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Rapidly formed quinalphos complexes with transition metal ions characterized by electrospray ionization mass spectrometry

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RATIONALE: Electrospray ionization tandem mass spectrometry (ESI-MS/MS) offers the unique opportunity to characterize complexes of the organophosphorus pesticide (OP) quinalphos (PA-Q) with transition metal ions immediately formed after contact. This study complements research looking at longer term kinetics of quinalphos hydrolysis in the presence of transition metal ions and gives insights into the structural features of the initial complex formation in solution. (Hydrolysis reaction: $PA-Q+H_2O \rightarrow PA-OH+HQ$, where PA-OH is the diethyl phosphate product and HQ is hydroxyquinoxaline.)

METHODS: Low micromolar PA-Q solutions with an approximately 3-fold molar excess of transition metal ions were immediately analyzed after mixing. Fragmentation of the transition metal ion complexes with PA-Q was accomplished in two different ways: first, in-source fragmentation by elevating the declustering potential and second, low-energy collision-induced dissociation (CID).

RESULTS: For Ag⁺, the [PA-Q-Ag⁺] and respective Ag⁺-containing degradation product ions are readily observed. For Cu²⁺, we observed the [PA-Q+Cu²⁺ + NO₃] complex ion with weak intensity and strong signals from both the [2PA-Q+Cu⁺] and the [PA-Q+Cu⁺] ions, the latter two attributable to charge-state reduction in the gas phase from Cu(II) to Cu(I), indicating that PA-Q fulfills specific structural requirements of the formed complex for charge-state reduction during transition from solution to the gas phase. For Hg²⁺, the [PA-Q+Hg²⁺ + (PA-OH-H)⁻] ion was the largest observed species containing one Hg²⁺ ion. No 1:1 species ([PA-Q] or other degradation products:Hg²⁺) was observable.

CONCLUSIONS: ESI-MS/MS of complexes formed from PA-Q and transition metal ions is a formidable technique to probe initial formation of these complexes in solution. Previous work from other groups established structural requirements that enable charge-state reduction from Cu(II) to Cu(I) in ligand complexes during transition into the gas phase, and these rules allow us to propose structural features of PA-Q complexes with copper ions in solution. Copyright © 2013 John Wiley & Sons, Ltd.

The synthesis of organophosphates was initiated by Boudet, Boullay, Lassaigne, Pelouze and Vögeli in the first half of the 19th century.^[1] This research area eventually evolved into many different important industrial production streams, one of them being the development of organophosphorus pesticides (OPs). Today, OPs are employed worldwide in widespread applications in agriculture as pesticides.^[2] OPs are often preferred to organochlorine pesticides, which show higher potential for bioaccumulation and longer persistence in the environment.^[3] Nevertheless, bioaccumulation of OPs and their toxic properties to non-target organisms are concerns, and the study of potential degradation pathways and their activation is of great interest.^[4,5]

Recent studies have demonstrated that heavy metals associated with colloidal particles are much more mobile in biogeochemical environments than previously thought.^[6] Release of metal-containing colloidal particles into soil suspensions and water streams depends on many factors including pH and ionic strength, which can be influenced by rainfall, soil contamination, fertilization or other chemical or physical soil perturbations.^[6] As a result, potential interactions of transition metal ions with pesticides either in surface soils, ground- or surface waters are very common and combined effects of heavy metal ions on pesticide adsorption to soil (or vice versa) have been reported in the current literature,^[7] including for organophosphates.^[8] A combined study on the presence of pesticides and heavy metals in a lake in India reported the presence of several heavy metals and 11 pesticides, including quinalphos, at medium to high ppb levels.^[9]

In the environment, OPs degrade mainly *via* photocatalytic,^[10] microbial,^[4,11] or hydrolytic^[12] pathways. Increased hydrolysis rates have been observed previously in the presence of transition

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metal ions.^[12,13] Other work has also demonstrated the ability of pesticides to form complexes with these metals, most likely at –S and –N sites in the respective molecules. For example, a recent nuclear magnetic resonance (NMR) study provided evidence of complex formation between Hg^{2+} and S atoms of the pesticide Demeton S.^[14] Interestingly, in that case the complexation process led to enhanced stability of the molecule with respect to its hydrolytic degradation, relative to the uncomplexed form. In contrast, our studies have shown that complexes of OPs with transition metal ions such as Hg^{2+} , Ag^+ , and Cu^{2+} show accelerated hydrolytic degradation, including diazinon and quinalphos.^[15,16] On the other hand, no degradation rate increase has been observed in the presence of Na⁺ and K⁺, which is likely due to the inability of these 'hard' metal ions^[17] to form similar complexes with the pesticide.

We and others have previously studied nucleophilic catalysis and degradation pathways of organophosphorus esters including OP pesticides.^[18–23] Importantly, both types of processes, H/D exchange and nucleophile catalysis, involve coordination of a metal ion into a Lewis base site (N, O, S) in the heterocyclic residue and ester moiety, respectively.

Establishing new models of the interaction between heavy metals and pesticides in the environment is an essential first step in filling current knowledge gaps on fate and distribution of pesticides and their metabolites. To expand and complement our previous catalysis studies we apply advanced mass spectrometric methods to look at complexes of transition metal ions and pesticides immediately formed on first contact. The soft nature of electrospray ionization mass spectrometry (ESI-MS) in combination with mass spectrometric detection has long been recognized as a formidable tool to characterize transition metal ion complexes in solution,^[24] as long as the species of interest survive the transfer into the gas phase and remain charged. Other spectroscopic techniques such as NMR are considered very powerful tools in structural studies of solvated molecules or complexes;^[25] however, due to dynamic solution equilibria or potential rapid degradation of formed complexes, interpretation of results is difficult or restricted to averaged properties of the investigated systems. The usefulness of ESI-MS results for interpretation or prediction of actual solution conditions is also under ongoing debate,^[26] and several studies have proposed the quantitative correlation of observed phenomena with ESI-MS gas-phase studies to actual solution conditions.[27-29]

Recently, Park *et al.* reported the ESI-MS structural characterization of several transition metal ion complexes formed with alachlor, an organochlorine pesticide, with the conclusion that in that case the transition metal ions exert their catalytic activity *via* an insertion mechanism into the C-Cl bond followed by Cl elimination.^[30]

The importance of OPs in agriculture and the scarcity of information currently available on associated OP degradation mechanisms induced by transition metal ion catalyzed complexation led us to carry out a study of the structural features of OP-transition metal ion complexes in solution with ESI-MS/MS, complementing our previous kinetic degradation studies, employing Ag⁺, Cu²⁺ and Hg²⁺ as examples.^[15]

An interesting aspect of mass spectrometric analysis of transition metal ion complexes are observations that during transfer of multiply charged metal ions and metal complexes from solution into the gas phase (i.e. here the electrospray ionization process), rearrangements of ion complexes and charge reduction processes take place, due to coulombic repulsion of localized charges.^[31–33] For complexes with a doubly charged metal ion, charge reduction typically involves elimination of a proton originating from one of its ligands [Me(II)L–H]⁺.^[33]

In the case of Cu(II), however, another phenomenon that involves reduction of oxidation states from Cu(II) to Cu(I) has also been described for some specific Cu(II) complexes with nitrogen-containing ligands.^[33] Reasons for this given by the authors are the high second ionization potential of Cu in the gas phase, the specific solvent environment, and the nature of the interacting ligands, and were attributed to direct occurrence in the gas phase and not to electrochemical redox reactions at the ESI tip: a complete understanding of the process is, however, still lacking.^[33]

For the above-mentioned alachlor complexation study, Park *et al.* pointed out the more stable d^{10} electron configuration for Cu(I) as an explanation for the observed Cu(II) to Cu(I) charge reduction.^[30]

Other studies also mention the much higher second ionization potential of Cu, compared to Co, Zn, and Ni, explaining why gas-phase charge reduction is only observed for Cu(II), and, in addition, they provide additional suggestions on the structural requirements of ligands that potentially enable charge-state reduction of Cu(II) to Cu(I) in the gas phase.^[34,35] For example, Tintaru *et al.* have shown that Cu(II) to Cu(I) charge-state reduction depends on the position of the benzoylamino substituent in (Nbenzoylamino)acridine isomers.^[35] For 4-(N-benzoylamino)acridine no charge-state reduction for Cu(II) to Cu(I) was observed and this was explained with activation of one of the N-H bonds in the benzoylamino substituents followed by elimination of a proton, due to the close proximity of Cu(II) and ligand binding sites. For 2-(N-benzoylamino)acridine, however, a charge-state reduction for Cu(II) to Cu(I) was observed, leading to a complex ion with one mass higher for this isomer complex compared to the previous case. As an explanation the authors propose that in the 2-(N-benzoylamino)acridine-copper ion complex the benzoylamino substituent is too far removed from the other acridine ligand binding sites so that N-H bond activation cannot take place and the Cu(II) charge state cannot be supported in the gas phase, subsequently forcing a charge-state reduction of Cu(II) to Cu(I) concurrent with the formation of an uncharged radical (in this case Cl[•]).^[35]

In other work of the same group, the authors propose that the electron-rich, multidentate and soft donor atoms containing a macrocyclic thiacrown ether ligand, as used in this gas-phase copper complexation study, provides the necessary environmental and structural requirements for the formation of 'soft' Cu(I) from 'hard' Cu(II), (adopted from Pearson's "Hard and Soft Acids and Bases" (HSAB) concept^[17]) following coordination of copper(II) ions to the ligand molecules.^[34] The above summarized principles for charge reduction during solution to gas-phase transfer will be addressed specifically for our observations with Cu²⁺ and Hg²⁺ complexation with quinalphos.

Silver and silver salts occur naturally and are also found as contaminants in environments polluted *via* wastes originating from photo-processing industries or in run-offs of silver mining

operations.^[36] Free silver ions are present in the environment and the bulk of them undergo rapid complexation with organic or inorganic ligands or reduction to metallic silver. Nevertheless, the proportion of free silver ions is significant and measurement of free silver ions in environmental samples is often used to estimate total silver content.^[36] Ag⁺, as an example for a singly charged transition metal ion, has the benefit of two almost equally abundant isotopes (¹⁰⁷Ag and ¹⁰⁹Ag) allowing for detailed fragment identification in MS/MS studies, as shown below.

 Cu^{2+} is an essential element ion for plant growth and often employed in fertilizers, e.g. foliar sprays (e.g. as $Cu(OH)_2$),^[37] making the combination of OPs and Cu^{2+} in nature very likely. Cu^{2+} ions are the most abundant form of copper found in surface waters.^[38] In this work we chose to focus only on Cu^{2+} species, albeit Cu(I) compounds are known to enter the environment due to their use as pesticides in agriculture.^[38] The presence of Cu^+ ions in water is likely very limited due to their low solubility;^[39] nevertheless, they might be present under reducing conditions such as in anoxic ponds, soils and sediments.

 Hg^{2+} is introduced into soils and waters mainly *via* deposition from atmospheric sources, principally coal combustion, and is thus a widespread contaminant in the environment.^[40] Other potential contamination from organic mercury-containing seed dressings in agricultural applications is in decline due to abolishment of mercury use in these products in the 1970s.^[41] Over 90% of the total mercury in the atmosphere is in the volatile metallic form (Hg⁰).^[42] Deposited metallic Hg is potentially oxidized to Hg²⁺ in soil and waters, albeit mechanisms are not yet fully understood and it is estimated that 3–4% is present in the divalent form (Hg²⁺) and 2–3% in methylated species.^[40] We chose to focus solely on the Hg²⁺ ions, due to their toxicity and higher solubility compared to Hg(I) species and metallic Hg.

Our particular interest presented here focuses on rapidly formed complexes after mixing of quinalphos (or *O*,*O*-diethyl *O*-quinoxalin-2-yl phosphorothioate) with Ag⁺, Cu²⁺, and Hg²⁺, since such a study is not always practical with NMR, due to the fast transition metal ion induced hydrolysis, especially for Hg²⁺. Our approach eliminates interferences of hydrolysis products *via* potential ionization suppression and allows discerning in-source fragments from hydrolysis products. ESI-MS also enables the study of these complexes at the micro-molar concentration level as compared to NMR studies, requiring high millimolar concentrations.^[12]

EXPERIMENTAL

Materials and reagents

Quinalphos (PA-Q, formula: $C_{12}H_{15}N_2O_3PS$, MW: 298.3 g/mol) and its hydrolysis products (PA-OH (the methyl ester was purchased since the ethyl ester was not commercially available), formula: $C_2H_7O_3PS$, MW: 142.1 g/mol, and hydroxyquinoxaline (HQ), formula: $C_8H_6N_2O$, MW: 146.1 g/mol) were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). Solvents (HPLC grade), transition metal salts and acids were from Fisher Scientific (Ottawa, Ontario, Canada). All chemicals were used without further purification. Quinalphos, its hydrolysis products and the transition metal

salts are toxic and were handled according to standard laboratory safety procedures employing personal protective gear and handling of the chemicals in a certified fume hood for dangerous chemicals.

Mass spectrometry

Low micromolar concentration quinalphos solutions with an approximately 3-fold molar excess of transition metal ions present in methanol/water (1:1) or for the case of Hg(NO₃)₂ in nitromethane, acidified to pH 4 with nitric acid, were infused into a QSTAR XL QqTOF mass spectrometer (Applied Biosystems/MDS Sciex, Toronto, Ontario, Canada) at a flow rate of 6 μ L/min. The instrument was equipped with a standard electrospray ionization (ESI) source and was operated at ambient temperature in positive mode.

Fragmentation of the transition metal complexes with quinalphos and its hydrolysis products was accomplished in two different ways: first, in-source fragmentation by elevating the declustering potential and second, low-energy collision-induced dissociation (CID) in the Q_2 quadrupole collision cell employing nitrogen as collision gas. Control experiments were done separately with the commercially available hydrolysis products of quinalphos.

Graphics

Chemical structures were drawn with ChemSketch Freeware (version 12.0, Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2011^[43]).

RESULTS AND DISCUSSION

Hydrolysis of quinalphos and its products

The structures of quinalphos and its hydrolysis products are shown in Fig. 1. The half-life for disappearance of PA-Q was determined to be 119 days in the absence of transition metal ions, but otherwise under employing the same conditions as for our ESI-MS studies (pH 4).^[15] Experiments were carried out in sealed quartz vessels under ambient light conditions at room temperature. Aliquots of sample solutions were compared by HPLC-UV to freshly prepared external



Figure 1. Hydrolysis scheme of quinalphos. (**PA-Q**, formula: $C_{12}H_{15}N_2O_3PS$, MW: 298.3 g/mol). The two hydrolysis products are 2-hydroxyquinoxaline (**HQ**, formula: $C_8H_6N_2O$, MW: 146.1 g/mol) and *O*,*O*-diethyl phosphorothioic acid (**PA-OH**, formula: $C_4H_{11}O_3PS$, MW: 170.2 g/mol).

standard solutions over several months.^[15] Our determined half-life for quinalphos in the absence of transition metal ions is longer than that recently reported by Gupta et al. (47 days at pH 6.3),^[44] which is most likely due to the lower pH employed in our setup. Introducing an excess molar amount of alkali ions (Na⁺, K⁺) did not affect the hydrolysis rate. Half-lives for disappearance of PA-Q in the presence of a 1–30 molar excess of Hg^{2+} , Ag^+ and Cu^{2+} were 19 s, 9 h and 27 days, respectively, confirming strong catalytic effects of these ions in the hydrolysis of PA-Q.^[15] The recent study of Gupta et al. on the decay pathways of PA-Q in waters and soils suggests that hydrolysis is the initial main pathway for PA-Q degradation in the environment.^[44] The study also suggests that some of the hydrolysis products are even more toxic and persistent than PA-Q itself,^[44] giving urgency to better understand transition metal ion induced hydrolysis of PA-Q.

Electrospray ionization mass spectrometry

Electrospray ionization in combination with hybrid tandem mass spectrometry, as for example in a QTOF mass spectrometer, has several advantages compared to conventional single-stage mass spectrometry. Fragmentation of analytes, i.e. the transition metal-pesticide complexes, can be achieved in two different ways: first, in-source fragmentation by elevating the cone voltage and second, low-energy CID in the Q₂ collision cell using nitrogen as collision gas. Combination of both methods allows for pseudo-MS³ analysis of the complexes.^[45] In addition, accurate mass measurements enable the correct assignment of fragments (see example given in Supplementary Fig. S1, Supporting Information). The employed QSTAR instrument from Applied Biosystems allows a narrow window selection less than 1 Da in the first quadrupole (Q1) for MS/MS experiments.^[46] This feature allows confirmation and correct assignment of fragment ions that contain transition metals having more than one abundant isotope (e.g. ¹⁰⁷Ag/¹⁰⁹Ag, or ⁶³Cu/⁶⁵Cu, see Supplementary Figs. S2 and S3, Supporting Information).

Our goal was to observe and characterize the quasi instantaneously formed transition metal ion–quinalphos complexes after contact, ideally before any hydrolysis is induced. This has the following advantages: (1) any interferences (e.g. ionization suppression from hydrolysis products) are avoided; (2) all observed signals are originating from PA-Q, the transition metal ions and/or their complexes, avoiding any confusion that might arise from the presence of hydrolysis products and confirming the identification of in-source fragments as shown in an example below for Cu²⁺. We therefore performed MS analysis directly after mixing of transition metal ion and quinalphos solutions with minimal incubation times, i.e. only the time required for mixing, experimental setup and injection into the ESI source.

The ESI-MS/MS study of the formed complexes enables a fundamental comparison to sodiated and potassiated pseudomolecular ions of PA-Q (i.e. $[PA-Q+Na^+]$, $[PA-Q+K^+]$). ESI-MS/MS studies of $[PA-Q+Na^+]$ and $[PA-Q+K^+]$ do not yield any meaningful fragments,^[15] except small signals for Na⁺ and K⁺ (data not shown), confirming the mere electrostatic attraction of these alkali ions to PA-Q, compared to the strong chelation of transition metal ions to PA-Q, yielding transition metal ion containing fragments during CID as shown below. Throughout our study we observed only singly charged ion species, which is in contrast to other studies (e.g. the alachlor study of Park *et al.*^[30]).

AgNO₃ interaction

Table 1 shows the observed species from solutions containing AgNO3 and quinalphos and its hydrolysis products (HQ and PA-OH), the largest complex observed being the 1:1 complex $[PA-Q+Ag^+]$ (Formula: $[C_{12}H_{15}N_2O_3PS+Ag^+]$; theoretical monoisotopic weight for ¹⁰⁷Ag⁺: 404.95920 u; experimental results: 404.96098 u; error: 4.4 ppm). Figure 2(A) shows the fragmentation spectra of the [PA-Q+Ag⁺] complex and a proposed fragmentation pathway for the major fragments. To determine which fragments contain Ag⁺, a direct MS/MS comparison of the complex ions $[PA-Q+^{107}Ag^+]$ and $[PA-Q+^{109}Ag^+]$ was performed employing the one unit resolution capability in the Q1 zone of the QTOF MS instrument (see Supplementary Fig. S2, Supporting Information). The MS/MS spectrum (Fig. 2(A)) shows fragment ions containing both HQ and PA portions of PA-Q and Ag⁺ as charge bearers $[HQ+Ag^+]$ and $[PA-C_2H_5+Ag^+]$, respectively), (i.e. confirming the retention of a strong coordination of Ag⁺ to both N and S in the PA-Q molecule in the gas phase, as is expected to occur already in solution.^[17,47] The observation of 'naked' Ag⁺ ions can be explained through neutral losses from or dissociation of two fragment ions (i.e. [HO+Ag⁺] and $[PA-C_2H_5+Ag^+]$) or directly through dissociation of the $[PA-Q+Ag^+]$ complex. We suggest that the occurrence of the $[HQ + H^+]$ ion with m/z 147 could be explained by a neutral loss of [PA-2H + Ag] from the precursor $[PA-Q + Ag^+]$ ion complex; however, our experiments cannot provide a further detailed explanation for such a mechanism. The observation of the [HQ + Ag⁺] ion already in the MS scan (see Table 1) could either be due to the interaction of Ag⁺ with HQ resulting from preceding PA-Q hydrolysis, or due to in-source fragmentation of the $[PA-Q+Ag^+]$ ion. We suggest that the latter option is the main cause, since increasing the cone voltage also increases

Table 1.	Related	ion	species	observed	in	MS	scan	for
AgNO ₃ a	ddition		•					

Compound	Major peaks observed	Proposed ion formula
PA-Q	<i>m/z</i> 405 and 407 <i>m/z</i> 377 and 379 <i>m/z</i> 299 <i>m/z</i> 253 and 255 <i>m/z</i> 147	$\begin{array}{l} [PA-Q+Ag^+] \\ [PA-Q-C_2H_4+Ag^+] \\ [PA-Q+H^+] \\ [HQ+Ag^+] \\ [HQ+H^+] \end{array}$
HQ	<i>m</i> / <i>z</i> 107 and 109 <i>m</i> / <i>z</i> 253 and 255 <i>m</i> / <i>z</i> 147 <i>m</i> / <i>z</i> 107 and 109	Ag^+ $[HQ + Ag^+]$ $[HQ + H^+]$ Ag^+
PA-OH	<i>m</i> / <i>z</i> 249 and 251	$[PA-OH + Ag^+]$
(methyl ester) s но Росн ₃	<i>m/z</i> 217 and 219 <i>m/z</i> 155 and 157 <i>m/z</i> 107 and 109	$[C_2H_7PO_3 + Ag^+]$ (loss of S) [HPO + Ag ⁺] Ag ⁺





Figure 2. MS/MS spectra and proposed fragmentation pathways of transition metal ion–quinalphos complexes: (A) for the observed complex $[PA-Q+^{107}Ag^+]$ with m/z 405 and (B) for the observed complex $[PA-Q+^{63}Cu^+]$ with m/z 361. The fragmentation pathways for both complexes are similar and differ only in observed fragmentation ion intensities.

the intensity of the [HQ + Ag⁺] ion signal (data not shown) and significant hydrolysis should not have happened at that point, albeit the first option cannot be completely excluded from consideration.

In summary, for Ag^+ we observed only a 1:1 complex with PA-Q and the fragmentation experiment indicated strong coordination of Ag^+ to N- and S-ligand binding sites, suggesting that the observed gas-phase complex retains the main structural features of the expected complex formed already in solution.

Cu(NO₃)₂ interaction

Table 2 shows the observed species from solutions containing Cu(NO₃)₂ and quinalphos and its hydrolysis products (HQ and PA-OH). All observed complexes are singly charged and a weak signal of the [PA-Q + Cu²⁺ + NO₃] complex is observed together with two complexes that involve Cu(II) to Cu(I) charge reduction in the gas phase ([2PA-Q+Cu⁺] and [PA-Q+Cu⁺]) (formula for [PA-Q+Cu⁺]: [C₁₂H₁₅N₂O₃PS+Cu⁺]; theoretical monoisotopic weight for ⁶³Cu⁺ complex: 360.98370 u; experimental results: 360.98444 u; error: 2.0 ppm). Figure 2(B) shows the fragmentation spectra of the [PA-Q+Cu⁺] complex and a proposed fragmentation pathway.

Applying the summarized principles in the Introduction for charge-state reduction during transfer from solution to the gas phase to PA-Q in our work, we propose that one Cu(II) ion coordinates with two PA-Q molecules in solution (see Fig. 3). This structural setup provides the Cu(II) ion with multiple electron-rich ligand binding sites (N- and S-) but no activatable N–H, O–H or S–H bond in close proximity that

Cu(NO ₃) ₂ addition				
Compound	Major peaks observed	Proposed ion formula		
PA-Q	<i>m</i> / <i>z</i> 659 and 661 <i>m</i> / <i>z</i> 423 and 425 <i>m</i> / <i>z</i> 361 and 363 <i>m</i> / <i>z</i> 333 and 335 <i>m</i> / <i>z</i> 299 <i>m</i> / <i>z</i> 209 and 211 <i>m</i> / <i>z</i> 147	$\begin{array}{l} [2PA-Q+Cu^{+}] \\ [PA-Q+Cu^{2+}+NO_{3}^{-}] \\ [PA-Q+Cu^{+}] \\ [PA-Q-C_{2}H_{4}+Cu^{+}] \\ [PA-Q+H^{+}] \\ [HQ+Cu^{+}] \\ [HQ+H^{+}] \end{array}$		
HQ	<i>m/z</i> 208 and 210 <i>m/z</i> 180 and 182 <i>m/z</i> 147	$[HQ - H^{+} + Cu^{2+}]$ [HQ-H ⁺ - CO + Cu ²⁺] [HO + H ⁺]		
PA-OH	<i>m</i> / <i>z</i> 204 and 206	$[PA-OH - H^+ + Cu^{2+}]$		
(methyl ester)	<i>m</i> / <i>z</i> 110 and 112	$[PO^{-} + Cu^{2+}]$		

Table 2. Related ion species observed in MS scan for

would allow subsequent proton elimination during transfer from solution to the gas phase. We further propose five pathways (i–v, Fig. 3) that suggest how the three main observed species in the MS scan could be formed (see Table 2): (i) Through charge-state reduction from Cu(II) to Cu(I) and elimination of an NO₃[•] radical, $[(PA-Q)_2 + Cu^+]$ is formed. Radical formation of both Cl^{•[34]} and NO₃^{•[48]} have been



Figure 3. Proposed structure for the Cu(II) complex with two PA-Q molecules in solution and possible reaction pathways (i–v) for the observed ion species in the MS scan after transfer to the gas phase (explanation in the text). There is no direct experimental evidence for pathway ii to occur, but CID MS/MS spectra (see Supplementary Scans 5 and 6, Supporting Information) for ion complexes [(PA-Q)₂+Cu⁺] with *m*/*z* 659/661 and [PA-Q+Cu²⁺ +NO₃] with *m*/*z* 423/425, yielding [PA-Q+Cu⁺] with *m*/*z* 361/363 as the main fragment, support the suggested pathways iv and v.

suggested before as a concurrent complementary reaction for Cu(II) to Cu(I) charge reduction in the gas phase, with NO₃. formation found to occur easier compared to Cl[•] formation,^[48] with no further explanation given. (ii) The formation pathway of $[PA-Q+Cu^+]$ follows exactly (i) but, in addition, involves a neutral loss of one coordinated PA-Q molecule during transfer to the gas phase. (iii) The formed $[PA-O+Cu^{2+}+NO_3]$ ion is the only observed species in the gas phase, in which Cu(II) retains its solution charge state, and could possibly be preceded by the formation of a gasphase $[(PA-Q)_2 + Cu^{2+} + NO_3^{-}]$ complex that subsequently loses a neutral PA-Q molecule or eliminates an NO3 radical with concurrent Cu(II) to Cu(I) charge reduction to form $[(PA-O)_2 + Cu^+]$; however, we do not have any experimental evidence for the presence of such a precursor ion. (iv) This pathway occurs most likely completely in the gas phase and involves a neutral loss of PA-Q from $[(PA-Q)_2 + Cu^+]$ to yield $[PA-Q+Cu^+]$. A CID MS/MS study of the $[(PA-Q)_2+Cu^+]$ ion yields [PA-Q+Cu⁺] as a main fragment, supporting this proposed pathway (see Supplementary Scan 5, Supporting Information). (v) Again, this pathway occurs most likely in the gas phase and involves a Cu(II) to Cu(I) charge-state reduction and elimination of a NO3° radical. A CID MS/MS study of the $[PA-Q+Cu^{2+}+NO_3^{-}]$ ion yields $[PA-Q+Cu^{+}]$ as the main fragment (see Supplementary Scan 6, Supporting Information), supporting our proposed pathway. This MS/MS experiment also suggests that Cu(II) to Cu(I) charge-state reduction can occur readily in the gas phase and is not linked to potential electrochemical processes at the electrospray tip. Previous reports have also proposed that potential electrochemical reactions at the ESI tip are not responsible for observed Cu(II) to Cu(I) charge-state reduction in mass spectrometric studies of Cu(II)-ligand complexes.^[34,35]

When added to the hydrolysis products HQ or PA-OH, Cu(II) remains in the di-positive state and charge reduction of the observed complex ions occurs via ligand-proton elimination ($[HQ-H^++Cu^{2+}]$, $[PA-OH-H^++Cu^{2+}]$, see Table 2) as described as one of the potential charge-reduction

mechanisms of multiply charged transition metals in the introduction. Applying the proposed principles of Schröder's group,^[35] this can be explained with the close proximity of the activatable O–H bonds in both cases, after Cu(II)–ligand coordination.

The MS/MS spectrum of the quinalphos-copper complex $([PA-Q+Cu^{+}])$ follows closely the same fragmentation pathway as proposed for $[PA-Q+Ag^+]$ (see Figs. 2(A) and 2(B)). The main difference is in the lower intensity of the $[PA - C_2H_5 + Cu^+]$ fragment ion compared to its counterpart the $[PA - C_2H_5 + Ag^+]$ fragment ion. This potentially indicates a stronger affinity of Cu(I) to N- rather than to S-binding sites on PA-Q compared to Ag⁺. The intensity of the 'naked' Cu⁺ ion is lower than that of the respective Ag⁺ ion under the similar experimental conditions. With increasing CID energy the intensity of this ion can be slightly increased, however not to the same extent as seen for Ag⁺, and it remains unclear if instrument/detector mass bias, high Cu(I)-ligand affinity and/or other unknown reasons are responsible for this observation. As was the case for $[HQ + Ag^+]$, the $[HQ + Cu^+]$ ion is observed both in the MS/MS spectrum of $[PA-Q+Cu^+]$ and already in the MS scan (see Table 2). It is clear that the observation of the [HQ+Cu⁺] ion with m/z 209 already in MS scan is due to in-source fragmentation of $[PA-Q+Cu^+]$, since direct interaction of Cu(II) with HQ (potentially present due to partial hydrolysis of PA-Q in solution) would yield a $[HQ-H^++Cu^{2+}]$ ion with m/z 208, as described above for the results from mixing HQ with Cu(II). Again as seen for $[HQ+Ag^+]$ above, further support for this finding is the observation of increased ion intensity at m/z 209 in MS mode, when the cone voltage is increased (data not shown).

Hg(NO₃)₂ interaction

Table 3 shows the observed species from solutions containing $Hg(NO_3)_2$ and quinalphos and its hydrolysis products (HQ and PA-OH). For the experiments with mercury, the solution system was changed from methanol/water to nitromethane,

to avoid complicated complex formations containing additional solvent molecules and/or sodium or potassium ions as observed with the $MeOH/H_2O$ solvent system (data not shown). Nitromethane has proven to be a formidable solvent

Table 3. Related ion species observed in MS scan for

$Hg(NO_3)_2$ addition					
Compound	Major peaks observed (for ²⁰² Hg)	Proposed ion formula			
PA-Q	m/z 909	$[3(PA-OH - H)^{-} + 2Hg^{2+}]$			
-	<i>m/z</i> 669	$[PA-Q+Hg^{2+}+(PA-OH-H)^{-}]$			
		see Fig. 4			
	<i>m/z</i> 541	[PA-OH + Hg + (PA-OH - H)] see Fig 4			
	m/z 405	$[PA-OH + HgSH^+]$ see Fig. 4			
	m/z 377	$[PA-OH - C_2H_4 + HgSH^+]$			
HQ	m/z 347	$[HQ - H^{-} + Hg^{2+}]$			
	$m/z \ 147$	$[HQ + H^+]$			
PA-OH	m/z 343	$[PA-OH - H^+ + Hg^{2+}]$			
(methyl ester)					
S OCH ₃					
HO OCH3					

for ESI-MS of metal-containing compounds and complexes.^[49] Figure 4 shows an MS spectrum of the observed species of a solution containing Hg(NO₃)₂ and quinalphos and Fig. 5 shows the fragmentation spectra of the [PA-Q + Hg²⁺ +(PA-OH – H)⁻] complex at *m*/*z* 669 (for ²⁰²Hg²⁺) and a proposed fragmentation pathway.

The interaction of mercury with quinalphos is more complex than with Cu or Ag. No 1:1 complexes of mercury: quinalphos or quinalphos fragments are observed, only 1:2 complexes (Fig. 4, peaks 405, 541 and 669). In contrast to Cu(II) there is no reduction of Hg(II) to Hg(I) observable and charge reduction of the complexes occur only via ligand-proton elimination. Neither doubly charged $[(PA-Q)_2 + Hg^{2+}]$ nor $[PA-Q + Hg^{2+}]$ are observed, although they probably exist in solution. This observation indicates that charge-state reduction of the complex is required for surviving the transfer from solution to the gas phase. Since there are no activatable N-H or O-H bonds for proton elimination in close proximity to the Hg²⁺-binding sites for intact PA-Q - Hg²⁺ complexes and Hg(II) to Hg(I) chargestate reduction with concurrent NO3 radical formation does not occur as it does in the case of Cu(II) (possibly due to the lower second ionization potential of Hg compared to Cu; $IE(Hg^+) = 18.7568 \text{ eV} < IE(Cu^+) = 20.2924 \text{ eV}^{[50]}$ the only observed gas-phase ion complexes contain at least one PA-Q hydrolysis product ($[PA-Q + Hg^{2+} + (PA-OH - H)^{-}]$ with m/z 669), namely PA-OH, which now allows proton elimination via activation of the O-H bond on the PA-OH moiety. In the case of the observed complex ion $[PA-OH + Hg^{2+} + PA-OH - H^+]$,



Figure 4. MS spectrum and proposed ion complex structures of species obtained by Hg(NO₃)₂ addition. Note, only the species with m/z 541 shows a sodiated or potassiated adduct form, since only this complex contains an easily exchangeable proton. Insert I shows a theoretical isotope model for the complex at m/z 669 and insert II reflects the zoomed-in real data. The complex at m/z 405 is most likely an in-source fragmentation product of the complex at m/z 541, as it is one of the main fragmentation products in a respective MS/MS study (see Supplementary Fig. S4). The lack of sodiated or potassiated signals for this complex indicates that the respective precursor sodiated/potassiated complexes (m/z 563 and 579) do not easily undergo in-source fragmentation. All observed complexes undergo in-source loss of ethyl groups (-C₂H₄).



Figure 5. MS/MS spectrum of the complex resulting from $Hg(NO_3)_2$ addition to quinalphos at m/z 669, proposed main fragmentation pathways, and an insert depicting a proposed structure for the fragment with m/z 411 (explanation in the text).

there is a second exchangeable proton present that can be easily replaced with sodium or potassium ions, likely present as ubiquitous trace contaminants (see Fig. 4).

Our observations agree well with the study of Burford's group in which mercury complexes with the one sulfurcontaining amino acid cysteine were observed either in 1:2 or 2:3 ratios, but not in a 1:1 ratio, and also no Hg(II) reduction to Hg(I) was observed.^[51] Further studies employing other techniques such as NMR and implementation of other solvent systems might be useful to unravel the detailed underlying mechanisms of mercury–quinalphos complexation: however, this is challenging due to the high hydrolysis rate of quinalphos in the presence of mercury ions and this is also beyond the scope of our presented work.

From the MS/MS spectra of the complexes at m/z 669 (Fig. 5) and at m/z 541 (Supplementary Fig. S4, Supporting Information) it becomes clear that Hg interacts strongly with the sulphur-containing moieties as mainly sulphur-containing fragment ions are observed (see Table 3 and Fig. 5). This is in contrast to the cases of Cu²⁺ and Ag⁺, where also metal ion complexes with the nitrogen-containing HQ moiety alone are observed (see Fig. 2 and Tables 1 and 2). The occurrence of [PA-Q+H⁺] can be explained with a neutral loss of $[PA-OH - 2H^{+} + Hg^{2+}]$; however, as was the case for Ag⁺ and Cu⁺, there is no experimental evidence for further details of such a mechanism. For the strongest observed fragment ions two main pathways are proposed: the first involves neutral loss of the HQ moiety from the precursor ion $[PA-Q+Hg^{2+}+(Pa-OH-H^{+})]$ at m/z 669 (for ²⁰²Hg²⁺) yielding an ion species with m/z 523 that subsequently loses all four C₂H₄ moieties connected to P, yielding a final ion fragment at m/z 411 for which we propose a plausible structure (see insert Fig. 5). The second main pathway involves the neutral loss of C₂H₅O₂PS from the precursor ion, yielding an ion complex with m/z 545. It might be possible that one of the ethoxy groups from the PA moiety in the ion complex is transferred to the Q moiety yielding 2-ethoxyquinoxyaline; however, there is no further experimental evidence currently available to support such an assumption. The fragment with m/z 517 could be formed via a neutral loss of C_2H_4 from the species with m/z 545 or

through a neutral loss of $C_2H_5O_2PS$ from the species with m/z 641, which was originally formed through a neutral loss of C_2H_4 form the precursor ion at m/z 669.

Further fragmentation of the species with m/z 517 can either yield a species with m/z 489 via a further neutral loss of C₂H₄ or a species with m/z 381 via a loss of C₄H₉PO₃ and a proposed formation of HgS. The species with m/z 381 could also be formed via a loss of C2H5PO3 from the species with m/z 489. Loss of neutral HgS could then yield the observed $[HQ + H^+]$ ion at *m*/*z* 147; this ion could also be a product of fragmentation of the observed [PA-Q+H⁺] ion. Finally, the minor fragment with m/z 505 could be formed through neutral loss of HQ and H₂O from the precursor ion or through H₂O loss from the observed ion with m/z 523. A few smaller fragments with m/z 175, 209, 241 and 269 can currently not be readily explained and could also not be reproduced with an MS/MS study of another isotope (i.e. $[PA-Q+^{200}Hg^{2+} + (Pa-OH - H)^{-}]$ with m/z 667). It is possible that these observed ions are due to unknown adduct formation or other unknown reactions due to potential collision cell contaminations.

In summary, for Hg²⁺ we observed only 1:2 and 1:3 complexes with PA-Q and its hydrolysis products, which agree well with observations from another group investigating Hg²⁺–thiol complexes.^[51] The fragmentation study of the main complex ions showed a strong interaction of Hg²⁺ with mainly S-binding sites, which again suggests that the gas-phase complex retains structural features of the expected complex formed already in solution.

CONCLUSIONS

In our study of quinalphos–transition metal ion complexes employing ESI-MS combined with CID fragmentation experiments, we have investigated structural features of the observed complexes, with the intent of providing complementary additional information to other studies that investigated OP degradation mechanisms in the presence of transition metal ions.

A major change from expected solution-formed complexes was found for the main gas-phase complexes observed for PA-Q interaction with Cu^{2+} . Here, we observed Cu(II) to

Cu(I) charge reduction in the complex, most likely occurring during the transfer from solution to the gas phase. Recently, Schröder's group proposed requirements for Cu(II) to Cu(I) charge reduction to occur:^[34,35] the ligand must provide electron-rich multidentate binding sites and easily activatable N–H or O–H bonds (for potential proton elimination) must not be in close proximity. Applying these principles to PA-Q enabled us to propose a novel structure for the 1:2 solution complex of Cu(II) with PA-Q.

Our study demonstrates the usefulness of ESI-MS/MS for the study of transition metal ion–organophosphate complexes. Especially the employment of Cu^{2+} as chelating ion is useful in complexes that undergo charge-state reduction from Cu(II) to Cu(I) during the transfer from solution to the gas phase, as it allows conclusions to be drawn on the original complex structure in solution.

The nature of our experiments under conditions that favor mass spectrometric analysis (e.g. low pH, no environmental matrices or other potentially interfering ingredients) emphasize that our work can only provide a simplified first model of transition metal ion–quinalphos interaction as it might occur in the environment. We anticipate that this work will be a valuable basis and guide for future investigations that look at pesticide interactions with heavy metals bound to colloidal particles (or vice versa), as these are known to occur in nature and facilitate the high mobility and distribution of these compounds, recently observed in soil and water samples.^[6]

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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