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Iodine binding to Humic Acid

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1 **1 Abstract**

2 The rate of reactions between humic acid (HA) and iodide (I^-) and iodate (IO_3^-) have been
3 investigated in suspensions spiked with ^{129}I at concentrations of 22, 44 and 88 $\mu g L^{-1}$ and stored at
4 $10^\circ C$. Changes in the speciation of $^{129}I^-$, $^{129}IO_3^-$ and mixed ($^{129}I^- + ^{129}IO_3^-$) spikes were monitored over 77
5 days using liquid chromatography inductively coupled plasma mass spectrometry (LC-ICP-MS). In
6 suspensions spiked with $^{129}I^-$ 25% of the added I^- was transformed into organic iodine (Org- ^{129}I)
7 within 77 days and there was no evidence of $^{129}IO_3^-$ formation. By contrast, rapid loss of $^{129}IO_3^-$ and
8 increase in both $^{129}I^-$ and Org- ^{129}I was observed in $^{129}IO_3^-$ -spiked suspensions. However, the rate of
9 Org- ^{129}I production was greater in mixed systems compared to $^{129}IO_3^-$ -spiked suspensions with the
10 same total ^{129}I concentration, possibly indicating $IO_3^- - I^-$ redox coupling. Size exclusion
11 chromatography (SEC) demonstrated that Org- ^{129}I was present in both high and low molecular
12 weight fractions of the HA although a slight preference to bond with the lower molecular weight
13 fractions was observed indicating that, after 77 days, the spiked isotope had not fully mixed with the
14 native ^{127}I pool. Iodine transformations were modelled using first order rate equations and fitted
15 rate coefficients determined. However, extrapolation of the model to 250 days indicated that a
16 pseudo-steady state would be attained after ~ 200 days but that the proportion of ^{129}I incorporated
17 into HA was less than that of ^{127}I indicating the presence of a recalcitrant pool of ^{127}I that was
18 unavailable for isotopic mixing.

19 Keywords: Humic acid; iodine; kinetics; speciation; iodine-129; soil.

20 **2 Introduction**

21 Iodine is an essential micro-nutrient for all mammals, with a recommended daily dietary intake of

22 100-150 $\mu\text{g d}^{-1}$ for humans (Johnson, 2003). Iodine deficiency diseases (IDDs) are a global health
23 problem, estimated to affect up to one third of the world's population (WHO, 2004). They are a
24 significant social and economic stress on developing countries and an area of concern for developed
25 countries (WHO 2008). Mechanisms that control the transfer of iodine from the terrestrial
26 environment to the food chain are a poorly understood component of global iodine cycles (Johnson
27 2003a).

28 Soil iodine concentrations in the UK are typically within the range 0.5 – 100 mg kg^{-1} (Whitehead,
29 1984, Johnson, 2003b). Seawater is considered to be the largest source of iodine to the terrestrial
30 biosphere (Muramatsu and Wedepohl, 1998, Fuge and Johnson, 1986) therefore higher soil
31 concentrations tend to occur in coastal areas. High soil iodine concentrations are also often
32 associated with high organic matter content, with humus the primary reservoir of soil iodine (Dai et
33 al., 2009, Smyth and Johnson, 2011, Xu et al., 2011b). In general, a larger soil iodine concentration
34 generally results in greater iodine uptake by plants (Weng et al., 2008, Dai et al., 2006), however
35 iodine associated with organic matter appears to be relatively unavailable for plant uptake (Keppler
36 et al., 2003, Xu et al., 2011b, Xu et al., 2012).

37 Humic acid (HA) is the colloidal fraction of humus, containing both aliphatic and aromatic moieties,
38 depending on the degree of humification and the original vegetation source of the organic matter.
39 Its large surface area and significant presence in soil organic matter mean that it is highly influential
40 in determining soil iodine dynamics (Xu et al., 2011b, Yamada et al., 2002, Francois, 1987, Hansen et
41 al., 2011, Allard, 2006). Although the composition of HA varies between soils, the functional groups
42 present are similar and therefore understanding iodine interactions with HA contributes significantly
43 to understanding its dynamics in soil (Schlegel et al., 2006, Warner et al., 2000, Saunders et al.,
44 2012). A few authors have investigated reactions between HA and iodine (e.g. Christiansen and
45 Carlsen, 1991, Reiller et al., 2006, Choung et al., 2013) but the majority of studies involve other
46 reaction components, often in complex media such as aerosols; the rate of reaction between I and
47 HA has generally not been explicitly considered. Iodination of HA is thought to occur mainly via
48 reduction of IO_3^- to produce reactive intermediate species, such as I_2 or HOI, followed by
49 electrophilic substitution reactions with electron donor groups on the HA (Francois 1987).
50 Whitehead (1974) identified weakly acidic phenolic and amino acid groups as the most likely
51 reaction sites. Christiansen and Carlsen (1991) concluded that HA reacted with a transient iodine
52 species resulting from reaction between I^- , peroxidase enzymes and hydrogen peroxide over 20
53 minutes, although they were unable to determine whether the transient species was an enzyme-
54 iodine complex or HOI/ I_2 produced by action of the peroxidase on I^- . They suggested three possible

55 types of sites in the HA were available for reaction: primary sites where I was weakly bonded,
56 secondary sites susceptible to nucleophilic iodide-iodide substitution and tertiary electrophilic sites.
57 They also observed that iodine appeared to be uniformly distributed across all molecular size
58 fractions. Schlegel et al. (2006) used Extended X-ray Absorption Fine Structure (EXAFS) to
59 investigate naturally iodinated marine-derived humic substances with 10% iodine by weight and
60 concluded that iodine was covalently bonded, primarily to aromatic rings, but probably present as
61 more than one type of organic species. Preferential bonding of iodine to polycyclic aromatic moieties
62 in black carbon was also demonstrated by Choung et al. (2013).

63 The objectives of this work were to (i) measure and model the dynamics of the reaction between IO_3^-
64 and I^- and HA (ii) establish whether HA contains a recalcitrant (unreactive) pool of iodine and (iii)
65 investigate whether iodine exhibits preferential association with high or low molecular weight
66 moieties in HA. This directly addresses our currently poor understanding of the interaction of iodine
67 with soil organic matter.

68 **3 Materials and methods**

69 **3.1 Preparation and characterisation of humic acid solutions**

70 Humic acid (HA) was extracted from a coniferous plantation soil, Leicestershire, UK (52° 42' N, 1° 14'
71 W; 195 m) by shaking for twelve hours with 0.1 M NaOH, followed by centrifugation (10,000 g,
72 15 min) and acidification to pH 2 of the supernatant, using concentrated HCl. Humic and fulvic
73 fractions were separated by centrifugation and the HA was purified using dialysis against 1 %v/v HCl
74 and HF then deionised water; the resultant HA was then freeze dried and finely ground (Marshall et
75 al., 1995). A portion of dried, ground HA was dissolved in 0.016 M NaOH and adjusted to pH 7.0
76 using NaOH to give a final concentration of 7.18 mg HA mL⁻¹. A neutral pH value was chosen to be
77 close to typical arable soil conditions and avoid volatilization of I₂. Dissolved organic carbon in the HA
78 solution was determined using a Shimadzu TOC-VCPH analyser. Each sample was acidified to pH 2-3
79 using HCl to remove inorganic carbon, before the remaining (organic) carbon was detected as CO₂ by
80 non-dispersive infrared detection after heating the sample to 720 °C with a platinum-coated alumina
81 catalyst. Samples were quantified against standards of 2.125 g L⁻¹ potassium hydrogen phthalate (C
82 concentration = 1000 mg L⁻¹), diluted to appropriate concentrations with Milli-Q water (18.2 MΩ
83 cm). Total iodine concentration in the HA suspension was measured by ICP-MS (Thermo-Fisher
84 Scientific X-Series^{II}) using internal detector cross-calibration with Rh and Re (10 µg L⁻¹) as internal
85 standards. Stock standards for ¹²⁷I were prepared at iodine concentrations of 1000 mg L⁻¹ from
86 oven-dried KI and KIO₃, and stored at 4 °C in 1 % tetra methyl ammonium hydroxide (TMAH).

87 Iodine-129 was obtained as a solution of sodium iodide ($^{129}\text{I}^-$) from the American National Institute of
88 Standards (NIST, Gaithersburg, Maryland, USA; CRM 4949C, $0.004 \text{ mol L}^{-1} \text{ Na}^{129}\text{I}$, 3451 Bq mL^{-1}).
89 Iodate-129 ($^{129}\text{IO}_3^-$) was prepared by oxidation of $^{129}\text{I}^-$ with sodium chlorite using a method adapted
90 from Yntema and Fleming (1939). Successful oxidation to IO_3^- was confirmed by ICP-MS with in-line
91 chromatographic separation using HPLC (Dionex, ICS-3000) operated in isocratic mode with an anion
92 exchange column (Hamilton PRP-X100; $250 \times 4.6 \text{ mm}$, $5 \mu\text{m}$ particle size). The mobile phase (1.3 mL
93 min^{-1}) was $60 \text{ mmol L}^{-1} \text{ NH}_4\text{NO}_3$, $1 \times 10^{-5} \text{ mmol L}^{-1} \text{ Na}_2\text{-EDTA}$, 2% methanol; pH was adjusted to 9.5
94 with TMAH.

95 Triplicate aliquots of HA solution (pH 7) were spiked with $^{129}\text{I}^-$ and/or $^{129}\text{IO}_3^-$ on 8 occasions to give
96 incubation times of 26, 79, 155, 328, 596, 992, 1404 and 1855 hr. Samples were stored in the dark
97 at $10 \text{ }^\circ\text{C}$, the average annual soil temperature measured at Armagh Observatory, Northern Ireland
98 (Garcia-Suarez and Butler, 2006). Final concentrations of spiked iodine were 22.1, 44.1 and
99 $88.2 \mu\text{g L}^{-1}$ (Table 1). The CRM 4949C contained ^{127}I equivalent to 12 % of the ^{129}I concentration; this
100 was accounted for as described in Electronic Annex A so that, for simplicity, all iodine added in the
101 spike ($^{129}\text{I} + ^{127}\text{I}$) is referred to as ^{129}I and native iodine is described as ^{127}I .

102 3.2 Iodine speciation

103 Iodine species were separated using HPLC (Dionex, ICS-3000), with a Superose 12 10/300 GL size
104 exclusion chromatography (SEC) column (GE Healthcare), linked to ICP-MS. A sample injection
105 volume of $25 \mu\text{l}$ and isocratic elution with 0.1 M Tris (Tris(hydroxymethyl)aminomethane) adjusted
106 to pH 8.8 using 50 % Trace Analysis Grade (TAG) HNO_3 at a flowrate of 1 mL min^{-1} was used. The
107 HPLC was controlled using Chromeleon software (Dionex, version 6.80SR12) and sample processing
108 was undertaken using Plasmalab software. Working standards of $^{127}\text{I}^-$ and $^{127}\text{IO}_3^-$, at iodine
109 concentrations of $0 - 100 \mu\text{g L}^{-1}$, were used. Species-specific quantification was carried out with
110 standards of $^{127}\text{I}^-$, $^{127}\text{IO}_3^-$, $^{129}\text{I}^-$ and $^{129}\text{IO}_3^-$ prepared in Milli-Q water and mean, isotope-specific,
111 sensitivity values (signal (integrated CPS) per ppb) calculated from iodide and iodate standards were
112 used to quantify HA-bonded iodine (HA-I); drift correction was applied by analysis of repeated
113 standards through the experimental run. Limits of detection were $0.047 \mu\text{g L}^{-1}$ for ^{127}I and $0.014 \mu\text{g L}^{-1}$
114 for ^{129}I . Humic acid controls, spiked with Milli-Q water, were analysed alongside ^{129}I -spiked samples
115 to determine the equilibrium speciation of native ^{127}I .

116 A correction factor for ^{129}Xe was determined for each run and applied to all ^{129}I results, according to
117 Equation 1:

$$118 \quad ^{129}\text{S} = ^{129}\text{S}_{\text{meas}} - (k \times ^{131}\text{S}_{\text{meas}}) \quad (1)$$

119 where ^{129}S = corrected signal (CPS) for ^{129}I ; $^{129}\text{S}_{\text{meas}}$ = measured signal at $m/z = 129$; k = a factor
 120 determined by iteration for each run (typically 1.08) to give an average ^{129}S baseline of zero; $^{131}\text{S}_{\text{meas}}$
 121 = measured signal for ^{131}Xe . Matrix matching and standard addition were used to calculate mean
 122 sensitivity for all samples in each run, from which concentrations of ^{129}I and ^{127}I in each peak were
 123 quantified.

124 3.3 Modelling

125 Iodine-129 transformations were represented as simultaneous ordinary differential equations (Fig.
 126 1):

$$127 \quad \frac{d[\text{IO}_3^-]}{dt} = -(k_1+k_4)[\text{IO}_3^-] + k_5[\text{Org-I}] \quad (2)$$

$$128 \quad \frac{d[\text{I}^-]}{dt} = -k_3[\text{I}^-] + k_1[\text{IO}_3^-] + k_2[\text{Org-I}] \quad (3)$$

$$129 \quad \frac{d[\text{Org-I}]}{dt} = -(k_2+k_5)[\text{Org-I}] + k_4[\text{IO}_3^-] + k_3[\text{I}^-] \quad (4)$$

130 Where k_1 , k_2 , k_3 , k_4 and k_5 are unknown rate coefficients (hr^{-1}) estimated by fitting the model to the
 131 observed concentrations of $^{129}\text{IO}_3^-$, $^{129}\text{I}^-$ and $\text{Org-}^{129}\text{I}$.

132 The differential equations were solved using 4th order Runge-Kutta and fitting was performed using a
 133 Marquardt procedure (Press et al, 1986) implemented in [OpenModel](http://openmodel.info/) (Tarsitano et al 2011,
 134 <http://openmodel.info/>) to minimize the residual sum of squares (RSS) between modelled and
 135 observed values over all the time points and different spike combinations. Alternative model
 136 structures were considered, the arrangement described (and shown schematically in Fig. 1) provided
 137 the best fit to the data.

138 4 Results and discussion

139 4.1 Iodine dynamics

140 Measured characteristics of the HA are given in Table 2. Concentrations of ^{127}I species represent
 141 iodine in equilibrium with HA suspensions with median values of $98.0 \mu\text{g L}^{-1}$ of $\text{Org-}^{127}\text{I}$ and $15.1 \mu\text{g L}^{-1}$
 142 $^{127}\text{I}^-$; iodate ($^{127}\text{IO}_3^-$) was not detected. The C:I mole ratio in the HA was 396,000.

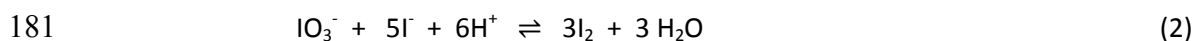
143 Figure 2 shows the change in speciation of spiked ^{129}I over time; model fits to the data are discussed
 144 in a later section. In suspensions spiked with I^- only (Table 1: I_{22} , I_{44} and I_{88}) a gradual decrease in $^{129}\text{I}^-$
 145 concentrations was observed over time with a concomitant increase in $\text{Org-}^{129}\text{I}$. By the final time
 146 point (1855 hr) ~25% of added $^{129}\text{I}^-$ had been transformed to $\text{Org-}^{129}\text{I}$ irrespective of initial spike

147 concentration. No generation of $^{129}\text{IO}_3^-$ was observed. Suspensions spiked with $^{129}\text{IO}_3^-$ (Table 1: IO₂₂,
148 IO₄₄ and IO₈₈) showed rapid initial loss of $^{129}\text{IO}_3^-$ with associated increases in both $^{129}\text{I}^-$ and Org- ^{129}I .
149 Concentrations of $^{129}\text{I}^-$ were always greater than Org- ^{129}I . By the end of the experiment (1855 hr)
150 concentrations of $^{129}\text{IO}_3^-$ in each system were < 5% of the initial concentrations and Org- ^{129}I
151 accounted for ~40% of the added iodine, again irrespective of initial spike concentration. In mixed
152 systems (Table 1; Mix₂₂, Mix₄₄ and Mix₈₈), where equal concentrations of $^{129}\text{I}^-$ and $^{129}\text{IO}_3^-$ were added,
153 results were similar to those observed when $^{129}\text{IO}_3^-$ alone was added (Figure 2). Concentrations of
154 $^{129}\text{IO}_3^-$ decreased rapidly with both $^{129}\text{I}^-$ and Org- ^{129}I increasing over time. Again no concentration-
155 dependence was observed, with similar proportions of $^{129}\text{I}^-$ and Org- ^{129}I observed regardless of spike
156 concentration.

157 Humic acid has been reported to both reduce IO_3^- and oxidise I^- in soils (Yamaguchi et al., 2010),
158 however oxidation of I^- by organic matter to form IO_3^- is expected to be much slower than reduction
159 of IO_3^- to I^- (Schlegel et al., 2006) unless Fe^{III} and Mn^{IV} oxides are present which may catalyse the
160 oxidation of I^- (Gallard et al., 2009, Allard et al., 2009, Fox et al., 2009). This difference in rate
161 (between I^- and IO_3^-), in the absence of metal oxides, was confirmed by the absence of $^{129}\text{IO}_3^-$ in $^{129}\text{I}^-$
162 spiked systems, in contrast to the rapid production of $^{129}\text{I}^-$ in HA solutions spiked with $^{129}\text{IO}_3^-$.
163 Shetaya et al. (2012), selected soils with a range of pH values and concentrations of Fe/Mn oxides
164 and organic matter for incubation with $^{129}\text{I}^-$ or $^{129}\text{IO}_3^-$ at 10°C and 20°C. In contrast to the current
165 study, and that of Choung et al. (2013), they found that conversion of added $^{129}\text{I}^-$ to (humic-bound)
166 Org- ^{129}I ($t_{1/2} = 0.38 - 45$ hr) was faster than conversion of $^{129}\text{IO}_3^-$ to Org- ^{129}I ($t_{1/2} = 9.0 - 412$ hr).
167 Comparison of these half-life values for whole soil with the data comparing iodide and iodate in Fig.
168 2 may indicate the importance of metal oxides in soils in rapidly adsorbing IO_3^- , and thereby slowing
169 its transformation into Org-I, or possibly enabling oxidation of I^- to an intermediate species capable
170 of reacting with humus. Additionally the large overall differences in reaction rates seen between
171 soils and HA may reflect enzyme activity in soils which is likely to be absent from isolated HA
172 fractions.

173 The rate of Org- ^{129}I production was greater in the systems spiked with $^{129}\text{IO}_3^-$ than those spiked with
174 $^{129}\text{I}^-$ at the same concentration (Fig. 2). However, in mixed systems the *initial* rate of Org- ^{129}I
175 production was greater than in solutions spiked with $^{129}\text{IO}_3^-$ alone, although a slightly lower
176 concentration of Org- ^{129}I was eventually present after 500 hr incubation (e.g. IO₄₄ and Mix₄₄).
177 However comparison of systems with the same initial concentration of $^{129}\text{IO}_3^-$ (e.g. IO₂₂ and Mix₄₄)
178 showed that Org- ^{129}I concentrations were substantially greater in the mixed systems where $^{129}\text{I}^-$ was

179 also present compared to the equivalent $^{129}\text{IO}_3^-$ -spiked system. This may suggest that redox coupling
180 between $^{129}\text{I}^-$ (or $^{127}\text{I}^-$) and $^{129}\text{IO}_3^-$ had occurred in the mixed spike solutions (Eq. 2):



182 When only $^{129}\text{I}^-$ was added, its transformation to Org- ^{129}I did not depend upon concentration (Fig. 3),
183 suggesting its oxidation mechanism was independent of the presence of another species. However,
184 considering the presence of native iodide ($^{127}\text{I}^-$) in the HA solutions, the *relative* rate of $^{129}\text{I}^-$
185 production, from $^{129}\text{IO}_3^-$, in the mixed spike system was greater when lower concentrations of $^{129}\text{IO}_3^-$
186 were added (Fig. 3); this result is consistent with faster reduction of IO_3^- (to I^-) at higher
187 iodide/iodate ratios. For the mixed-spike system the ratio of measured iodide to added iodide
188 progressively exceeded 1.0, reflecting the production of I^- from reduction of added IO_3^- .

189 The *relative* rates of $^{129}\text{IO}_3^-$ loss in both $^{129}\text{IO}_3^-$ - and mixed-spike systems were slightly greater at
190 lower IO_3^- concentrations with similar rates observed for systems with the same total ^{129}I
191 concentration (Fig. 4). Again, this suggests that the rate of iodate reduction is limited, either by the
192 concentration of iodide (Eq. 2) or some other reductive mechanism associated with the HA.

193 Figure 5 compares the concentrations of species measured in systems where a mix of species was
194 added with the *sum* of species from the appropriate $^{129}\text{I}^-$ and $^{129}\text{IO}_3^-$ treatments spiked with a single
195 species (e.g. Mix₄₄ compared with I₂₂+IO₂₂). Although it could be argued that this comparison is not
196 completely valid because the ^{129}I :HA ratio differed, the rate of $^{129}\text{I}^-$ transformation to Org- ^{129}I (Fig. 3,
197 open symbols) indicated that HA concentration is not a limiting factor. Figure 5 suggests that
198 initially there was less $^{129}\text{I}^-$, and consequently greater concentrations of $^{129}\text{IO}_3^-$ and Org- ^{129}I , in the
199 mixed spike systems (circles fall below the 1:1 line). However, the difference in species composition
200 between the mixed and single-species systems generally declined with time - eg highest values for
201 iodide (open and closed circles).

202 Comparison of the concentrations of the species present in single species spiked systems with $^{129}\text{I}^-$ -
203 spiked systems at an equivalent total iodine concentration (e.g. IO₄₄ + I₄₄ compared with I₈₈) also
204 show that $^{129}\text{I}^-$ was only transformed to Org- ^{129}I and that this happened faster when $^{129}\text{IO}_3^-$ was
205 present, perhaps reflecting the 5:1 ratio in Eq. 2.

206 **4.2 Iodine distribution within the HA**

207 The distribution of iodine within the HA was examined using SEC-ICP-MS. Lower molecular weight
208 (LMW) HA may be expected to react more easily with iodine due to its greater surface area which
209 may provide greater accessibility to reactive sites. Conversely, the greater negative charge density

210 expected on LMW fractions may act to exclude or delay I^- or IO_3^- ions from interaction. Xu et al.
211 (2011a), in a study where IO_3^- was added to HA at pH 3, observed that the LMW fractions (3 – 50
212 kDa) sorbed more iodine than the > 50 kDa fractions after 72 hr. By contrast, Christiansen and
213 Carlsen (1991) observed no dependence on MW size fraction following a rapid (20 min) reaction of
214 Aldrich HA with I^- in the presence of peroxidase enzymes. Figure 6 shows the cumulative integrated
215 counts per second (ICPS) for the organic portion of the SEC chromatogram for added ^{129}I and pre-
216 existing ^{127}I in the HA after a 77 day incubation of the Mix₈₈ solution. Higher molecular weight
217 (HMW) molecules elute first due to exclusion from the column matrix - shown by the prominent
218 peak around 400 s. The lines representing the cumulative integrated signals for ^{127}I and ^{129}I in Figure
219 6 suggest that a greater proportion of ^{127}I than ^{129}I was present in larger HA molecules.
220 Approximately 50% of the cumulative integrated signal for ^{127}I had been detected by 530 s,
221 compared to 600 s for ^{129}I . This indicates that ^{129}I had not fully mixed with the pre-existing HA-bound
222 ^{127}I and had reacted preferentially with the LMW fractions of HA. It also indicates the presence of
223 recalcitrant iodine (^{127}I), unavailable to mix with the added ^{129}I , and with a greater presence in the
224 HMW fractions of HA. Comparison of the Mix₈₈ 77 day chromatogram for ^{127}I and ^{129}I (Figure 6) also
225 shows a smaller excluded peak maxima compared to the main organic peak for ^{129}I and a shift in the
226 ^{129}I peak maxima towards LMW fractions, again suggesting preferential binding of freshly added
227 iodine to LMW fractions of HA in contrast to (native) ^{127}I .

228 4.3 Modelling

229 Fitted model (Fig. 1) predictions are compared to the observations in Fig. 2 and estimated rate
230 coefficients are given in Table 3. Overall the model fit was good (for all data: $r^2 = 0.96$, $p < 0.001$),
231 supporting the model structure. The values of the (apparent) rate constants broadly reflect the
232 dynamics of the system. Iodate is rapidly converted to humic-bound forms ($k_4 = 2.62 \text{ hr}^{-1}$) but the
233 optimal model fit required a reverse reaction ($k_5 = 0.157 \text{ hr}^{-1}$) suggesting an approach to an
234 equilibrium position rather than a zero sink for iodate. The further reduction of iodate to iodide was
235 slower ($k_1 = 4.11 \times 10^{-3} \text{ hr}^{-1}$) and both the 'direct' conversion of iodide to humic-bound iodine and the
236 re-mineralisation of iodide were comparatively very slow reactions ($k_3 = 3.16 \times 10^{-4}$ and $k_2 = 4.67 \times$
237 10^{-4} hr^{-1}).

238 In order to investigate the availability of ^{127}I for mixing with ^{129}I , modelling was extended to 250 d. At
239 this point a pseudo-steady-state was apparent, with no significant changes in species concentrations
240 from 200 d after spiking. After 250 d contact between ^{129}I and HA the model predicted that $^{129}I^-$
241 /Org- $^{129}I = 0.24$, whereas the measure ratio $^{127}I^-$ /Org- ^{127}I was lower, at 0.17. Therefore although the
242 model prediction suggested that ^{129}I was at equilibrium, a greater proportion of ^{127}I than ^{129}I existed

243 as Org-I, suggesting the presence of a recalcitrant pool of ^{127}I , unavailable for isotopic mixing. From
244 the $^{129}\text{I}^- / \text{Org-}^{129}\text{I}$ ratio and measured $^{127}\text{I}^-$ in solution, the estimated labile Org- ^{127}I was $62.9 \mu\text{g L}^{-1}$,
245 suggesting that 64% of the HA bound iodine was labile and 36% was incapable of isotopic mixing
246 within 250 d.

247 Keppler et al. (2003) and Xu et al. (2011b) suggested that iodination of HA occurs early in the
248 humification of fresh plant material with fewer binding sites available as humification progresses.
249 Steric hindrance by aliphatic chains may also make some aromatic binding sites less accessible to
250 freshly added iodine, while effectively 'fixing' native iodine (Xu et al., 2012). Schwehr et al (2009)
251 also observed, in an experiment where natural sediments were spiked with I^- , that recently added I^-
252 was less strongly sorbed than native iodine, and that greater added concentrations resulted in a
253 smaller proportion bound. Therefore although organic-rich soils may well contain higher total
254 concentrations of iodine much of that iodine may not be readily accessible to plants.

255 **5 Conclusions**

256 Inorganic iodine (both I^- and IO_3^-) reacted with HA to produce Org-I. The reaction was slower with I^-
257 compared to systems spiked with IO_3^- or a mixture of IO_3^- and I^- . In mixed and IO_3^- -spiked systems I^-
258 concentrations increased rapidly and remained higher than Org-I concentrations throughout the
259 duration of the experiment.

260 Native iodine in the HA solutions was present only as I^- and Org-I; no IO_3^- was observed. There was
261 evidence that the presence of native $^{127}\text{I}^-$ allowed more rapid reduction of spiked $^{129}\text{IO}_3^-$, potentially
262 by redox coupling with I^- . This was supported by the relative rates of reaction of the two species,
263 with a faster reaction observed when a mix of inorganic species (I^- and IO_3^-) was added, rather than a
264 single species (I^- or IO_3^- alone). In soils, by contrast, I^- has been reported to transform to Org-I more
265 quickly than IO_3^- , possibly due to the presence of metal oxides or enzymatic oxidation. In a 'purified'
266 HA solution the same reaction mechanisms are not available and I^- oxidation was relatively slow.

267 Size exclusion chromatography showed that ^{129}I became associated with both high and low
268 molecular weight HA although a slight preference for lower molecular weight fractions was
269 suggested. The native and spiked isotopes were not fully mixed after ~ 2 months of reaction
270 indicating the presence of a recalcitrant pool of ^{127}I ; this was also strongly indicated by modelling
271 over longer timescales.

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275

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Tables

Table 1: Summary of added iodine (^{129}I) species in incubated HA solutions.

Table 2: Measured characteristics of a humic acid (HA) isolated from the Ah horizon of a coniferous plantation soil in Leicestershire (UK); ($52^{\circ} 42' \text{ N}$, $1^{\circ} 14' \text{ W}$; 195 m).

Table 3: Optimised parameter values (first-order rate constants) describing HA-iodine dynamics in the model shown in Figure 1.

Table 1: Summary of added iodine (^{129}I) species in incubated HA solutions.

Solution	$^{129}\text{I}^-$ added ($\mu\text{g } ^{129}\text{I L}^{-1}$)	$^{129}\text{IO}_3^-$ added ($\mu\text{g } ^{129}\text{I L}^{-1}$)
I ₂₂	22.1	0
I ₄₄	44.1	0
I ₈₈	88.2	0
IO ₂₂	0	22.1
IO ₄₄	0	44.1
IO ₈₈	0	88.2
Mix ₂₂	11.0	11.0
Mix ₄₄	22.1	22.1
Mix ₈₈	44.1	44.1

Table 2: Measured characteristics of a humic acid (HA) isolated from the Ah horizon of a coniferous plantation soil in Leicestershire (UK); (52° 42' N, 1° 14' W; 195 m).

Characteristic	Value	Units	Source
Ash content	0.66*	%	Marshall (1992)
Total acidity	6.49*	mol _c kg ⁻¹	Marshall (1992)
Dissolved organic carbon	3.67	mg ml ⁻¹	DOC analysis
¹²⁷ Iodide [#]	15.1	µg l L ⁻¹	SEC analysis
Org- ¹²⁷ I [#]	98.0	µg l L ⁻¹	SEC analysis

*Ash content and total acidity are the mean of two measurements quoted by Marshall (1992).

[#] Measured in HA suspensions

Table 3: Optimised parameter values (first-order rate constants) describing HA-iodine dynamics in the model shown in Figure 1.

Rate constant	Mean (hr⁻¹)	S. D.
k1	0.00411	0.00010
k2	0.000467	0.00004
k3	0.000316	0.00002
k4	2.62	0.00000
k5	0.157	0.00003

Figures

Figure 1: Conceptual model describing transformations of spiked ^{129}I in the presence of HA. Rate constants k_1 – k_5 describe first-order rate equations.

Figure 2: Modelled (lines) and measured (symbols) change in ^{129}I concentrations with time following spiking with ^{129}I at a range of concentrations and species compositions. Species measured included $^{129}\text{I}^-$ (black circles ●, black lines), $^{129}\text{IO}_3^-$ (open squares □, dashed lines) and Org- ^{129}I (grey triangles ▲, grey lines). Error bars show standard error of triplicate measurements.

Figure 3: Change in the ratio of measured iodide to added iodide over time, following addition of iodide (*open* symbols) and mixed iodide/iodate ^{129}I spikes (*closed* symbols). Total concentrations of ^{129}I added were: $22.1 \mu\text{g L}^{-1}$ (circles), $44.1 \mu\text{g L}^{-1}$ (squares) and $88.2 \mu\text{g L}^{-1}$ (triangles).

Figure 4: Change in ratio of measured iodate to added iodate over time, following addition of iodate (*open* symbols) and mixed iodide/iodate ^{129}I spikes (*closed* symbols). Total concentrations of ^{129}I added were: $22.1 \mu\text{g L}^{-1}$ (circles), $44.1 \mu\text{g L}^{-1}$ (squares) and $88.2 \mu\text{g L}^{-1}$ (triangles).

Figure 5: Comparison of total concentrations of iodine-129 species in solution in mixed and single spiked systems at total iodine concentrations of $44 \mu\text{g L}^{-1}$ (*open* symbols) and $88 \mu\text{g L}^{-1}$ (*closed* symbols). Dashed line = 1:1, square symbols = iodate, circles = iodide, triangles = Org-I.

Figure 6: Cumulative ICPS (integrated counts per second) as a percentage of total counts, from size exclusion chromatograph of ^{129}I (thick black line) and ^{127}I (thick grey line); 25%, 50% and 75 % of cumulated ICPS are indicated by circles, squares and diamonds, respectively. The Org-I section of ^{127}I (thin black line) and ^{129}I (thin grey line) SEC chromatograms (2^o Y-axis) are also shown. All data are for the Mix₈₈ solution (Table 1) after 77 days incubation.

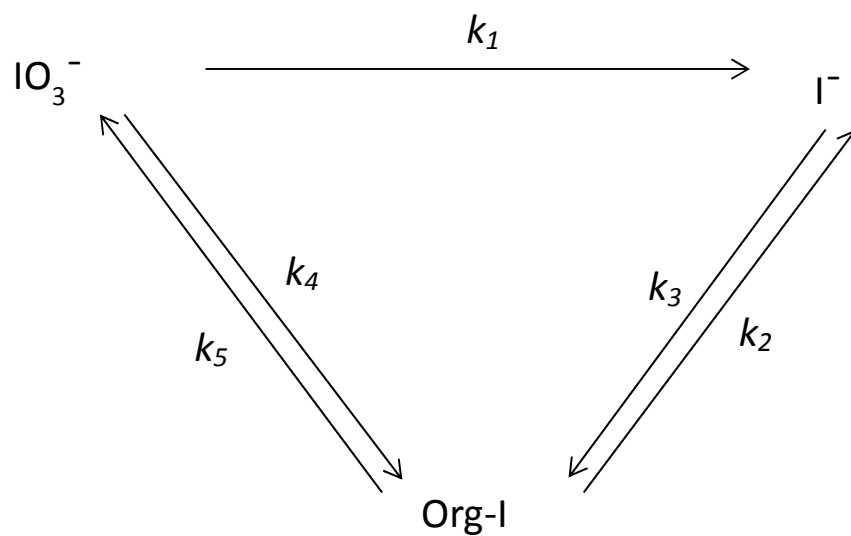


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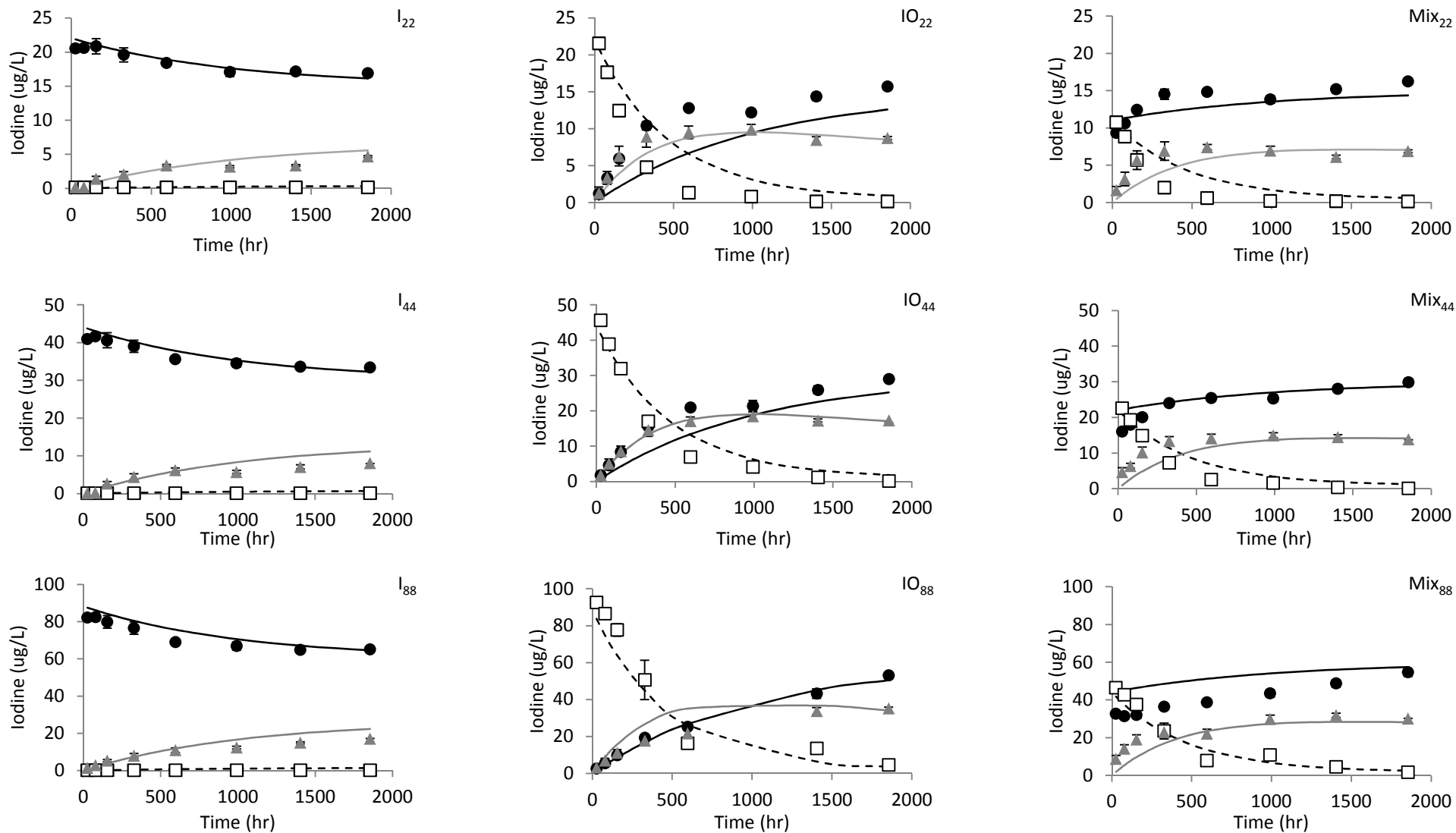


Figure 2: Modelled (lines) and measured (symbols) change in ^{129}I concentrations with time following spiking with ^{129}I at a range of concentrations and species compositions. Species measured included $^{129}\text{I}^-$ (black circles ●, black lines), $^{129}\text{IO}_3^-$ (open squares □, dashed lines) and $\text{Org-}^{129}\text{I}$ (grey triangles ▲, grey lines). Error bars show standard error of triplicate measurements.

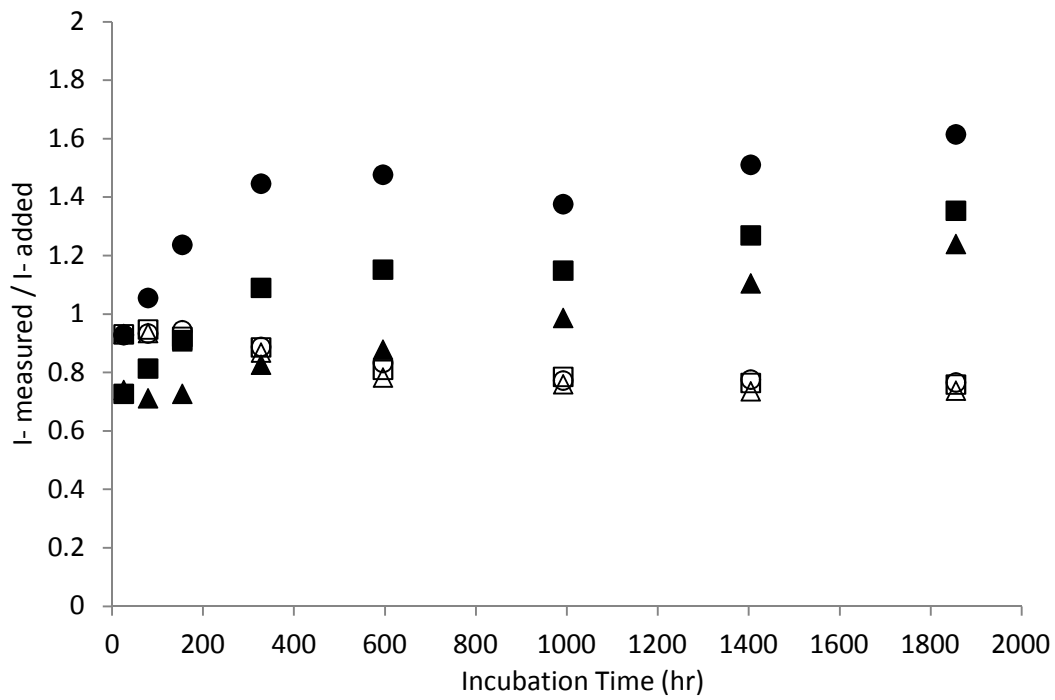


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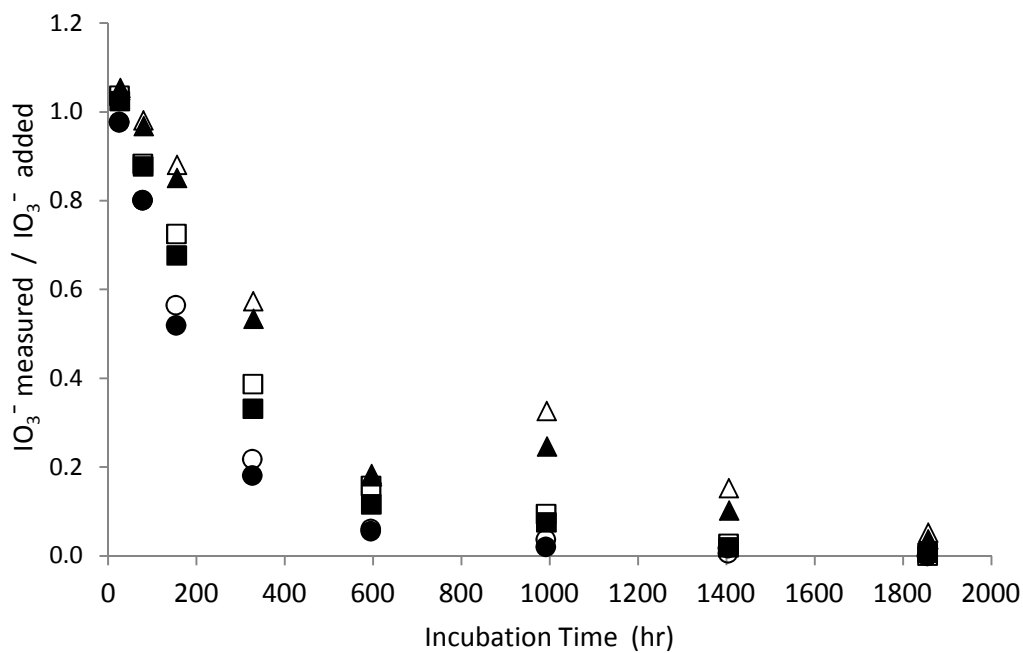


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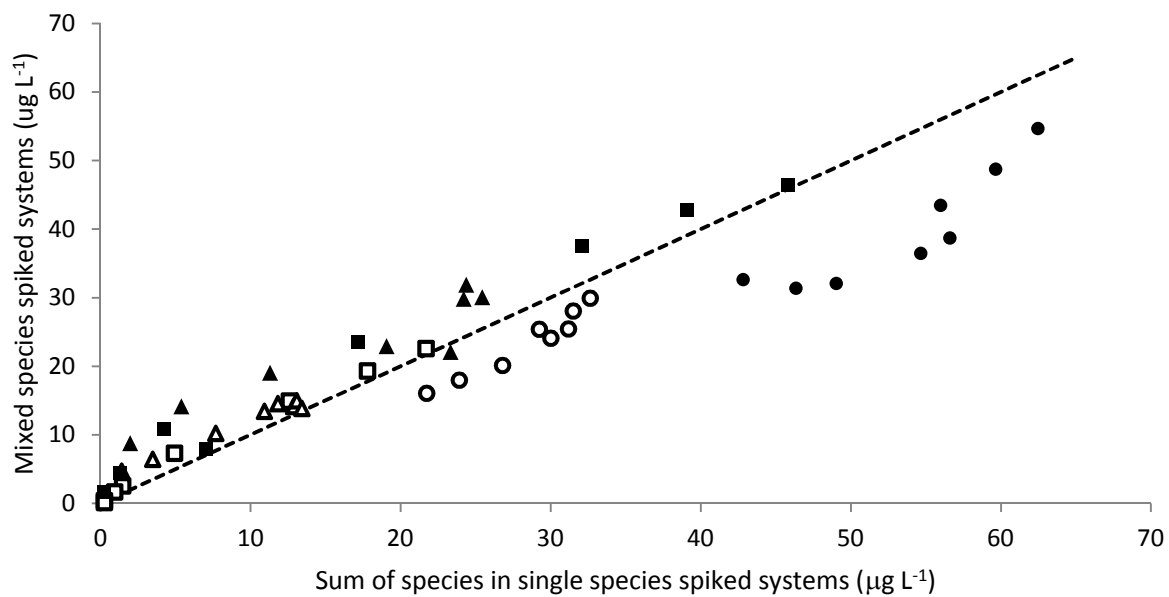


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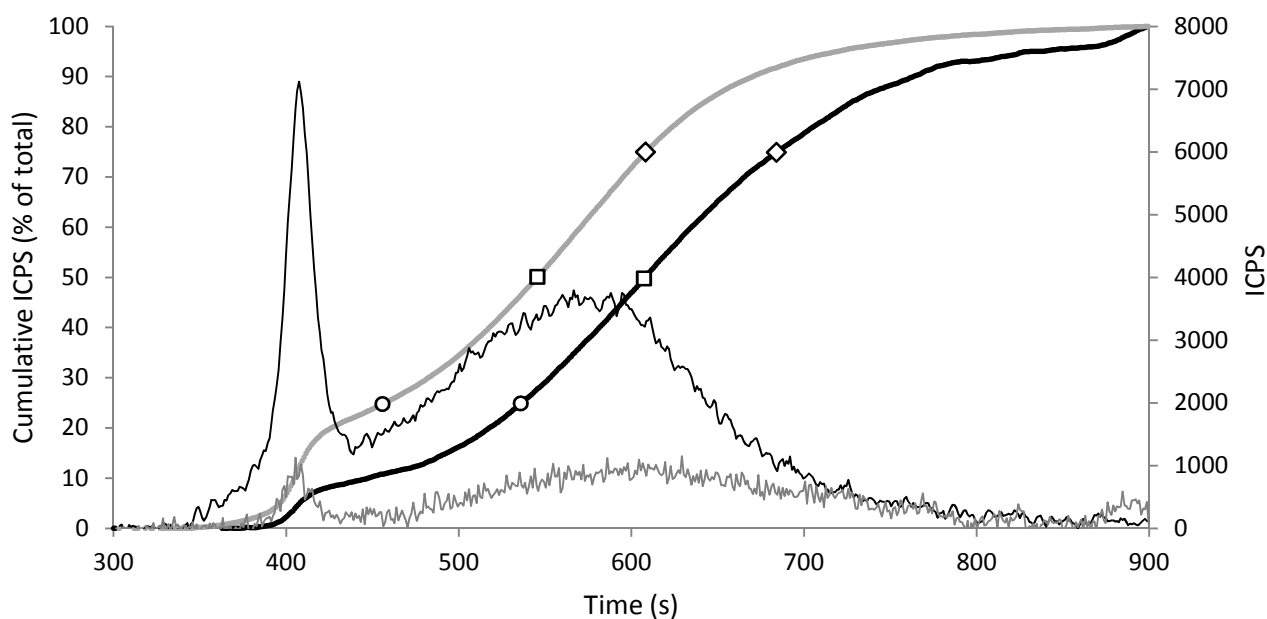


Figure 6: Cumulative ICPS (integrated counts per second) as a percentage of total integrated counts, from size exclusion chromatograph of ^{129}I (thick black line) and ^{127}I (thick grey line); 25%, 50% and 75 % of cumulated ICPS are indicated by circles, squares and diamonds, respectively. The Org-I section of ^{127}I (thin black line) and ^{129}I (thin grey line) SEC chromatograms (2^o Y-axis) are also shown. All data are for the Mix₈₈ solution (Table 1) after 77 days incubation.

Electronic Annex A: Correction applied for the presence of ^{127}I in the ^{129}I SRM 4949C:

All measured ^{129}I concentrations had a correction applied to nominally ascribe *all* iodine added in ^{129}I spikes ($^{129}\text{I} + ^{127}\text{I}$) to ^{129}I , for ease of description:

$$^{129}\text{I} = ^{129}\text{I}_{\text{meas}} \times 1.12 \times (127/129) \quad (\text{A1})$$

Where ^{129}I = 'corrected' concentration of I from the spike in solution ($\mu\text{g L}^{-1}$) i.e. ($^{129}\text{I} + ^{127}\text{I}$ present in the spike),
 $^{129}\text{I}_{\text{meas}}$ = measured concentration of ^{129}I ($\mu\text{g L}^{-1}$), 1.12 corrects for the presence of 12 % ^{127}I in the ^{129}I and (127/129) is a gravimetric correction. The corresponding correction was also applied to measurements of ^{127}I , according to Eqn. A2:

$$^{127}\text{I} = ^{127}\text{I}_{\text{meas}} - (0.12 \times ^{129}\text{I}_{\text{m}}) \quad (\text{A2})$$

Where ^{127}I = 'corrected' concentration of ^{127}I in solution ($\mu\text{g L}^{-1}$) i.e. ^{127}I minus the ^{127}I present as a result of the spiking, and $^{127}\text{I}_{\text{meas}}$ = measured concentration of ^{127}I ($\mu\text{g L}^{-1}$).