) showed that non-biological chemical processes do not play an important role in the loss of paraquat from the soil. In the absence of any microbial activities their estimated paraquat half-life was >9 years. Most commonly reported half-life values range from about 7 years (Hance et al. 1980; Cheah et al. 1998) up to 26 years when paraquat is applied at a rate corresponding to five times the sorption capacity of the soil (Kookana and Aylmore 1993) (Table 3). Among the most extreme values reported in the literature are those for paraquat applied in tropical soils of Thailand with extremely short half-lives of 36–46 days (Amondham et al. 2006).

6.2 Water

Adsorbed paraquat can potentially be found in surface water systems associated with soil particles carried by erosion (Leonard et al. 1979). Other possible mechanisms include paraquat
drift onto aquatic ecosystems, when paraquat is applied near surface waters, such as reservoirs, canals, and rivers, in countries with less stringent regulations (Wijeyaratne and Pathiratne 2006).

In the United States, a monitoring program conducted by the USEPA found paraquat in 11 out of 971 water wells sampled between 1983 and 1990 having concentrations greater than 100 μg/L in wells located in extremely permeable coarse-grained glacial soils, whose saturated hydraulic conductivities are about 20,000 ft/day (USEPA 1992). In a similar monitoring program as part of the National Water-Quality Assessment Program’s first decade of water-quality assessments by the USGS, paraquat was never detected in water samples collected from 5,047 groundwater wells distributed in 51 major hydrologic systems in the United States (Gilliom et al. 2006).

In California, paraquat has not been detected in groundwater as a non-point source pollutant. The CDPR Well Inventory Database lists three unconfirmed detections of paraquat in 1993 and 1997, all concentrations were below laboratory reporting limits. Subsequent monitorings conducted by the CDPR to confirm these detections resulted in no measurable concentrations of paraquat (C. Nordmark, personal communication, May 8, 2017).

Few peer-review reports in the literature for California or the United States support findings that paraquat can be detected in surface waters resulting from non-point source pollution. A search in the USGS database of the National Water-Quality Assessment Program, which includes data collected by over 400 state, federal, tribal, and local agencies, did not return any detection (Gilliom et al. 2006). Furthermore, similar searches of the CDPR surface water database and the California Environmental Data Exchange Network (CEDEN) database maintained by the California State Water Resources Control Board returned seven detections (0.42–3.6 μg/L, Irrigated Lands Regulatory Program) out of 1450 samples, between 16 May and
2006 and 16 September, 2014 (CSWRCB 2017). No additional detections were reported nationwide by other agencies contributing to the water quality data of the National Water Quality Monitoring Council (NWQMC 2017).

6.3 Air

The main pathways by which paraquat can travel into the air are through adhesion to particulate matter that is transported in the air or from drift during application (Seiber and Woodrow 1981; Chester and Ward 1984; Ames et al. 1993; Lee et al. 2005). These pathways can lead to adverse effects due to dermal and respiratory exposure, particularly to field applicators or people residing near an application (Weinbaum et al. 1995).

Seiber and Woodrow (1981) monitored a field application in two mature cotton fields in Kings County, California, using air samplers, and installed additional paired samplers in two enclosed-cab harvesters (inside and outside the operator’s cabin) to monitor the airborne dust generated during the harvesting operations. Paraquat concentrations measured in the air downwind regularly decreased from values ranging 4.31–10.7 μg/m³ at 1 m from the downwind border of the two fields to <50 ng/m³ at about 400 m downwind. The post-application concentrations decreased to 1%–10% of the initial values two–four h since the end of the application, and to non-detectable values five–seven h afterwards. Paraquat was also present in the airborne particulate matter during harvesting operations at one of the sites (1.245 and 506 ng/m³ outside and inside the open cab, respectively). They concluded that such values were well below most acute and sub-acute LD₅₀s recorded in animals.

7 Environmental degradation

In surface soils or biological materials paraquat may undergo microbial degradation during the time period from immediately after an application until it becomes inactivated and protected via
adsorption onto soil particles (Carr et al. 1985). This bioavailable paraquat may also photo-decompose over several weeks (Funderburk and Bozarth 1967; Roberts et al. 2002). Degradation on plant materials generally occurs much more rapidly than the degradation in soil (Lee et al. 1995). When paraquat becomes protected/immobilized in subsurface soils it may persist for several years or longer, and currently there is no effective degradation method to degrade paraquat that is strongly bound in soil (Ye and Lemley 2008). Ultraviolet or solar radiation alone are insufficient to ultimately mineralize paraquat and serve as a method for depolluting contaminated waters or soils, and microbiological processes are slow and require long incubation times (Moctezuma et al. 1999).

7.1 Microbial

A vast array of bacteria and fungi in a soil’s solution are capable of slowly degrading the bioavailable paraquat that is believed in equilibrium with the paraquat strongly adsorbed onto the soil mineral phase (Funderburk and Bozarth 1967; Ricketts 1999; Roberts et al. 2002; Wu et al. 2013), and/or the paraquat that is weakly adsorbed onto SOM (Burns and Audus 1970) (Table 2). Both laboratory and field incubation studies have confirmed that microbial degradation is responsible for the generally slow paraquat degradation if/when paraquat becomes bioavailable (Funderburk and Bozarth 1967; Burns and Audus 1970; Lee et al. 1995; Murray et al. 1997; Cheah et al. 1998; Ricketts 1999; Ismail et al. 2011). Paraquat-degrading microorganisms include the bacteria *Pseudomonas* spp. and *Flavobacterium* spp. (Murray et al. 1997), the fungi *Neocosmospora vasinfecta* (Funderburk and Bozarth 1967) and *Lipomyces starkeyi* Lod. and Rij (Burns and Audus 1970; Carr et al. 1985), and the patented microbial mat (consortium of cyanobacteria [*Oscillatoria* sp.] and bacteria) used by Murray et al. (1997).
The bioavailable paraquat can be degraded by soil fauna when in presence of sufficient carbon (C) and nitrogen (N) sources, or only C sources because the herbicide can act as the sole source of N to sustain microbial activities (Carr et al. 1985). Degradation can occur both under aerobic (Ismail et al. 2011) and anaerobic conditions (Lee et al. 1995), laboratory in vitro experiments (Carr et al. 1985), or in field dissipation studies (Amondham et al. 2006). The degradation rate has been shown to be higher for plant- than soil-associated paraquat, under aerobic than anaerobic conditions, and directly proportional to a plant’s C/N ratio (Lee et al. 1995); it increases with the addition of C sources, such as sucrose, that enhance bacterial degradation under aerobic (Ricketts 1999) and anaerobic conditions (Wu et al. 2013).

Funderburk and Bozarth (1967) isolated paraquat-tolerant microorganisms from soil under laboratory conditions. They proposed that the pathway of paraquat degradation in soil by a non-identified bacterial includes the de-methylating of the parent molecule, splitting of one of the two hetorocyclic rings, followed by formation of a carboxilated 1-methylpyridinium ion (Figure 6). A similar pathway with initial de-methylating of the parent molecule was reported also by Ricketts (1999). In incubation experiments, those authors showed that no paraquat remained in solution at the end of each incubation, and the analysis of the degradation products showed almost identical metabolite profiles between the different microorganisms. They could also identify \(^{14}\)C-oxalic acid as the main degradation product (85 % of total remaining radioactivity in the incubating solution) and other, non-identified products.

### 7.2 Photochemical

Degradation due to UV radiation can be expected to act as an important mechanism on paraquat bound to the surface of treated plants and paraquat-laden soil particles on the topsoil exposed to solar radiation (Calderbank and Slade 1976). Using paper chromatography and other techniques,
Slade (1965) found that paraquat under Ultraviolet (UV) irradiation is degraded to 1-methyl-4-carboxypyridinium ion and methylamine hydrochloride (Figure 7). That author observed that paraquat under solar light is degraded only when adsorbed to a surface, but not when in aqueous solution. These findings on photo-degradation were later evaluated by other authors (Table 2), who also identified additional products using the same or similar experimental conditions (Funderburk and Bozarth 1967; Kearney et al. 1985; Nguyen and Zahir 1999; Florêncio et al. 2004). Similarly, the work by Kearney et al. (1985) showed limited photodegradation of aqueous paraquat under UV or solar radiation under normal aerobic conditions.

8 Ecotoxicology

The main focus of this section will be on new toxicological data available for terrestrial invertebrates, because they are important indicators of ecosystem pollution for risk assessment (Edwards and Bohlen 1992; Wang et al. 2012; Givaudan et al. 2014), and the model species identified with a relatively high level of concern (LOC) on an acute basis by the USEPA (USEPA 1997). In small mammals, endangered species LOCs are exceeded for large herbivorous and insectivorous. As reported in the previous sections, paraquat is used primarily in agricultural and forestry settings, including along rights-of-way, fence lines, pipelines. These uses particularly increase the risk of exposing wild birds and mammals to paraquat (Hoffman et al. 1987; Berny 2007). Despite strong evidence in favor of paraquat deactivation/immobilization by soil, Eisler (1990) has also underscored that long-term fluxes of paraquat from the soil-adsorbed phase into the food chain remain poorly understood.

It is unlikely that paraquat may cause chronic effects in birds due to accumulation in the food chain, whereas the main concern for avian populations is that direct application to eggs may cause birth defects. The risk is the greatest immediately after an application and tails off
overtime due to wetting and drying cycles that may re-solubilize the paraquat cation. Similarly the risk for insects, such as honey bees (Apis mellifera), would be greatest during an application, but paraquat is typically applied when honey bees are not active in the field (USEPA 1997). From the time Eisler (1990) conducted his synoptic review of the literature available up until the late 1980s, new information has become available particularly on earthworms, honeybees, amphibians, and fish.

8.1 Mode of action: paraquat redox chemistry

The work by Farrington et al. (1973) analyzed and elucidated the main chemical reactions responsible for paraquat herbicidal action in plants. These reactions occur because, in aqueous solution, the paraquat cation, PQ$^{2+}$, acts as a terminal electron acceptor to form the monovalent cation PQ$^{+}$ that is stable in the absence of oxygen. Researchers coined the word “viologen” specifically for the BP salts, because of their blue-violet color corresponding to the one-electron reduction (Michaelis and Hill 1933; Ross and Krieger 1980). Assuming a continuous supply of electrons to paraquat and aerobic conditions, paraquat will cycle from reduced (PQ$^+$) to oxidized form (PQ$^{2+}$) with continuous production of the superoxide anion free radical O$_2^-$ (Michaelis and Hill 1933; Smith 1985). When in contact with any biological tissues, such as plant or animal tissues, the superoxide radical O$_2^-$ reacts with any biological targets leading to the formation of hydrogen peroxide (H$_2$O$_2$) and secondary hydroxyl radicals (OH$^-$) particularly superoxide radicals OH$^-$ (Farrington et al. 1973; Calderbank and Slade 1976; Beloqui and Cederbaum 1985; Hassett et al. 1987). These reactive species are then reduced by any metal ions that are part of the biological targets/tissues acting as electron donors (Goldstein et al. 2002).
8.2 Plants

It is more likely that paraquat may move offsite due to drift during ground or aerial application, rather than through volatilization (USEPA 1997), thereby affecting both target and non-target plant species. Main effects include wilting and general collapse in herbaceous plants. Perennial plants may regrow and the most resistant plants may be affected by temporary scorch (Eisler 1990).

8.2.1 General physiological effects

There is evidence that paraquat enters the plant through the leaf cuticle rather than through stomata (Brian 1967) and depending on which kind of plant tissue is sprayed some amounts may also be adsorbed onto cell walls before reaching the membranes (Funderburk and Lawrence 1964). When leaves are treated with paraquat, the formation of extremely reactive free radicals leads initially to damage due to the polymerization of the unsaturated lipids of the cell membranes. Thus, cell membranes lose integrity, which favors water loss and rapid desiccation or in other words tissue necrosis (Dodge 1971; Farrington et al. 1973). Because of the redox processes involved and their strong tendency to act as an electron acceptors, the BPs are also referred as “electron diverters” of the Photosystem I (PSI).

When paraquat is present in illuminated chloroplasts, it acts as an electron acceptor that traps all electrons from PSI at a diffusion-controlled rate (Farrington et al. 1973) to form its cation radicals, generating $O_2^-$ at a rapid rate via oxidation. Thus, ferridrin and NADP+ cannot be photo-reduced. This causes accumulation of $H_2O_2$ in the chloroplast. Paraquat radical is formed within the thylakoids of the chloroplast, and then diffuses into the stroma, where initially there are aerobic conditions. Given that the BPs have a redox potential more negative than NADP+
and ferredoxin, they interfere with NADP reduction in the chloroplast (Dodge 1971; Asada 1999).

The production of $\text{O}_2^-$ and $\text{H}_2\text{O}_2$ may then lead to formation of more reactive oxygen radicals, which may be more toxic to the cell and initiate cell membrane disruption and destruction of chlorophyll, interfering with the activities of the chloroplasts. These harmful byproducts cause breaches in the integrity of the membranes surrounding cell organelles, resulting in uncontrolled electrolyte leakage and cell death (Calderbank and Slade 1976). The BP herbicides damage plants mainly in the presence of light, whereas in darkness damage develops more slowly and only at high application rates as shown by Slade and Bell (1966). Based on the findings of those authors, paraquat movement into the plant was limited when the plant was exposed to light due to tissue damage, whereas in darkness the translocation of paraquat occurred over greater distances into the xylem through undamaged tissue. It is well established that paraquat translocation throughout the main plant organs is the greatest under dark conditions (Brian 1967). Under experimental conditions it has been shown that paraquat is normally reduced in green tissues by energy derived from light, even though such activity is also present in the dark in a reduced form (Homer et al. 1960). Paraquat may also be taken up by a plant’s root systems, although this is an uncommon occurrence given that paraquat mobilization under illumination in a plant tissue is minimal due to the redox reactions leading to the destruction of cell membranes and consequent desiccation of plant tissues (Hawkes 2014).

Since the 1970s, researchers have investigated the possible mechanisms responsible for plant resistance. Two prevailing mechanisms have received most research attention since the early 1980s: (1) sequestration of paraquat away from its site of action in the chloroplast (Fuerst et al. 1985), and (2) an increase in the activity of oxygen radical-scavenging enzymes in resistant
(R) biotypes which foster tolerance against active oxygen species formed by paraquat (Hart and Di Tomaso 1994). The first mechanism involves restricted rate of herbicide movement throughout the plant (Preston et al. 1994; Yu et al. 2007; Powles and Yu 2010) and has not yet been established unambiguously for any biotype (Hart and Di Tomaso 1994; Hawkes 2014). Researchers have hypothesized that only limited amounts of paraquat may reach the site of action in the chloroplasts (limited penetration has been detected in *Hordeum glaucum* Steud. and *Conyza bonariensis* (L.) Cronq.), and if paraquat does reach the chloroplasts, it is rapidly transferred and sequestered into a metabolic inactive compartment via a sequestration mechanism (Hart and Di Tomaso 1994; Jóri et al. 2007).

Most recent studies in *Lolium rigidum* suggest that resistance to paraquat may be associated with a mechanism of the cell cytoplasm that causes a greater rate of vacuolar sequestration, but the precise biochemical and molecular basis of this sequestration mechanism remains unclear (Busi and Powles 2011; Hawkes 2014). Other proposed mechanisms include (3) the lack of penetration due to epicuticular wax, (4) an alteration in the redox potential of the PSI primary electron acceptor, (5) adsorption of paraquat to lignified areas, (6) the binding of paraquat onto cell walls, or (7) developed ability to prevent paraquat from entering the symplast. To date, there is not enough empirical evidence in favor or against mechanisms 3–7 (Hart and Di Tomaso 1994). Since paraquat rapidly diverts electrons from the PSI, acting as an electron acceptor in the chloroplast, there likely are no binding-site mutation-based mechanisms that foster paraquat resistance. Hawkes (2014) indicated that currently there is no report of paraquat resistance dependent on site-specific mutations, whose identification would have important implications to develop paraquat resistant crops.
8.2.2 Dose-response studies

The doses for lethal and sub-lethal effects vary depending on species and biotype. In his review of the literature, Eisler (1990) reported that in sensitive species of terrestrial plants and soil microflora adverse effects occur at 0.28–0.6 kg/ha. This is in agreement with other findings by Preston et al. (1994), who reported that adverse effects start to occur in sensitive species at 0.2 kg/ha and that the difference in toxicity indices between susceptible (S) and R biotype varied 10–50 times. Those authors studied paraquat effects on capeweed (Arctotheca calendula [L.]) biotypes collected from an alfalfa (Medicago sativa L.) field near Ararat, Australia that had received 24 consecutive annual applications of diquat and paraquat. They estimated LD$_{50}$ ~ 4 kg a.i./ha and 0.4 kg a.i./ha for the R and S biotype, respectively. Busi and Powles (2011) studied paraquat resistance in Wimmera ryegrass (Lolium rigidum Gaud.) R and S biotypes that were previously selected for glyphosate resistance at 0.15, 0.25, or 0.35 kg glyphosate/ha. In their work, glyphosate-selected and unselected plants were treated at 0, 0.006, 0.013, 0.025, and 0.050 kg paraquat ion/ha both under laboratory and field conditions to estimate 15-day LD$_{50}$s. They found that biotypes that were selected for glyphosate resistance showed also a concomitant paraquat resistance, and the LD$_{50}$ of a three-time glyphosate-selected progeny (0.044 kg/ha) was fourfold greater than the unselected parent (0.011 kg/ha).

Similar findings about concomitant resistance among biotypes were reported by Moretti et al. (2016), who studied accessions that were found resistant to both paraquat and glyphosate in orchards and vineyards of the Central Valley in California. Plants were collected from separate genetic groups located at distances up to 160 km. In a randomized complete block design experiment, they considered genetic group and application rate (0, 0.019, 0.056, 0.167, 0.5, 1.5, 4.5, 13.5, and 40.5 kg PD/ha) as the two main experimental factors, to assess paraquat dose-
responses. They estimated that the 28-day LD$_{50}$ varied 50–393 times in hairy fleabane and 418 times in horseweed. The highest and lowest overall LD$_{50}$s were 0.01 and 15.5 kg/ha, respectively, for hairy fleabane and 0.02 and 21.6 kg/ha, respectively, for horseweed.

8.3 Soil fauna and flora

Earthworms are important indicators of ecosystem functioning and health, as well as soil contamination from agrochemicals (Edwards and Bohlen 1992; Givaudan et al. 2014). Most reports underscore that paraquat adsorbed through the gut of earthworms and other invertebrates or microarthropods is rapidly excreted without any significant bioaccumulation in tissues (Summers 1980). Past reviews comparing paraquat to other agrochemicals indicated that paraquat is less toxic to earthworms than other herbicides, fungicides, and insecticides—it is ranked as one of the least toxic (Haque and Ebing 1983; Edwards and Bohlen 1992).

8.3.1 Toxic effects

Adverse effects on growth in soil start to occur at extremely high concentrations: at about 1000 ion mg/kg soil or 1500 kg/ha (assuming a soil bulk density of 1 g/cm$^3$ and depth of 15 cm) based on Van Gestel et al. (1992). Haque and Ebing (1983) estimated 14-day LC$_{50}$ values of >200 mg ion/kg soil substrate for Eisenia foetida and Lumbricus terrestris (assuming a soil bulk density as before, the corresponding content would be ~ 300 kg ion/ha).

Roberts et al. (2002) revised the results from long-term research trials about monitoring changes in soil properties following a single or annual paraquat applications (Table 2). Those studies showed that an increase of paraquat in soil had caused temporary changes in soil fauna composition within the first decade since cessation, but levels were well below values that would cause marked effects on crops or soil fauna, indicating that repeated paraquat applications, at recommended application rates of about 1 kg/ha, will not have any negative impact on the
number or activity of soil microorganisms. Those authors concluded that the overall changes were more the indirect result of eliminating the competing vegetation, rather than direct action of paraquat on soil fauna.

The findings about low toxicity in earthworms reported by Roberts et al. (2002) were confirmed most recently by Papini et al. (2006), who studied the influence of two substrates, representing a clayey and sandy soil type, on paraquat bioaccumulation in the earthworm (*Eisenia foetida*) under laboratory controlled conditions. The two substrates were treated to obtain a paraquat concentration of 1.2, 12, and 120 μmol *14C*-paraquat/g substrate. Ten worms were added to each glass vessel containing the substrate and maintained in dark at 20 ± 1 °C for 90 days. The bioassay was then dismantled. At the end of the experiment, they found that the paraquat mass bio-accumulated was always less than 1 % of total biomass. No mortality due to paraquat was detected, supporting the general belief that paraquat is non-toxic to these worms at commonly adopted field application rates.

However, when the action of paraquat is not buffered by soil inactivation/sequestration, toxic effects do occur also in earthworms as shown by Muangphra et al. (2014) through contact toxicity tests. Those authors conducted both acute and chronic toxicity studies on *Pheretima peguana*, the most common earthworm species found in agricultural fields of Thailand. They also evaluated the genotoxicity of glyphosate and paraquat as indicated by chromosomal aberrations, DNA damage, and cytoskeleton damage in coelomocytes (immune cells in the coelomic cavity) of *P. peguana*. Using probit analysis, they estimated a 2-day LC₅₀ of 0.39 kg ion/ha, and found that even the chronic dose of 0.0039 kg ion/ha induced genetic (clastogenic and aneugenic) effects on earthworm coelomocytes.
A comparison between LD$_{50}$ estimates on filter paper and artificial soil is provided for the earthworm *Eisenia fetida* by Wang et al. (2012). Their estimated 2-day LD$_{50}$ value in filter paper was 23.5 kg ion/ha indicating moderate toxicity; whereas the 7-day and 14-day LD$_{50}$ values in artificial soil were >1000 mg/kg (or 1500 kg ion/ha, assuming a soil bulk density of 1 g/cm$^3$ and depth of 15 cm), underscoring, that paraquat is among the least toxic herbicides when applied to soil as indicated by Edwards and Bohlen (1992).

### 8.3.2 Teratogenic effects

Van Gestel et al. (1992) described both toxic and teratogenic effects in the earthworm *Eisenia foetida* under laboratory controlled conditions. They monitored cocoon production in earthworms that were placed in artificial soil for an adaptation period without paraquat, followed by a three-week period of exposure to paraquat. Dry PD and water were added to the soil (55 % gravimetric water content) and earthworms were exposed for three weeks. Cocoon production diminished at 450 mg/kg dry soil (or 675 kg ion/ha) and was significantly reduced at 1000 mg ion/kg dry soil. Cocoons had abnormal shapes, and the percentages of the small ones passing through a 2-mm sieve were 0.7%, 4.5%, and 10.8% for the 0, 450, and 1000 mg/kg (control) rate, respectively. There was no paraquat effect on the number of juvenile fertile cocoon. Instead, paraquat applied at 1000 mg/kg significantly reduced the total number of offspring (juvenile/worm/wk). The difference between the no observed effect concentration for reproduction (NOEC = 56 mg/kg dry soil) and the median lethal dose (LC$_{50}$ = 1000 mg/kg dry soil) was a factor of >100. Those authors concluded that their results were in agreement with previous findings, indicating that paraquat produces long-lasting and irreversible effects on earthworms.
8.3.3 Soil bacteria

Paraquat can cause negative impacts on organisms responsible for nutrient cycling. Gadkari (1988) studied the influence of atrazine and paraquat on a mixed culture of nitrifying bacteria in a laboratory aqueous system. Paraquat caused a complete inhibition of ammonium and nitrate oxidation: at 5 μg/mL and 10 μg/mL ammonium oxidation was completely inhibited for over 40 days; nitrate oxidation was also completely inhibited at the same concentrations for 28 days; even at 1 μg/mL ammonium and nitrate oxidation were inhibited for >18 days.

8.4 Birds

Paraquat is among the most embryotoxic environmental contaminants for bird eggs (LC₅₀ ~ 1.7 kg/ha, day 18) at the customary application rates as reported by Hoffman and Albers (1984), who refers also to Lutz-Ostertag and Henou (1975) (chicken and Japanese quail [Coturnix japonica] eggs) and Hoffman and Easting (1982) (mallard [Anas platyrhynchos] eggs). Bird nestlings also appear sensitive as reported for the American kestrel (Falco sparverius) by Hoffman et al. (1985), and bobwhite quail (Colinus virginianus), Japanese quail, ring-necked pheasant (Phasianus colchicus) and mallard by Heath et al. (1972). At the same time, adult bobwhite quail (Colinus virginianus) could tolerate a diet containing paraquat at 100 ppm (mixture of water and mash) for 60 days without showing signs of toxicity or impaired learning, indicating that paraquat is highly toxic to avian embryos but not to adults (Bunck et al. 1986).

8.4.1 Toxic and teratogenic effects

Hoffman and Eastin (1982) conducted a series of experiments to investigate the effects of two insecticides (lindane and toxaphene) and two herbicides (paraquat and 2,4,5-T) by treating mallard eggs in paraquat formulations at actual field concentrations. The eggs were immersed in different mixtures, even though paraquat is generally applied in aqueous emulsion, containing
different herbicidal concentrations and different solvents. Paraquat was the most toxic to the embryos regardless of the type of mixture in agreement with previous findings (Lutz Ostertag and Henou 1974). When treatment with aqueous paraquat emulsion was on three-day-old mallard embryos the corresponding LC$_{50}$ (day 18) was 1.5 lb a.i./acre (1.5 times the recommend field application rate). Paraquat in aqueous emulsion caused impaired growth and was slightly teratogenic at half the field level of application, causing 23 % mortality. Brain defects (anencephaly and exencephaly) occurred only at higher concentrations (1.5–3 times the field level).

These results were confirmed by the work of Hoffman and Albers (1984), who conducted a subsequent comparison of the effects on mallard eggs exposed to 42 pesticides, including herbicides, on mallard. They used the same methods as described by Hoffman and Eastin (1982). Their results showed again that paraquat, both in the aqueous emulsion and organic mixtures, was the most toxic. The LC$_{50}$ was about 1.5 lb/acre in the aqueous emulsion, causing grown inhibitions and teratogenic effects: They found that in comparison to the other herbicide studied, that is, trifuralin, paraquat was more teratogenetic, causing extensive edema and brain defects (anencephaly and exencephaly), as well as growth inhibition at treatment doses less than the LC$_{50}$. The LC$_{50}$ of the eight considered pesticides were greater in the organic solvent than in the aqueous emulsion likely due to enhanced penetration through the eggshell and its membranes. Similar effects on bird reproduction were reported by Dunachie and Fletcher (1967), who found an extremely high rate of mortality in longhorn chickens ($Gallus$ domesticus) from eggs previously injected with paraquat at 0.15 ppm and a generally reduced hatchability.

Predatory birds like kestrels feed on other species, such as grasshoppers, small rodents, and passerine birds, which may come into contact with paraquat during agricultural spraying or by
ingestion (Hoffman et al. 1985). Those authors studied the effects of paraquat oral administration in distilled water to American kestrel nestlings by randomly assigning each hatchling to a control, 10, 20, or 60 mg/kg treatment level. They found that 44% of the nestlings receiving the highest dose died after four days, and the growth rates of the paraquat-treated groups were significantly lower than all controls. These results indicated that American kestrel nestlings are particularly sensitive to paraquat exposure: Histopathological examinations showed liver, kidney, brain, and lung damages, suggesting greater sensitivity to paraquat in altricial nestling kestrels than in young or adult birds of precocial species.

8.4.2 Reproductive effects

Northern bobwhite (Colinus virginianus) appeared less sensitive to paraquat use as reported by Bauer (1985), who investigated the effects on reproduction and growth of a paraquat-contaminated diet at levels equal or lower than those that could be found in paraquat-treated fields (0, 20, 60, and 180 ppm for six weeks). The studied variables included egg production, fertility, hatchability, chick abnormalities, and chick survival and weights up to age seven days. The parental (P) hens fed at 180 ppm laid fewer eggs than at lower rates and had lower ovary and oviduct weights. However, there were no significant differences in P fertility, chick abnormalities, or survival compared with controls. First generation hens from paraquat treated parents laid eggs with a one-week delay, lower rate of egg production, and 10-day delay in clutch completion compared with controls.

8.5 Honeybees

Habitat reduction has often led beekeepers to pasture their bees on insecticide-treated fields (Johansen 1977). Paraquat caused extremely toxic effects to honey bees (Apis mellifera L.) in small cages that were exposed to paraquat at 4 lb a.i./acre as a water spray: 90% of bees died
three days after exposure (Moffett et al. 1972). Paraquat was extremely toxic in a sucrose syrup diet at concentrations of 100 mg/L (Morton et al. 1972). In honey bee colonies placed in isolated desert apiaries and fed exclusively with aqueous paraquat at 1000 ppm of a.i., large numbers of bees died immediately and all were dead within five weeks (Morton et al. 1974). Based on the available information, it had been concluded by the USEPA (USEPA 1997) that paraquat was non-toxic to slightly toxic to adult bees.

The most recent information available since that time supports instead the conclusions by those previous authors (Moffett et al. 1972; Morton et al. 1972). The survival rate of paraquat-injected queens was greater than that of paraquat-injected workers, due to the presence of the protein vitellogenin acting as an antioxidant (Corona et al. 2007). In a most recent work by Cousin et al. (2013), honeybee combs were transported to the laboratory and the newly hatched larvae were exposed to paraquat (0, 0.001, 0.01, 0.1, and 1 μg/kg of food) for two days. These exposures caused a reduction in embryonic cells, that is, oenocytes, occurring at concentrations as low as 1 ng/kg. Reduction in cell size was concentration dependent.

8.6 Amphibians

Amphibians are generally more susceptible than mammals and birds to the negative effects of agrochemicals, because they have evolved adapting to both aquatic and terrestrial habitats; and their skin is highly permeable because it is involved in water, gas, and electrolyte exchange with the environment. In California there is concern that paraquat may affect the federally threatened California red-legged frog (Rana aurora draytonii), whose habitat includes both coastal and interior mountain ranges (USEPA 2009).
8.6.1 Toxic, teratogenic, and reproductive effects

Several researchers have investigated the sub-lethal and lethal effects of paraquat on amphibians. The Frog Embryo Teratogenesis Assay-Xenopus (FETAX) is a well-established screening method to identify the potential developmental toxicity of single or multiple chemicals in amphibians, and is considered less protective than using more traditional aquatic test species, because it is relatively insensitive. It provides a tool not only to assess mortality, but also malformation and growth inhibition using a standardized method. While there is general agreement on the embryotoxic effects of paraquat in amphibians, there is disagreement regarding its teratogenic effects. This is likely due to the challenges in explaining the number and true cause of the observed malformations in tadpoles (Dial and Bauer 1984; Vismara et al. 2000; Osano et al. 2002).

Dial and Bauer (1984) investigated exposure to paraquat of northern leopard frog (*Rana pipiens*) embryos at field application rates (0, 0.1, 0.5, 2.0, and 10 mg/L). They found that eggs were very resistant to paraquat because development proceeded regularly to hatch up to day four in all exposed groups. However, with the exception of the 0.1 mg/L group, there was an increase in mortality of tadpoles starting three days post hatch in all other groups; and survival rates to day 12 were 75.5, 69.1, 5.5, 0, and 0 % for the 0, 0.1, 0.5, 2.0, and 10 mg/L rates, respectively. Growth rates decreased in all groups and were inversely proportional to rate. They interpreted their data as a clear sign of teratogenic effects, starting at as low as the rate of 0.5 mg/L. After hatch on day four, paraquat caused death, retardation of growth, multiple tail malformations, reduced head development, and reduced motor ability.

The same authors found tadpole survival is a function of age, i.e. there are greater survival rates in older tadpoles. Dial and Dial (1987) compared developing embryos of the northern
leopard frog and 15-day-old tadpoles that were treated with diquat or paraquat. Paraquat treatments (at 0.5 and 2 mg ion/L) were replicated, and occurred when the eggs were at the early gastrula phase for the first group, and when the tadpoles were 15 days old for the second group. The treated eggs were found to be resistant to both herbicides because their development proceeded normally to hatch (day four) in all groups. However, survival of the tadpoles in the first group showed increased mortality. On day 16, survival rate was 6.3 % and 0 % for the first group receiving the 0.5 mg/L and 2.0 mg/L paraquat ion dose, respectively; for the same doses it was 66.7 % and 5 %, respectively, for the 15-day-old tadpoles.

Lajmanovich et al. (1998) studied the acute tolerance to paraquat in *Scinax nasica* tadpoles, as well as conducting morphological analyses to detect alterations in the internal gill structure. Their LC$_{50}$ estimates were 38.96, 29.97, 24.95, and 21.99 mg/L for the 24, 48, 72, and 96 h interval, respectively. The corresponding 120-h LC$_{50}$ (~ 15 mg/L) calculated via interpolation was about 100 times higher than that computed by Vismara et al. (2000) or by Linder et al. (1990), who reported a 96-h LC$_{50}$ of 1.3 mg/L in leopard frog embryos, which would indicate that adverse effects occur at much lower levels.

Paraquat was found embryotoxic but not teratogenic on amphibian development based on FETAX bioassay studies by Vismara et al. (2000). Those authors used probit analysis on the percentage of mortality and the percentage of malformed larvae to investigate toxicity and teratogenic effects. Tadpoles of *Xenopus laevis* were treated with aqueous paraquat at 0.0625, 0.125, 0.18, 0.25, and 0.5 mg/L, and were studied at specific developmental stages, from 8 to 47 h and 120 h post fertilization (PF). They observed significant reductions in larvae growth that were proportional to the treatment concentration. Histological examinations of the larvae that received the 0.125 dose revealed that 29 % of the treated larvae were affected by a specific
malformation, identified as ventral tail flexure. There were statistically significant differences in larvae length, between larvae treated at the lowest rate of 0.0625 mg/L and the control. However, they concluded that such differences were more the result of growth retardation rather than teratogenic effects, because no significant teratogenic effects were noticeable from the time of hatching until day three at the onset of lethal effects. Paraquat was only highly embryolethal with a 120-h-PF LC$_{50}$ of 0.138 mg/L and TC$_{50}$ of 0.267 mg/L. Mortality rates at 120 h PF were 12.4, 41.9, 83.5, 88.8, and 96.6 % for the rates of treatment reported above, respectively.

Osano et al. (2002) studied the teratogenic effects of three pesticides, including paraquat, on *Xenopus laevis* tadpoles using the FETAX bioassay. They found a drastic increase in mortality from 24 h to 96 h with corresponding 96-h LC$_{50}$ estimate of 0.67 mg/L. Malformation in all embryos occurred at concentrations greater than 0.2 mg/L, whereas growth reduction was apparent at all test concentrations (0.1–5 mg/L). Those authors concluded that paraquat should be classified as teratogens as suggested also by the work of Quassinti et al. (2009), showing that paraquat interferes also in amphibian reproductive processes.

8.7 Fish and other aquatic species

Paraquat can contaminate aquatic environments as water runoff due to its high aqueous solubility, and surface or marine waters in countries that allow its use for the control of aquatic plant species (Tortorelli et al. 1990; Ayanda et al. 2015; Ling et al. 2017), where fish mortality may indirectly increase also due to oxygen consumption by dead plants (Summers 1980).

8.7.1 Toxic effects

The susceptibility of fish and other aquatic species in waters treated with paraquat was studied by Fytizas (1980), who simulated under laboratory settings the accidental discharge of paraquat in a water body and its effects on marine species. They estimated the lethal time necessary to kill
50% of the fish exposed ($LT_{50}$) in bony fish ($Mugil cephalus$), the small decapos crustaceous ($Pagurus$ sp.), and the gastropod mollusc ($Murex brandaris$) treated with Gramoxone (20% a.i.) through a bioassay, that is, in all-glass aquaria of 75 L for fish and 35 L for all other species. They found that the $LT_{50}$ at 10 mg/L for fish was 1 h, whereas it was 36 h and 24 h for the crustaceans and gastropods, respectively. The sensitivity was inversed when considering low concentrations instead: $LT_{50}$ values were 16, 10, and 18 days for fish, crustacean, and gastropod, respectively, at the 1 mg/L dose. The fish exposed to the highest doses that were killed within 24 h showed damaged gills covered in mucus, hemorrhage and necrosis of the liver and kidney, and hemorrhagic ulcerations of the digestive tract.

Paraquat action on fish is species dependent as shown by the early work of Earnest (1971), who studied the effects of paraquat applied during 30 min to a pond at a concentration of 1.14 mg/L (using a boat-mounted bar spreader) near the town of Morrison, Colorado. Acute effects were more evident one and two days post application. The most affected species was bluegill ($Lepomis macrochirus$), and least affected was channel catfish ($Ictalurus punctatus$). Paraquat caused distress in 50 bluegills three h following the application, 89 dead bluegills and one dead trout one day post treatment, and at least 34% mortality of the bluegills within two days. Most common malformations were the presence of granuloma involving pancreatic cells.

Toxicity also varies with fish development stages. For example, the Louisiana crayfish ($Procambarus clarkia$) has also been often used as bioassay organism to study pesticide effects due to its commercial role as food supply. Leung et al. (1980) studied adult and juvenile crayfish individuals exposed to six different paraquat concentrations (adult: 0, 15, 25, 35, 50, 75, and 100 mg/L; juvenile: 0, 0.5, 2.0, 4.0, 7.0, and 8.0 mg/L) and used probit analysis to estimate $LC_{50}$s for different time intervals. Differences in mortality between treated and untreated group started to
be detected 48 h post treatment at the lowest doses, that is, 15 mg/L for the adult and 0.5 mg/L for the juvenile group. Adult mortality was 10 % in the 15 mg/L-treated group and 0.9 % in the corresponding control; juvenile mortality was 9.3 % in the 0.5 mg/L-treated group and 6.7 % in the control. The 48-h LC50 estimates were 5.2 mg/L and 39 mg/L for juvenile and adult, respectively.

The 48-h LC50 estimate for juveniles reported by Leung et al. (1980) was the same (i.e. 5.2 mg/L) as that reported by Tortorelli et al. (1990) for catfish fry (*Plecostomus commersoni*), but about 74 times higher than the 96-h LC50 of 0.07 mg/L reported most recently by Ayanda et al. (2015), who studied the effects of paraquat and glyphosate acute exposure in African catfish (*Clarias gariepinus*) juveniles. Discrepancies in these estimates may vary also due to the effect of temperature, an important factor influencing the immune response in fish exposed to paraquat as shown by Salazar-Lugo et al. (2011).

The estimates for the toxicological indices reported above agree also with the very comprehensive review conducted by Summers (1980), indicating that in general adverse, sub-chronic effects to adult fish start to occur at aquatic concentration of <1 mg ion/L for varying durations; and most recent work have indicated that acute effects occur at concentration >10 mg ion/L (Figueiredo-Fernandes et al. 2006; Parvez and Raisuddin 2006; Salazar-Lugo et al. 2011; Ma et al. 2014).

### 8.7.2 Oxidative stress indicators

In the 1980s, the main research focus has shifted to also include the oxidative stress induced by paraquat on fish species due to its redox properties, following the early work of Bus et al. (1976), who hypothesized lipid peroxidation as one of the main mechanisms of toxicity (Tortorelli et al. 1990). Thus, paraquat has been widely used as a model agent of oxidant injury (Gabryelak and
Klekot 1985). A large number of publications have focused on changes in biochemical and histological parameters useful for monitoring environmental exposure of fish to contaminants and laboratory and field studies (e.g. Parvez and Raisuddin 2006). Ayanda et al. (2015) found that paraquat also caused changes in the activities of aminotransferase, aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase, supporting a wealth of other findings that it interferes with the normal functions in fish (Gabryelak and Klekot 1985). Figueiredo-Fernandes et al. (2006) monitored the effects of temperature and gender on selected oxidative stress parameters (hepatic levels of superoxide dismutase[SOD], glutathione reductase [GST], and glutathione S-transferase) in Nile tilapia (Oreochromis niloticus) exposed to 0.5 mg ion/L at 17 and 27 °C for 45 days paraquat. They found that the activities of SOD and GST were sex dependent, and were greater in males compared to females at both experimental temperatures. There was no temperature dependence.

8.7.3 Behavioral analyses

Most recent research has been focused also on paraquat effects on zebrafish (Danio rerio), which is currently considered as a promising experimental model to investigate neurological diseases or for validation of psychopathological models (Bortolotto et al. 2014; Nunes et al. 2017). In their work, Bortolotto et al. (2014) conducted behavioral analyses (locomotion, social interaction, and Y-maze task) on adult zebrafish treated with paraquat at 10 mg/kg and 20 mg/kg (i.e. six paraquat repeated injections, one every 3 days during 16 days) compared to a control. They found that locomotion and distance travelled decreased after 24 h following each injection for both doses, but no significant differences were observed in non-motor behavior (i.e. anxiety-related behavior and social interactions).
A similar study was conducted by Nunes et al. (2017) on four- to six-month-old zebra fish assigned to two experimental groups: the treated (20 mg/kg) and the untreated one following the same injection procedure described in Bortolotto et al. (2014). The two groups were monitored for behavioral changes and biochemical parameters (tissue analysis, mitochondrial viability assay, lipid peroxidation bioassay, reactive oxygen species [ROS] levels, antioxidant enzymes, non-protein thiols, and protein determination). They found that paraquat caused an increase in aggressive behavior, alters non-motor patterns associated with defensive behaviors, and changes in redox parameters of the brain. There were significant changes in most parameters associated with the antioxidant defense system, an unexpected decrease in lipid peroxidation, and no change in ROS concentration.

9 Summary and Conclusions
Review of the literature reveals gaps, considerations, and recommendations for additional research work. These research inquiries will improve our understanding of paraquat environmental fate, and ultimately our ability to develop more accurate strategies for environmental risk assessment.

1. In California, paraquat remains an important post-emergence herbicide that is used to eliminate competing vegetation alone or in combination with other herbicides, such as glyphosate, on the main cash crops cultivated in the state, including almond, alfalfa, and grapes. The increasing number of reported paraquat-resistant species, such as hairy fleabane and horseweed, is currently leading to the development of new strategies for vegetation control.

2. The available monitoring studies in California support findings that paraquat may contaminate surface waters—in very limited cases—through runoff and/or erosion due to
its high aqueous solubility or adsorption onto soil particle. However, conclusions about
the specific mechanism driving these detections remain speculative, because available
experimental studies support the hypothesis that paraquat can be found in surface waters
due to soil erosion only, as paraquat bound to soil or sediment particles—not in an
aqueous solution form. The percentage of reported detections was <1% of the total
sample size.

3. Most recent studies have confirmed that aqueous paraquat can be degraded biologically
and photo-chemically, but such degradation is extremely slow and cannot be considered
as an efficient and viable method to remediate paraquat-contaminated soils or waters.
There are no existing technologies to reclaim contaminated soils containing strongly
bound paraquat that is considered environmentally unavailable. Some transport may
occur due to erosion and runoff processes, but these or similar fluxes from the soil into
terrestrial food chains remain poorly studied often due to the complexity in designing
long-term field studies.

4. Paraquat is considered one of the least toxic pesticides to earthworms. It is moderately
toxic to birds and moderately to highly toxic to many species of fish. Most recent studies
have confirmed that paraquat causes both teratogenic and toxic effects in amphibians. In
California the overall highest reported concentration (3.6 μg/L) detected in surface waters
was about 17 times lower than the value (62.5 μg/L) associated with adverse effects in
amphibians, one of the most sensitive species and an important bio-indicator of pesticide
contamination. Similarly, this concentration was about 278 times lower than the reported
limit in the literature shown to cause adverse effects in fish, i.e. 1 mg/L.
Acknowledgments

Funding for this work was provided by the California Department of Pesticide Regulation (CDPR), California Environmental Protection Agency. The statements and conclusions are those of the authors and not necessarily those of the CDPR. We also appreciate the valuable revisions of Pamela Wofford and Madeline Brattesani during the initial phases of this work. Any reference to commercial products, their source, or their use in connection does not imply actual or implied endorsement of such products.

Disclosure statement

No potential conflict of interest was reported by the authors.
References


45
2013. "Size Changes in Honey Bee Larvae Oenocytes Induced by Exposure to Paraquat at

http://ceden.waterboards.ca.gov/AdvancedQueryTool.


Table 1. Selected physical and chemical properties of paraquat dichloride (C$_{12}$H$_{14}$Cl$_2$N$_2$) (IUPAC name: 1,1'-dimethyl-4,4'-bipyridinium dichloride; CAS Registry number: 1910-42-5).

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (g/mol)</td>
<td>257.2</td>
<td>Molecular weight of paraquat ion is 186.3</td>
<td>Kidd and James (1991)</td>
</tr>
<tr>
<td>Density (g/cm$^3$) at 20 °C</td>
<td>1.24–1.26</td>
<td></td>
<td>Kidd and James (1991)</td>
</tr>
<tr>
<td>Solubility in water</td>
<td></td>
<td>Almost completely dissociated in aqueous solution as cation and anion</td>
<td>Haley 1979; Weed Science Society of America 2014</td>
</tr>
<tr>
<td>Melting point</td>
<td>Approximately 300 °C</td>
<td>Paraquat dichloride</td>
<td>Kidd and James (1991)</td>
</tr>
<tr>
<td>Stability</td>
<td></td>
<td>Degrades under UV light, stable in neutral and acid media, and rapidly hydrolyzes in alkaline solutions</td>
<td>Calderbank and Slade (1976)</td>
</tr>
<tr>
<td>Photochemical degradation in water</td>
<td>3 days</td>
<td>0.1 % paraquat dichloride solution</td>
<td>Slade (1965)</td>
</tr>
<tr>
<td>Vapor pressure</td>
<td>$&lt;1.0 \times 10^{-7}$ (mm Hg at 25 °C)</td>
<td>Negligible at room temperature. Practically non-volatile</td>
<td>Lock and Wilks (2010)</td>
</tr>
<tr>
<td>Octanol-water distribution coefficient, log(K$_{ow}$)</td>
<td>-4.5 (at 20 °C)</td>
<td></td>
<td>Weed Science Society of America (2014)</td>
</tr>
<tr>
<td>Linear K$_d$</td>
<td>1520–2516 (L/kg)</td>
<td>Texture: loam to sandy loam (clay content, 11–22 %)</td>
<td>Pateiro-Moure et al. (2009)</td>
</tr>
<tr>
<td>K$_d$</td>
<td>$7.3 \times 10^4$ (mL/g)</td>
<td>Li-montmorillonite suspension</td>
<td>Vintén et al. (1983)</td>
</tr>
<tr>
<td>Redox system potential</td>
<td>$E = -0.446$ V</td>
<td>Redox potential for one-electron reduction (blue reduced form) measured at pH ranging 8.4–13</td>
<td>Michaelis and Hill (1933)</td>
</tr>
</tbody>
</table>
Table 2. Selected studies on the microbial degradation of bioavailable paraquat, photochemical degradation of aqueous paraquat, and combined mechanisms by environmental media.

<table>
<thead>
<tr>
<th>Degradation type/media</th>
<th>Experimental</th>
<th>Main findings/ by-products</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial degradation/soil</td>
<td>Laboratory incubations over 16 days. Selected microorganisms from paraquat-treated soil (Cahaba loamy fine sand) were grown on paraquat and thin-layer substrate. Autoradiographs of thin-layer electrophoresis plates to track $^{14}$C-methyl-labeled paraquat</td>
<td>The fungus <em>Neocosmospora vasinfecta</em> was capable of reducing paraquat to the colored free radical without any paraquat degradation. A non-identified bacteria degraded paraquat. Identified product was 1-methyl-4,4'-dipyridinium ion</td>
<td>Funderburk and Bozarth (1967)</td>
</tr>
<tr>
<td>Microbial/soil</td>
<td>Laboratory incubation. A $^{14}$C-paraquat dichloride (PD)/soil complex was added to a sucrose medium inoculated with the soil fungus <em>Lipomyces starkeyi</em> Lod. and Rij to monitor the degradation of PD over 72 h. Separation of organic and inorganic soil fractions. Determination of $^{14}$C-PD in soil extracts and emitted $^{14}$C-CO$_2$. Two silt loam soils had 2.9 % and 0.91 % soil organic carbon (SOC); two sandy loam soils had 2.5 % and 1.4 % SOC</td>
<td>Slow transfer of $^{14}$C-paraquat from SOM onto clay minerals. Microbial decomposition occurs only when paraquat is weakly adsorbed (reversible adsorption) onto SOM: no degradation when paraquat is adsorbed onto mineral fraction</td>
<td>Burns and Audus (1970)</td>
</tr>
<tr>
<td>Microbial/in vitro</td>
<td>The soil yeast <em>Lipomyces starkeyi</em> was added to a salt solution free of any N sources except N contained in the $^{14}$C-paraquat that was added as the sole N source</td>
<td>Paraquat degradation was associated with CO$_2$ evolution. Loss of integrity of the cell wall. Removal of the wall resulted in complete loss of degradative capacity</td>
<td>Carr et al. (1985)</td>
</tr>
<tr>
<td>Microbial/soil and plant residues</td>
<td>Laboratory incubation studies using aerobic and anaerobic soil microbes, $^{14}$C-paraquat and non-labelled paraquat, three plant residues (rice straw (<em>Oryza sativa</em> L. cv. Aoinokaze), dropwort (<em>Oenanthe jauanica</em> DC), and Chinese milk vetch (<em>Astragalus sinicus</em> L.), and paraquat treated and non-treated soil. Four conditions were tested: sterilized plants, sterilized with a soil suspension, intact plant residue, and intact plant with soil suspension</td>
<td>No degradation of paraquat adsorbed to sterilized plant residues. Plant-associated microorganisms had higher degradation rates than the soil-associated ones. Higher paraquat degradation under aerobic conditions and for plant residues with higher C/N ratio. Suppression when urea was added. Degradation products from rice straw experiment: monopyridone (1',2'-dihydro-1,1'-dimethyl-2'-oxo-4,4'-bipyridinium ion) and $^{14}$CO$_2$ for rice straw spiked with $^{14}$C-paraquat</td>
<td>Lee et al. (1995)</td>
</tr>
<tr>
<td>Microbial/soil and plant residues</td>
<td>Soils from a banana farm near St. Vincent (West Indies). Laboratory incubation experiment of soils incubated with only 100 mg ion/kg soil, or combined with other two pesticides at 50 mg/kg each. Twenty-one day exposure to indigenous soil bacteria or microbial mat (consortium of cyanobacteria [Oscillatoria sp.] and bacteria) vs. sterilized control under 12 h dark-12 h light and 25 °C</td>
<td><em>Pseudomonas</em> spp. and <em>Flavobacterium</em> spp. were identified as the pesticide-resistant soil bacteria. Rapid microbial degradation in both experiments. In the experiments with indigenous soil microorganisms: paraquat recovery was 40 % and 89.7 % species in non-sterile vs. sterilized soil, respectively, in the paraquat only experiment; it was 52 % at day 21 in the three-pesticide experiments.</td>
<td>Murray et al. (1997)</td>
</tr>
</tbody>
</table>
Lowest overall degradation was for the microbial mat (i.e., 60% recovery).

Extremely slow paraquat degradation due to strong adsorption. Sixty days after treatment, the $^{14}$CO$_2$ evolution was 4.73% in the aerobic sandy loam, 8.18% in the aerobic muck, and 1.94% in the anaerobic muck. Greater $^{14}$CO$_2$ evolution in the non-sterilized soils, indicating a slow rate of microbial degradation.

Cheah et al. (1998)

Microbial/soil

Laboratory incubation experiment over 60 days under controlled conditions (temperature and moisture) using Malaysian agricultural soils (sandy loam and muck soils, Malaysia, with soil organic C of 1.3% and 30%, respectively. Rate: 0.6–0.8 kg ion/ha with $^{14}$C-paraquat. Comparison sterilized vs. non-sterilized

Extremely slow paraquat degradation due to strong adsorption. Sixty days after treatment, the $^{14}$CO$_2$ evolution was 4.73% in the aerobic sandy loam, 8.18% in the aerobic muck, and 1.94% in the anaerobic muck. Greater $^{14}$CO$_2$ evolution in the non-sterilized soils, indicating a slow rate of microbial degradation.

Cheah et al. (1998)

Microbial/soil

Laboratory incubations in a mineral salts medium to foster microbial degradation of bioavailable $^{14}$C-paraquat using microbial cultures (from two UK sandy loam agricultural soils) under dark and aerobic conditions. Sucrose was also added as a C source. The paraquat-soil-culture mixture was incubated for 20–36 days with regular sampling of volatile products

Degradation of bioavailable paraquat is rapid: 50% mass was mineralized to $^{14}$CO$_2$ in three weeks. No paraquat could be detected at the end of the incubations, and the main identified products were $^{14}$C-oxalic acid (85%) and other, non-identified products.

Ricketts (1999)

Microbial degradation/soil. Investigations on the deactivation and sorption capacity of the soil through field and laboratory experiments

Paraquat applied well above the recommended application rates as a one-time or annual application in long-term research trials in four countries. The Frensham (U.K.) trials were the most intensively studied. They received, within the 0–15 cm soil depth increment, a one-time application at rates ranging 90–720 kg ion/ha. The fate of paraquat residue was then monitored over the following 20 years during which fields were maintained under cereal or grassland cultivation. Quantification of earthworm, micro-arthropod (some Collembola and Gamasina species), microbial population (number of microorganisms, total propagules, algae, bacteria, fungi, and actinomycetes population), and biomass (via ATP determination)

The detected paraquat residue indicated that dissipation was extremely slow. Significant decrease in the number of earthworms for the high rate treatments compared to control one year since application; a change in the composition of the earthworm population and no significant difference in the overall number of earthworms compared to control six years since application; no paraquat sorption by earthworms; a significant decrease in the number of micro-arthropod at the highest rates one year after application; no statistically significant differences in total microbial biomass and the number of microorganisms (total propagules, algae, bacteria, fungi, actinomycetes, and the yeast Lipomyces starkeyi) or in the ATP concentration; the population of L. starkeyi significantly decrease in the 720 kg/ha treatment compared to the control

Roberts et al. (2002)

Microbial/soil

Greenhouse experiment under controlled conditions (soil temperature and moisture) over 60 days (clay loam and clay soils from Malaysian agricultural soils). Non-sterilized vs. sterilized soils. Use of $^{14}$C-paraquat to estimate degradation

Faster paraquat degradation for the non-sterilized soils compared to the sterilized ones

Ismail et al. (2011)

Microbial/soil

Field soil cores monitored for 1 month (aplic. rate of 0.75 and 1.725 kg/ha). Incubated laboratory soil columns for 12 weeks (Application. rate of 3.45 kg/ha, twice normal use). Laboratory

Paraquat degradation was faster under field than under laboratory conditions. High degradation rates compared to other studies may depend on

Amondham et al. (2006)

Photochemical and biological degradation/soil
<table>
<thead>
<tr>
<th>Microbial degradation under anaerobic conditions using anthraquinone-2,6-disulphonate (AQDS) (in place of humic substances) to simulate no-tillage paddy fields conditions</th>
<th>Use of three facultative anaerobic bacteria (PQ-1, PQ-2, and PQ-3) isolated from vegetable soil in Sanya city, China. Laboratory batch equilibrium experiments to monitor degradation of paraquat anaerobically with AQDS as the sole electron acceptor with and without sucrose addition (source of energy)</th>
<th>About 100 % paraquat degradation in four days in the presence of AQDS plus sucrose. About 20 % degradation in the presence of AQDS without sucrose. Sucrose addition can significantly enhance paraquat degradation by anaerobic bacteria under anaerobic conditions</th>
<th>Wu et al. (2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^a)Photochemical degradation/aqueous solution</td>
<td>Laboratory experiment. Paraquat in aqueous solution was irradiated with Ultraviolet (UV) light generated by a lamp or under sunlight in the presence of oxygen. Autoradiograph of thin-layer chromatograph of UV-irradiated (^1)C-methyl paraquat</td>
<td>UV light degrades paraquat in presence of O(_2) over three days. However, decomposition of paraquat under sunlight appears to occur only when adsorbed to a surface, e.g. paraquat adsorbed on filter paper or thin layer silica gel, but not when in aqueous solution. Methyl quaternary pyridinium (i.e. 4-carboxy-1-methylpyridinium ion) and methylamine hydrochloride</td>
<td>Slade (1965)</td>
</tr>
<tr>
<td>Photochemical degradation/aqueous solution</td>
<td>Laboratory experiment. Paraquat in (1) solid (dry) and (2) aqueous form under UV radiation</td>
<td>(1) About half dry paraquat was degraded after two days and (\frac{3}{4}) after four days to volatile compounds. (2) Paraquat concentration decreased with increased UV exposure. Very little aqueous paraquat was present after two days. 1-methyl-4-carboxypyridinium chloride and two unidentified degradation products</td>
<td>Funderburk and Bozarth (1967)</td>
</tr>
</tbody>
</table>

\(^a\) Paraquat has a strong UV signal at 257 nm wavelength corresponding to a \(\pi-\pi^*\) transition of the electrons in the pyridinium ring.
<table>
<thead>
<tr>
<th>Overall objectives</th>
<th>Experimental</th>
<th>Half-life estimate</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined soil field and laboratory dissipation study and phytotoxicity study</td>
<td>Paraquat dichloride (PD) applied annually (1967–1972) on a sandy loam soil as one-time single dose of 4.48 kg/ha or</td>
<td>All paraquat applied over a period of six years could be extracted in 1971 and 1973. No degradation took place over seven years. No phytotoxic effects could be observed despite paraquat build up in soil</td>
<td>Fryer et al. (1975)</td>
</tr>
<tr>
<td>by the Weed Research Organization, Oxford, England</td>
<td>four separate doses of 1.2 kg/ha in plots under <em>Medicago sativa</em> L. Soil digestion using H₂SO₄ for paraquat determination. Laboratory incubation experiments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Re-sampling of the study plot described by Fryer et al. (1975) at the Weed Research</td>
<td>Re-analysis of the same plots under the same paraquat dose treatments (see above) during 1975–1978</td>
<td>Based on the 1975–1978 extractions, some paraquat disappeared from plots, i.e. 10% yearly loss regardless of when paraquat was applied. Estimated half-life was 6.6 years</td>
<td>Hance et al. (1980)</td>
</tr>
<tr>
<td>Organization, Oxford, England</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Determination of the rate and by-products of degradation for paraquat (2,4-D, lindane, and glyphosate) in two agricultural soils (sandy loam and muck, i.e. about 30 % soil organic C).</td>
<td>Use of ¹⁴C-paraquat to track degradation. Laboratory soil incubations under aerobic conditions for sandy loam, muck, and anaerobic for muck (Tanjong Karang and Cameron Highlands, Malaysia)</td>
<td>839.2–1072 days (2.6 years), 405.1–650.9 days (1.4 years), and 2289–3652 days (7.2 years), respectively</td>
<td>Cheah et al. (1998)</td>
</tr>
<tr>
<td>Assess degradation, mobility, sorption/desorption of paraquat in agricultural soils of the Yom River basin, Thailand</td>
<td>Field incubation of soil cores in polyvinyl chloride collected at 1, 4, 8, and 12 weeks following application. Two application rates of 0.75 kg/ha and 1.7 kg/ha</td>
<td>Degradation was faster under field conditions than under laboratory conditions with half-life estimate of 36–46 days</td>
<td>Amondham et al. (2006)</td>
</tr>
<tr>
<td>Monitoring paraquat degradation and testing the effect of soil temperature and moisture on paraquat degradation in Malaysian agricultural soils</td>
<td>Use of ¹⁴C-paraquat to estimate degradation comparing non-sterilized vs. sterilized soils. Application rate was 0.6–1 kg/ha</td>
<td>Clay loam soil: 187 days (non-sterilized) and 1386 days (sterilized). Clay soil: 231 days (non-sterilized), 1733 days (sterilized)</td>
<td>Ismail et al. (2011)</td>
</tr>
</tbody>
</table>
List of Figures

Figure 1. Synthesis of the paraquat dichloride salt. In the paraquat cation, the number-4 carbon atom joins the two pyridine rings and each nitrogen atom has a methyl group (Based on Cairns and Case [1975]).

Figure 2. Annual paraquat dichloride mass used in California during 2000–2014.

Figure 3. Cumulative frequency plot of paraquat dichloride-treated area (a) and mass per application (b) and rate of application vs. percentile (c) by year (2000–2014) in California (p5 through p95 are the 5th through the 95th percentile, respectively, of the frequency distribution of interest, CDPR [2016]).

Figure 4. Map of the average annual mass of paraquat applied primarily on agricultural commodities in different California counties during 2000–2014 (CDPR 2016).

Figure 5. Total paraquat dichloride mass applied to the top 20 crops (based on use) in California during 2000–2014 (CDPR 2016).

Figure 6. Proposed pathway of paraquat dichloride (1) degradation by soil microfauna, leading to the formation of a 4-carboxilated-1-methylpyridinium ion (2) followed by oxalic acid (3) and other non-identified products (not shown), and ultimately to mineralization products (4) (modified after Funderburk and Bozarth [1967] and Ricketts [1999]).
Figure 7. Pathway of paraquat dichloride (1) degradation by ultraviolet light in presence of oxygen. Identified degradation compounds were amino-aldehyde (likely) (2), 4-carboxy-1-methylpyridinium ion (3), and methylamine hydrochloride (4). Compounds (3) and (4) do not form in the absence of oxygen (modified after Slade [1965]).
The initial reaction is between the product resulting from the metal-pyridine interaction and an alkylene oxide in liquid ammonia at temperatures normally not exceeding -33 °C. In its usually oxidized form, paraquat is ionized with two positive charges. Thus, paraquat is usually manufactured as a salt containing two chloride anions.
Sacramento Valley
San Joaquin Valley

Average mass (kg/yr)
- 0.001 - 6
- 7 - 58
- 59 - 419
- 420 - 5911
(1)  \[ \text{H}_3\text{C}-\text{N}^+\text{CH}_3 \] \{ 2 \text{Cl}^- \}

\[ \text{H}_3\text{C}-\text{N}^+ \] \[ \text{COO}^- \] \text{Cl}^-

(2)  \[ \text{H}_3\text{C}-\text{N}^+ \] \[ \text{COO}^- \]

(3)  \[ \text{HO} \] \[ \text{OH} \]

(4)  \[ \text{NH}_3 + \text{CO}_2 + \text{H}_2\text{O} \]