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1 **The risk of chlorpropham cross-contamination of grain in potato stores.**

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8

9 **Abstract**

10 The contamination of food with pesticide residues is of significant concern to consumers and
11 legislation has been implemented worldwide to ensure compliance with Maximum Residue
12 Levels of chemicals in food. The occurrence of the potato sprout inhibitor, isopropyl N-(3-
13 chlorophenyl) carbamate (chlorpropham or CIPC) residues on cereals, such as wheat, is of
14 concern as this chemical is not authorised for use on cereals, and therefore the route of
15 unintentional contamination warrants further investigation. This study reports on the risk of
16 CIPC cross-contamination of grain that was stored in a commercial potato store and provides
17 a method for low level quantification of CIPC in cereals. A High Performance Liquid
18 Chromatography (HPLC UV/VIS) method for quantifying residues of CIPC in grains was
19 successfully validated and the presence of CIPC was confirmed by Gas Chromatography
20 Mass Spectrometry (GCMS).

21 The magnitude of contamination in the grain was influenced by: (I) direct contact with the
22 flooring of the store; (II) the headspace directly above the concrete surface and within the
23 store itself and (III) contaminated dust/CIPC particles in the store atmosphere. Cross
24 contamination is feasible irrespective of the CIPC concentrations in the concrete flooring and

25 even with storage of grain at an elevated height above the concrete, suggesting that the route
26 of cross contamination is a complex process involving physical and chemical (volatilization)
27 factors. The results are significant for recommendations involving the storage of grain in
28 buildings with a history of CIPC use and for remedial strategies for decontamination of these
29 buildings.

30

31 **Highlights**

32 CIPC cross contamination of grain occurs in potato stores.

33 Contact with CIPC contaminated concrete contributes to cross contamination of grain.

34 CIPC in the headspace within a store contributes to cross contamination.

35 HPLC UV/VIS and GCMS were used to confirm CIPC contamination in grain.

36

37 **Keywords**

38 HPLC UV/VIS, GCMS, MRL, concrete, CIPC.

39 Conflict of Interest: The authors declare that they have no conflict of interest.

40 **1. Introduction**

41 The high quality of food crops may be maintained by the use of pesticides which can remain
42 as residues and enter the food supply chain (Mondy *et al.*, 1992). These residues may
43 constitute a risk to consumers and may have human health concerns (Łozowicka *et al.*, 2012).
44 Pesticides can also unintentionally contaminate food products via storage surfaces (Garcia-
45 Febrero *et al.*, 2014) and the reduction or elimination of pesticide residues during storage of
46 agricultural products is a new challenge (Han *et al.*, 2016). Pesticides that serve a purpose on
47 a specific crop require regulatory approval and a Maximum Residue Level (MRL) may be set
48 (EFSA, 2011). Compliance with MRLs confirms pesticides have been used appropriately
49 and helps to ensure chemical residues are within statutory limits (Łozowicka *et al.*, 2012; Han
50 *et al.*, 2016). The MRL for the potato sprout inhibitor, isopropyl N-(3-chlorophenyl)
51 carbamate (chlorpropham or CIPC) on potatoes within the European Union (EU) is 10 mg kg⁻¹
52 ¹ (EC Regulation 1107/2009). Where there is no purpose for a chemical on a specific crop,
53 the MRL is set at the limit of quantification (LOQ); for example the MRL for CIPC on wheat
54 is 0.01mg kg⁻¹ (HSE, 2017).

55

56 The occurrence of CIPC residues on cereals such as wheat is of concern within the EU.
57 Detection of CIPC in wheat flour, used in a limited number of manufactured cereal products,
58 resulted in their withdrawal from shops in the United Kingdom (UK) (Curtis, 2006). It was
59 considered that the cross contamination was caused by the storage of wheat in potato stores
60 with a history of CIPC usage. Indeed, some wheat growers are using vacated potato storage
61 facilities for other crop commodities. In certain instances, stores may be rented out or
62 ownership may change, and often there are no records of the history of the stores pertaining
63 to the use of CIPC. Subsequently, the use of these contaminated potato stores for storage of

64 other crops results in the risk of cross contamination of valuable commodities (AHDB-
65 Potatoes, 2012).

66 In the UK, CIPC is commonly applied as a thermal fog, potentially contaminating the fabric
67 of the store roof, walls and concrete flooring (Boyd and Duncan, 1986; Smith *et al.*, 2013;
68 Khan *et al.*, 2012; Smith and Bucher 2012), hence; crops stored in direct contact with the
69 store fabric may become cross contaminated. In addition, CIPC is volatile and may cause
70 cross contamination via the atmosphere (AHDB-Cereals and Oil Seeds, 2015). Previous
71 research has established High Pressure Liquid Chromatography (HPLC) with Ultra
72 Violet/Visible (UV/VIS) detection and Gas Chromatography Mass Spectrometry (GCMS)
73 methodologies for the detection of CIPC in concrete (Douglas *et al.*, 2018) and these methods
74 were subsequently used to determine the degree of CIPC contamination in four potato stores
75 with different histories of CIPC usage (Douglas *et al.*, in press). The latter study
76 demonstrated CIPC penetrated to a depth of four centimetres (cm) in the concrete flooring
77 and persisted for decades after the last application. In one store, greater than 90% was held in
78 the surface one centimetre while, in contrast, a second store had less than 47% in the first cm.
79 The retention of CIPC to a depth of four cm into the flooring and the high percentages found
80 in the surface layer indicate the possible risk of cross contamination of crops in contact with
81 the concrete and the need to investigate this.

82 The quantification of CIPC in cereal grains, using HPLC UV/VIS, at very low levels close to
83 the limit of detection (LOD) and LOQ values is sometimes ambiguous due to possible matrix
84 effects caused by co-extracted natural products in grains. This may be problematic in terms of
85 determining the suitability of grain for use in the manufacturing of food products. Therefore,
86 alternative, higher sensitivity analytical methods are required to allow informed
87 recommendations to be made about residue fate within the food chain. The routes of CIPC

88 cross contamination will also provide an insight into measures that could be taken to prevent
89 this occurrence. The objectives of this research were to: 1. develop a method to quantify and
90 confirm the presence of CIPC in wheat at levels close to the MRL of CIPC in cereals and 2.
91 investigate the mechanisms for contaminant transfer and degree of contamination of grain in
92 a former potato store that had been exposed to CIPC applications.

93

94 **2. Experimental**

95 2.1 Materials.

96 Isopropyl N-(3-chloro-phenyl) carbamate (CIPC, 98% purity) was obtained from Sigma-
97 Aldrich (Dorset, United Kingdom). A Millipore Elix[®] 5 water purification system
98 (Molsheim, France) was used to produce HPLC grade water. HPLC-grade solvents, acetone
99 and acetonitrile, were purchased from Fisher Scientific (Loughborough, United Kingdom).
100 Syringe filters (13 mm) with 0.2 µm polytetrafluoroethylene (PTFE) membrane (VWR
101 International, USA), HPLC grade vials with PTFE screw caps (Agilent technologies, USA),
102 20 ml glass vials (PerkinElmer, USA) and HPLC grade vials with PTFE screw caps (Agilent
103 technologies, USA) were obtained from Crawford Scientific Ltd, UK. Non organic wheat
104 grain (*Triticum aestivum*) samples were obtained from a farm in Scotland (used in section
105 2.4) and organic special spelt wheat grain (*Triticum spelta*) was purchased from Holland &
106 Barrett (Germany) (used in section 2.5).

107

108 2.2 Standard solutions.

109 A standard stock solution of 1000 µg mL⁻¹ of CIPC was prepared in acetonitrile and stored at
110 4°C. Standard solutions (0.01-1.0 µg mL⁻¹), used for calibration curves, were prepared by

111 diluting the standard solution with appropriate volumes of acetonitrile. A spiking solution of
112 $100 \mu\text{g mL}^{-1}$ was prepared from the stock solution.

113

114 2.3 Extraction and analytical procedures.

115 Five grams (g) of CIPC-free non-organic wheat grain was spiked with 20 μl of a stock
116 solution of $10 \mu\text{g CIPC mL}^{-1}$ and extracted in 20 mL of acetonitrile to give a concentration
117 of $0.04 \text{ mg CIPC kg}^{-1}$ grain. Five replicates were prepared at this spiking level. A 30-minute
118 period was allowed for the CIPC to interact with the surface of the grains and for solvent
119 evaporation. The spiked samples were extracted and analysed by HPLC UV/VIS as outlined
120 in Douglas *et al* (2018). Three sub samples from one batch of contaminated grains (section
121 2.4) were homogenized by manual mixing and analysed to determine the effectiveness of the
122 mixing method (see Section 2.7). Based on the results, subsequent batches were extracted and
123 analysed once. This also applied to wheat samples collected from the second store trial
124 (section 2.5), which were also homogenized by manual mixing prior to extraction. Where
125 required, extracts from cross contaminated wheat samples were diluted to a concentration
126 range of 0.01 to $1.0 \mu\text{g mL}^{-1}$ prior to analysis by HPLC UV/VIS. The spiked recovery
127 samples and cross contaminated samples were also analysed by GCMS as outlined in
128 Douglas *et al* (2018), with the exception that the samples with CIPC concentration levels
129 below the limit of detection of the GCMS instrument were concentrated prior to analysis.
130 CIPC-free wheat grains were treated in the same way as those exposed in the store and
131 analysed by GCMS as a negative control. CIPC solutions ($0.01 \mu\text{g mL}^{-1}$) and solutions from
132 extractions of spiked wheat grains ($0.04 \text{ mg CIPC kg}^{-1}$) were concentrated and analysed by
133 GCMS as positive controls. A total volume (300 mL) of grain extract and CIPC stock
134 solution with CIPC levels of $0.01 \mu\text{g mL}^{-1}$ were evaporated to dryness at 20°C using a rotary

135 evaporator coupled to a Büchi Vac® V-500 pump. The evaporated samples were re-dissolved
136 into 500 µL of acetonitrile, giving a final concentration of 6 µg mL⁻¹ prior to GCMS analysis.

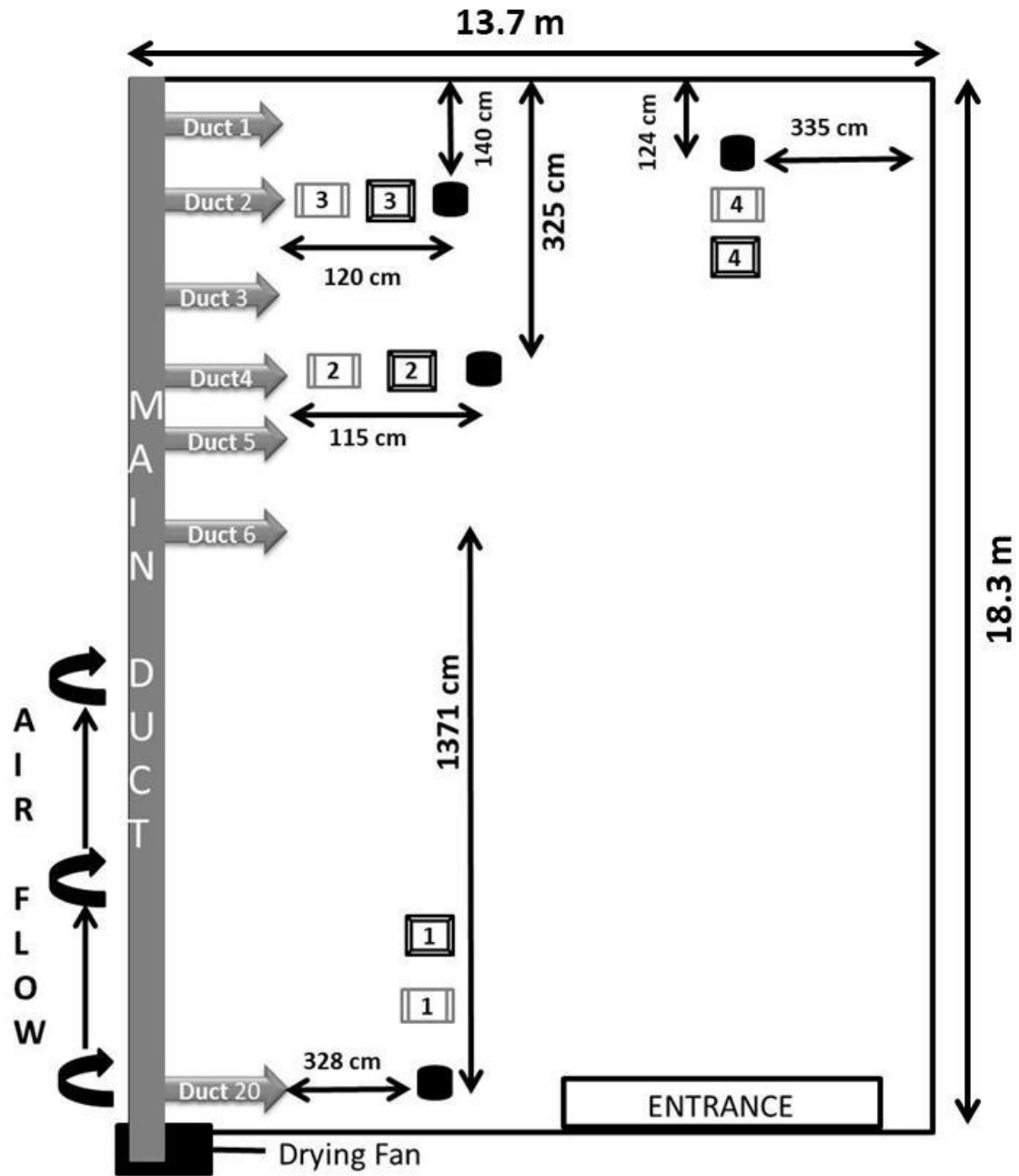
137

138 2.4 Preliminary store trial to assess the importance of the contaminated concrete and store
139 atmosphere on CIPC contamination of grain.

140 A commercial bulk potato store, with dimensions of 18.3 m × 13.7 m (length × width) and
141 capacity of 200 tonnes, was used in this study. Storage of crop commodities in the store had
142 ceased and the final CIPC application was in 1990 (five seasons of application). The point of
143 CIPC entry into the store was via galvanized steel ducts, where lateral ducts 2 and 4 were
144 primarily used and lateral duct 6 was occasionally used (Figure 1). Three ducts were used
145 because the store was not filled to its maximum potato capacity during CIPC application. The
146 estimated total amount of CIPC applied to the store, based on its history, was 319 kg (63.75 g
147 t⁻¹ × 25 years × 200 t). The distribution of CIPC in the concrete flooring of this store was
148 previously assessed, with concentrations ranging between 0.58 and 304 mg kg⁻¹ in the top
149 four cm, with around 47% within the top cm (Douglas *et al.*, in press). The temperature and
150 humidity was not monitored or regulated in this abandoned store during the trials. The trials
151 were conducted under natural environmental conditions (humidity and temperature) of the
152 store.

153 Wheat samples (130 g) were placed in duplicates in close proximity to four areas where the
154 CIPC in the concrete flooring had been determined (Figure 1). The wheat samples were
155 placed directly on the surface of the concrete flooring and were covered with either an
156 upturned plastic box (length 27 cm × 20 cm width × 17 cm height) secured to the floor with
157 duct tape (Gaffer, UK), which isolated the grain from the atmosphere or a mesh box that
158 allowed the air in the store to circulate over the grain (Figure 2). The grain remained there for

159 seven months, after which, CIPC was extracted and analysed by HPLC UV/VIS and GCMS
160 as outlined in Section 2.3.



161

162 Figure 1.

163

164 2.5 Full experimental trial to assess route of CIPC contamination of grain.

165 Following the initial trial described in section 2.4, the route of cross contamination of grain in
166 the same commercial store was further investigated in a second trial using custom-made
167 glassware. Wheat samples (100 g, n = 1) were placed in each experimental design.
168 Borosilicate glass beakers (3 L) were modified to produce five experimental designs (A, B,
169 C, D and E) used to determine the effect of the headspace, contaminated dust and the
170 concrete flooring on the cross contamination of grain (Figure 2). The spouts of the beakers
171 were removed, and the edges were turned up and flattened to form flanges. This was followed
172 by annealing at 500 °C and manual lapping with carborundum to smooth the flanges and aid
173 the sealing of adjoining containers to each other and to the concrete surface (Figure 2). The
174 beakers were modified so that adjoining and single containers had the same 20 cm headspace
175 height. Sieve cloth (355 µm) (Plastok Associates Ltd, UK) was fixed to containers A and B to
176 act as a support for the grain, to prevent direct contact with the contaminated concrete
177 surfaces, while the bases were removed to allow air circulation from the concrete to the
178 suspended grain. In design A (Fig. 2A), the effect of both the headspace above the concrete
179 surface and the store headspace was assessed while in B (Fig. 2B), the interaction of the store
180 headspace was restricted by an enclosed top container. In designs C and D (Fig 2C and Fig
181 2D), grains were in contact with the base of the glass beakers preventing direct contact with
182 the contaminated concrete surfaces. Samples in design C could interact with the store
183 headspace whereas in D the interaction between the store headspace was restricted by a clock
184 glass sealed with Blu tack (Bostik). Grains in design E (Fig. 2E) were in direct contact with
185 the concrete surface and restricted from the store headspace. The trial was executed in the
186 vicinity of a previously assessed core. Designs A and B were 20 cm from the centre of the
187 assessed core while C and E were 45 cm from A and B, respectively. Design D was 15 cm

188 from the assessed core (Figure 3). The trial was conducted for three months after which,
189 grains were extracted and analysed for CIPC as outlined in Section 2.3.

190

191 2.6 Sample collection and storage.

192 Grain samples retrieved from the trials were stored in resealable food and freezer bags (17 cm
193 × 21 cm Zip Lock) which were wrapped in aluminium foil (15 µm thick) and subsequently
194 triple wrapped in Zip Lock bags (S C Johnson) to prevent cross contamination between
195 samples. Samples that were expected to be CIPC free, i.e. in experimental design D, were
196 transported to the analytical laboratory where they were stored separately to prevent cross
197 contamination between samples. All grain samples were extracted for CIPC within two days
198 of collection. The clock glasses that were firmly sealed to the beakers to prevent air
199 interactions with the grain samples in experimental design D were washed twice with 20 mL
200 of acetonitrile using a Pasteur pipette. Each 20 mL sample collected was stored securely in 20
201 mL glass vials and analysed using the method outlined in Section 2.3. The average of the
202 respective values for each clock glass was taken to determine the CIPC concentration in the
203 dust which accumulated on them.

204

205 2.7 Quality control and quality assurance.

206 Quality control and quality assurance measures were incorporated throughout the study to
207 ensure the validity of the data. Qorpak extraction jars (PTFE lined caps; 16 oz) and other
208 glassware used for extractions were soaked overnight in Decon 90 solution, thoroughly rinsed
209 with tap water and dried at 25 °C in an oven. Prior to extractions, the glassware was rinsed
210 with acetonitrile and allowed to air dry for 15 minutes.

211 Procedural blanks (20 mL acetonitrile) and spiked samples (4.0 mg CIPC kg⁻¹) were routinely
212 analysed with each batch of samples, ensuring the absence of interfering substances and
213 satisfactory recovery of CIPC (greater than 95%). Instrument performance was verified using
214 CIPC standards which were analysed prior to sample analyses and between sample runs,
215 ensuring satisfactory day to day agreement of results. Background contamination was
216 prevented by: (I) flushing residual CIPC from the injector between analyses using 3 mL of
217 acetonitrile and (II) repeat injections with acetonitrile between analyses until an acetonitrile
218 blank gave no peak at the retention time of CIPC to ensure that there was no carryover.

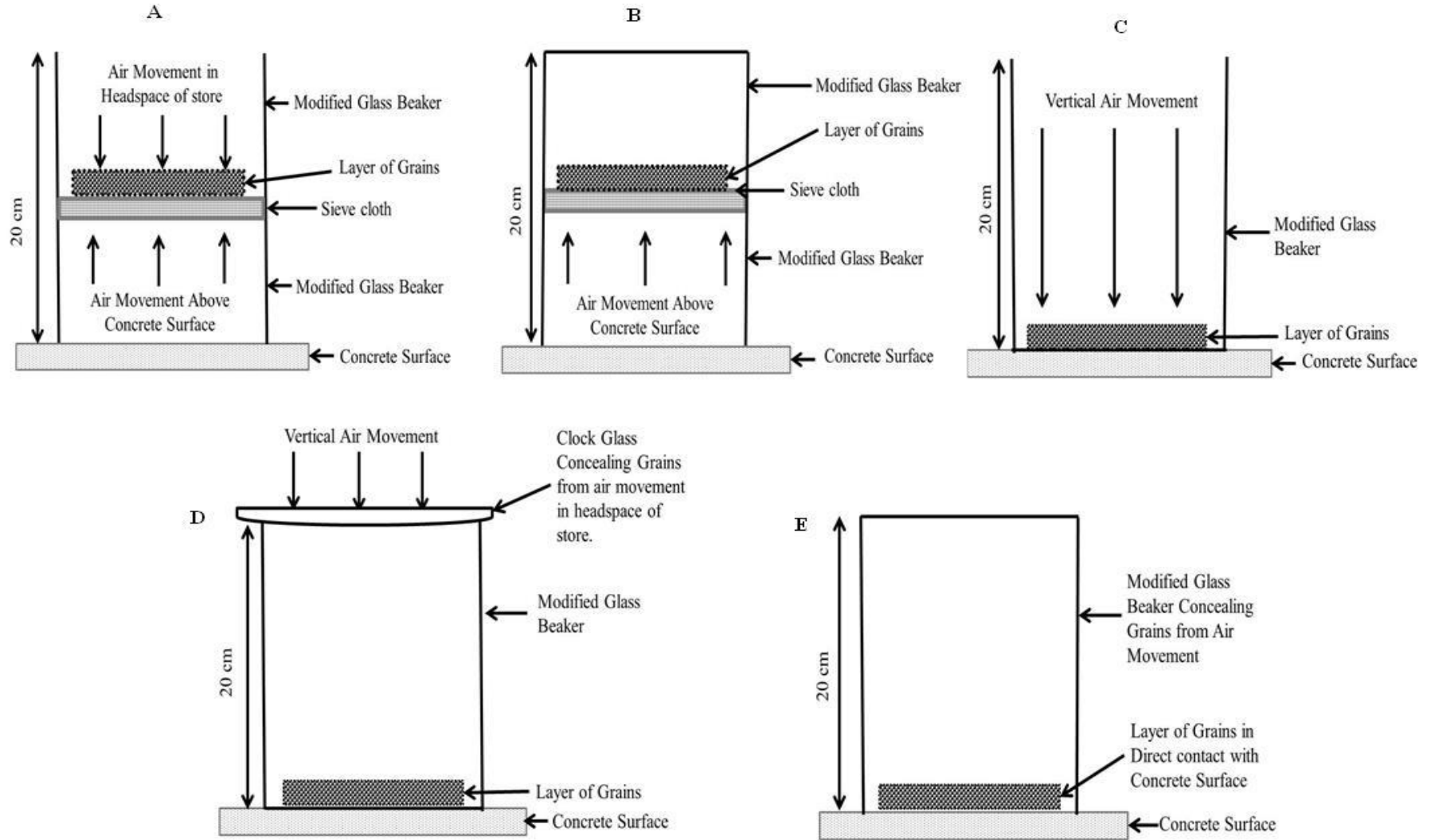
219 A contaminated grain sample was homogenised, split into three subsamples and extracted to
220 test the reproducibility of the processing and extraction methods. The similarity of CIPC
221 concentrations obtained for the contaminated sample (mean = 0.21 mg kg⁻¹ ± 2.59% Relative
222 Standard Deviation (RSD), n=3) indicated that the procedure for processing the grains
223 (manual mixing) prior to analysis was sufficient for sample homogenization and that the
224 method was precise. A clean clock glass, which was not taken into the store, was sequentially
225 washed and analysed as a negative control. This sample was CIPC free and was compared
226 with samples from clock glasses that were exposed to the store environment, thus confirming
227 the presence of CIPC in the accumulated dust.

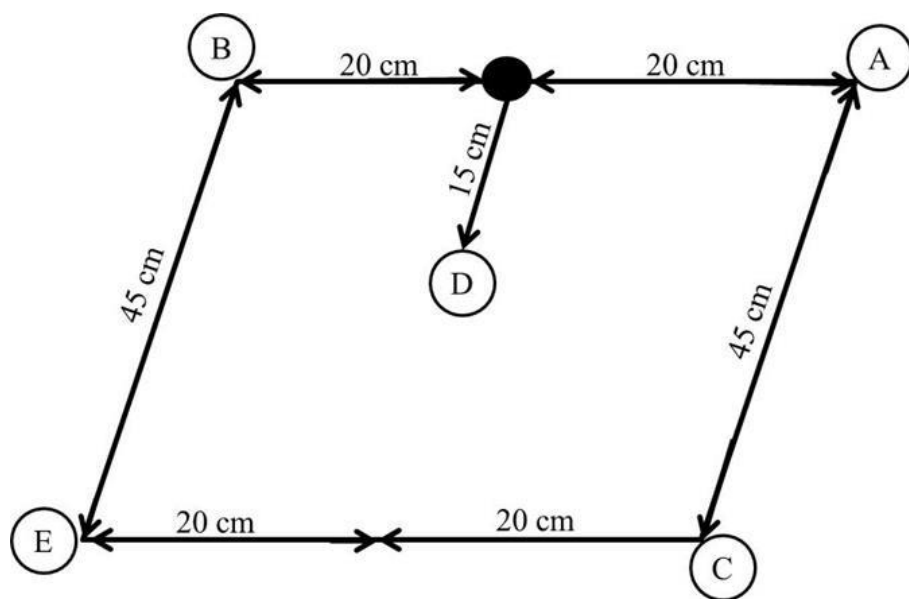
228 Sensitivity was evaluated for the non-organic and organic wheat grains used in the
229 preliminary and full experimental trials respectively. Sensitivity was determined by
230 estimating the LOD and LOQ using a repeat injection method (n=10) (Douglas et al., 2018).
231 The LOD and LOQ values were evaluated for CIPC-free non-organic wheat (n=10) and
232 spiked CIPC-free non-organic wheat (nominal level 0.04 mg kg⁻¹; n=10). The Standard
233 Deviation (SD) was calculated for both spiked (n=10) and non-spiked (n=10) samples, as
234 depicted in the following equation: $SD = \sqrt{[(SD_s)^2 + (SD_b)^2]}$ where spiked and non-spiked
235 grains are designated SD_s and SD_b , respectively. The LOD and LOQ values of 0.016 mg kg⁻¹

236 and 0.054 mg kg^{-1} are equal to 3 and 10 times the SD. This method was used for non-organic
237 wheat because the CIPC free non-organic wheat samples had an interfering peak very close to
238 the retention time of CIPC (Figure 4) and confirmation or otherwise of CIPC at this level was
239 difficult. Mass spectrometry was required to confirm the absence of CIPC in these samples,
240 ensuring that the interfering peak was not attributable to CIPC. This was achieved using
241 GCMS (Figure 5).

242 The CIPC-free organic wheat used in the full experimental trial outlined in Section 2.5 had no
243 interfering peak in the HPLC UV/VIS analysis and therefore mass spectrometry was not
244 required for these samples. The LOD and LOQ values were therefore evaluated with respect
245 to the instruments response. The LOD ($0.001 \text{ } \mu\text{g mL}^{-1}$) and LOQ ($0.004 \text{ } \mu\text{g mL}^{-1}$), with
246 respect to the instrument response, were previously evaluated using $0.01 \text{ } \mu\text{g mL}^{-1}$ CIPC
247 solution (Douglas et al., 2018). These values were used to determine the LOD and LOQ for
248 CIPC in the organic wheat samples. The instrument response LOD and LOQ values were
249 multiplied by a factor of four (to represent 5 g of wheat extracted in 20 mL acetonitrile) to
250 produce the LOD ($0.001 \text{ mg L}^{-1} \times 0.02 \text{ L}/0.005 \text{ kg} = 0.004 \text{ mg CIPC kg}^{-1}$) and LOQ (0.004
251 $\text{ mg L}^{-1} \times 0.02 \text{ L}/0.005 \text{ kg} = 0.016 \text{ } \mu\text{g g}^{-1}$) values for the organic wheat.

252 The instrument LOD and LOQ values were used for the evaluation of the accumulated dust
253 (particulate material) samples.





255

256 Figure 3.

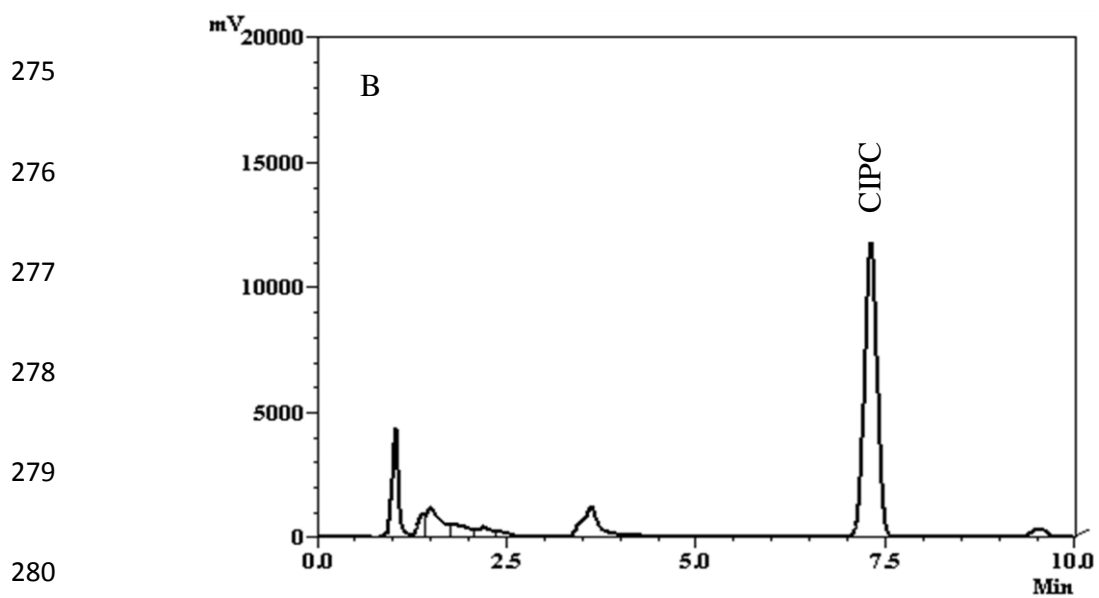
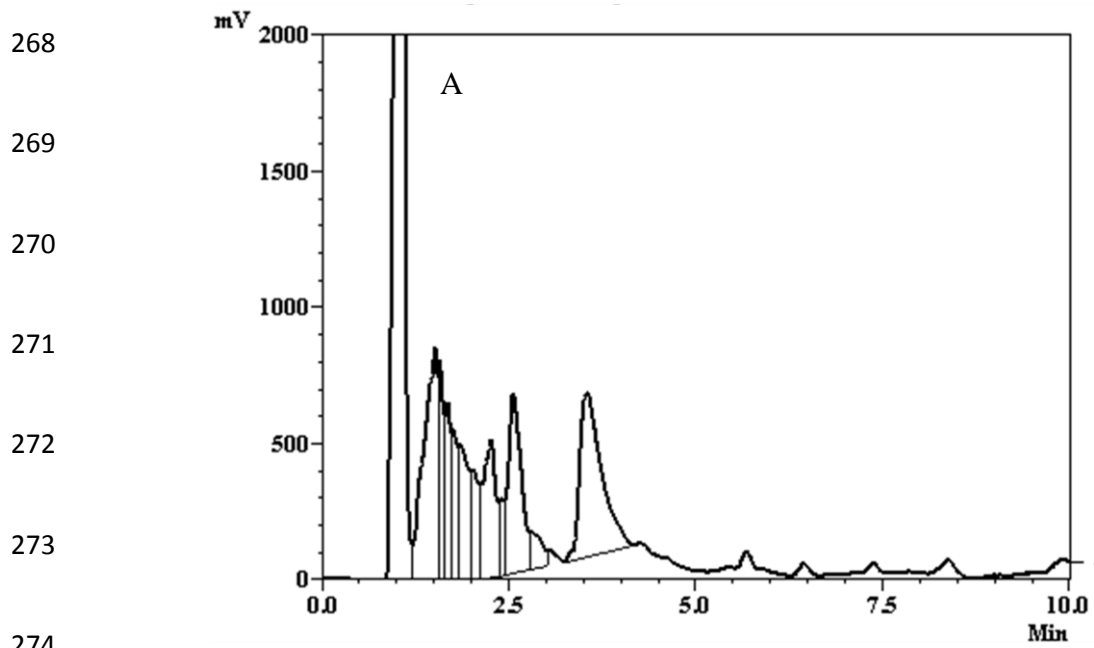
257

258 3. Results and discussion

259 3.1 Quantification of CIPC in grains using HPLC UV/VIS.

260 The method used for quantifying CIPC in concrete (Douglas *et al.*, 2018) was adapted and
 261 used in wheat. The accuracy and precision of the method with respect to wheat was
 262 determined by recovery tests conducted at two concentrations, using CIPC-free wheat spiked
 263 at 0.04 and 4.0 mg kg⁻¹. The percentages of CIPC recovered were 65 ± 5.6% RSD and 95 ±
 264 3.4% RSD for wheat spiked at 0.04 and 4.0 mg kg⁻¹, respectively. The correlation coefficients
 265 (R^2) for the calibration curves in the range 0.01 to 1.0 µg mL⁻¹ were greater than 0.99
 266 confirming linearity.

267



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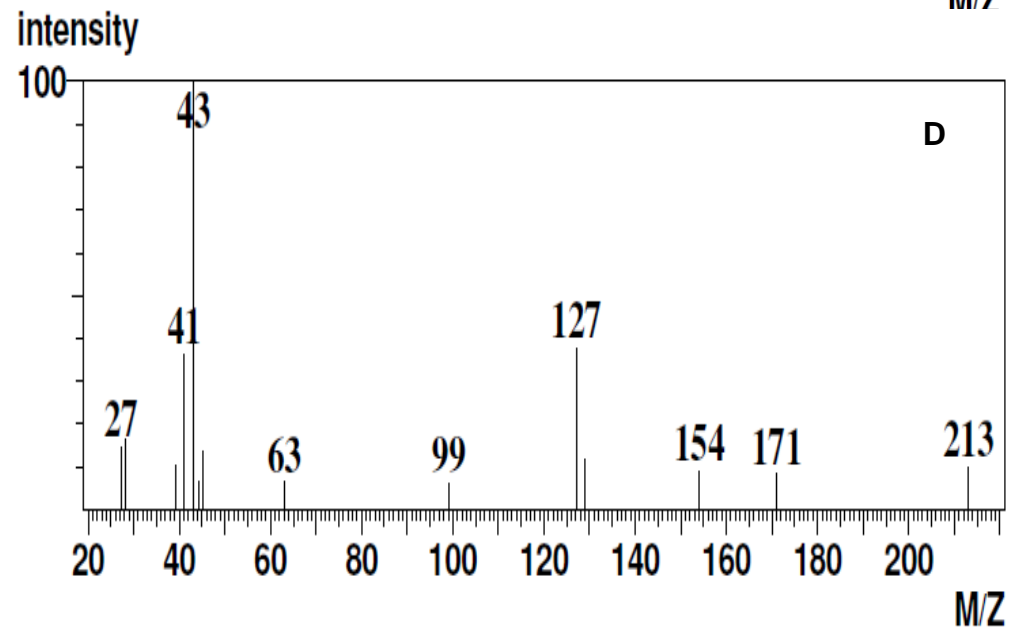
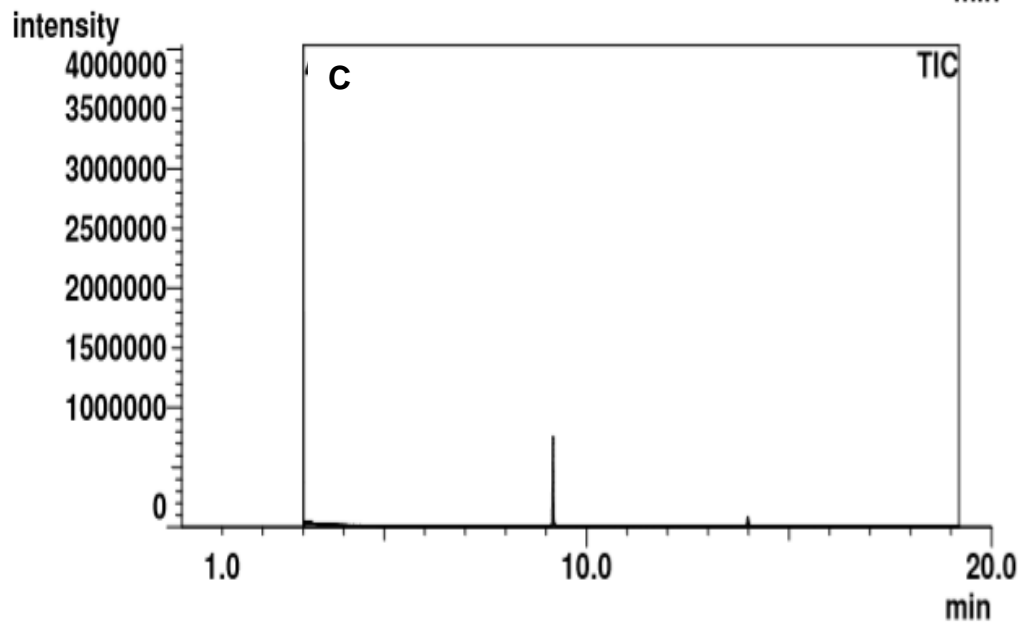
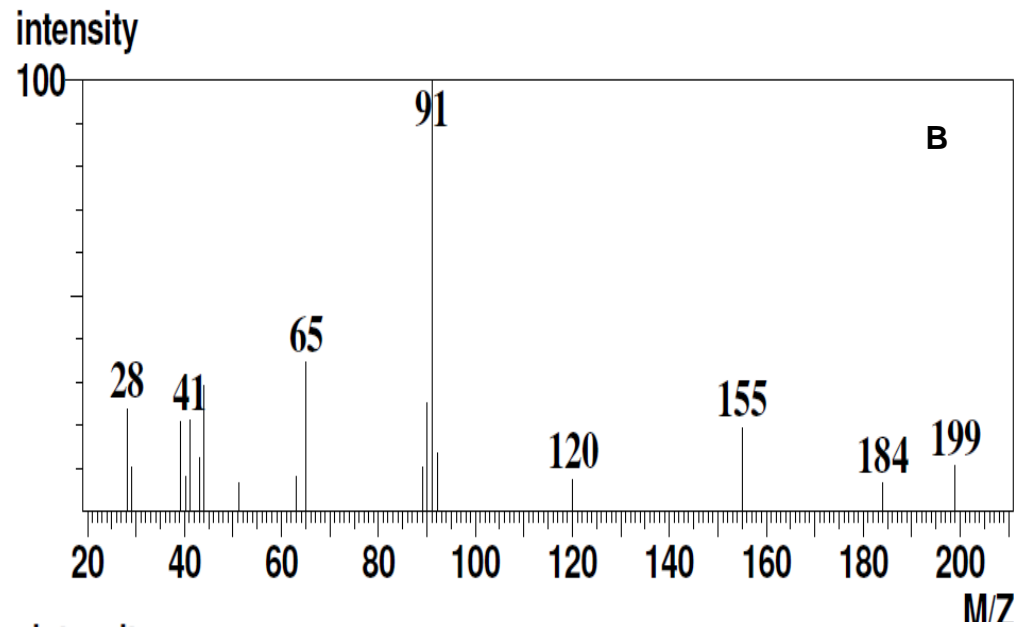
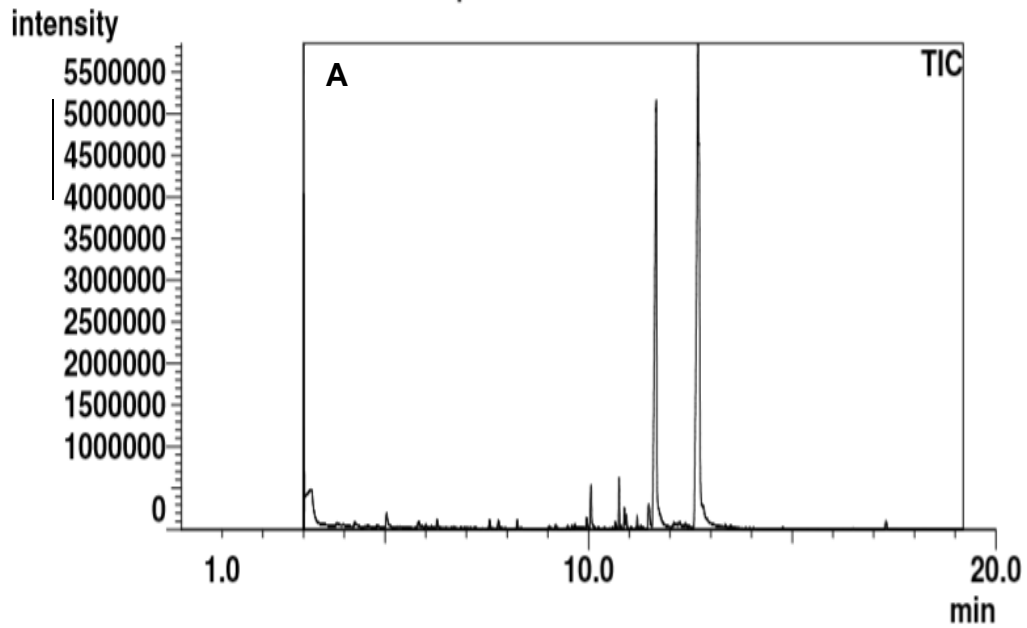
282 Figure 4.

283 3.2 Qualitative analysis of CIPC in spiked and cross contaminated wheat using GCMS.

284

285 The GCMS spectral patterns for CIPC-spiked and cross contaminated wheat samples (Figure
286 5C & D) were both consistent with the expected CIPC spectrum (213/215 m/z: parent ion;
287 153/154 m/z: m-chlorophenyl isocyanate; 171/173 m/z: free acid formed from isopropyl
288 residue; 127/129 m/z: chloraniline) as outlined on the NIST database:
289 (<http://webbook.nist.gov/cgi/cbook.cgi?Name=chlorpropham&Units=SI&cMS=on#Mass-Spec>)

290 This demonstrates that this method of detection is suitable for very low CIPC concentrations
291 in cross-contaminated wheat. Extractions obtained from CIPC-free wheat were also
292 concentrated prior to GCMS analysis. The spectral pattern for the CIPC-free wheat was
293 inconsistent with that of CIPC (Figure 5A & B). Similarity search, using the NIST/EPA/NIH
294 mass spectral library (NIST 05) and NIST mass spectral search program version 2.0d,
295 confirmed the peak spectral pattern of the CIPC-free wheat to be consistent with
296 benzenesulfonamide (molecular weight 199). The presence of this compound may be due to
297 chlorsulfuron; a selective herbicide used to control broadleaf weeds and some grasses in
298 wheat (Royuela *et al.*, 1990). This confirms the robustness of the GCMS method in
299 distinguishing between CIPC-free wheat and cross-contaminated wheat, in the presence of
300 other pesticides, at levels close to the limit of detection.



302 Figure 5.

303 3.3 Preliminary trial to assess the importance of contaminated concrete and store atmosphere
304 on CIPC contamination of grain.

305 The results demonstrate that the magnitude of cross contamination was influenced by the
306 CIPC concentration in the adjacent concrete (Table 1). Grains that were in contact with the
307 most contaminated concrete (304 mg kg⁻¹; position 2; Figure 1 and Table 1) had the highest
308 concentrations (25 mg kg⁻¹ and 111 mg kg⁻¹). Also, grain samples exposed to the atmosphere
309 as well as the concrete had higher CIPC concentrations relative to their counter-part samples
310 that were isolated from the atmosphere. For example, grain samples that were equidistant
311 from lateral duct 2 (position 4; Figure 1 and Table 1) had values of 4.3 mg kg⁻¹ (exposed to
312 atmosphere) and 0.53 mg kg⁻¹ (not exposed to the atmosphere). Since the distances of both
313 the exposed and non-exposed samples were the same with respect to lateral duct 2, and
314 assuming that CIPC was evenly distributed across the store from lateral duct 2, it may be
315 assumed that the atmosphere had an influence on the levels of cross contamination. In a case
316 where the exposed and non-exposed grain samples were aligned linearly with respect to the
317 CIPC point of entry into the store, the difference in concentration in the grains could be
318 attributed to the heterogeneity of CIPC distribution in the concrete flooring. For example,
319 grain samples that were aligned linearly to duct 2 (position 3, Figure 1 and Table 1) had
320 values of 43 mg kg⁻¹ (exposed to atmosphere) and 17 mg kg⁻¹ (not exposed to the
321 atmosphere). In this case, the concrete closest to the point of CIPC entry, where the exposed
322 samples were located, is more likely to have higher levels of CIPC. Nevertheless, in all cases,
323 the grains exposed to the concrete and the atmosphere had consistently higher levels of CIPC
324 by factors between 2.5 and 8 approximately, compared to those exposed only to the concrete.
325 The effect of the atmosphere on the cross contamination was subsequently investigated
326 further by eliminating direct contact with the contaminated concrete (section 3.4).

327

328 Table 1. CIPC concentrations (mg kg^{-1}) in grains that were in contact with the surface of
329 CIPC contaminated concrete for 7 months and either exposed or not exposed to the
330 atmosphere.

331

CIPC concentrations in grains (mg kg^{-1})			
Position in store	Total CIPC concentration in concrete core closest to sample (mg kg^{-1})	Mesh Box (exposed)	Plastic Box (not exposed)
1	0.58	0.25	0.079
2	304	111	25
3	216	43	17
4	10.5	4.3	0.53

332

333 3.4 Full experimental design to assess route of CIPC contamination of grain.

334 The preliminary assessment of the route of cross contamination suggests that in addition to
335 direct contact with the contaminated concrete flooring, other factors such as CIPC in the
336 headspace of the store contributes to the process (Table 1). An experimental design with five
337 types of custom-made glassware was subsequently used to investigate the influence of the
338 headspace and the physical contact of the grain with the flooring of a store on the route of
339 cross contamination.

340

341 3.4.1 The possible effect of accumulated dust (particulate material) on the route of cross
342 contamination of grains.

343 The presence of CIPC was confirmed in dust that accumulated on the clock glasses in
344 positions 2 to 4 (on experimental design D) which were in the vicinity of the point of CIPC
345 entry into the store (Table 2). The samples in position 1, which was remote from the point of
346 entry, had no CIPC present. The magnitude of CIPC in the dust and concrete flooring seemed
347 to be dependent on the point of CIPC entry into the store; suggesting that deposition and
348 accumulation of CIPC in the roofing and flooring at these points may have a more
349 pronounced influence on cross-contamination. This suggests that contamination of grain
350 samples in designs A and C may be attributed to both the headspace and dust particles.

351

352 Table 2. Concentration of CIPC in accumulated dust from a commercial store.

Position in store	Total CIPC concentration in concrete core closest to sample (mg kg ⁻¹)	Average CIPC concentration in acetonitrile washes (µg mL ⁻¹)
1	0.58	0.000
2	304	0.018
3	216	0.015
4	10.5	0.014

353

354

355 3.4.2 The effect of the headspace and physical contact with contaminated surfaces on the
356 route of cross contamination.

357

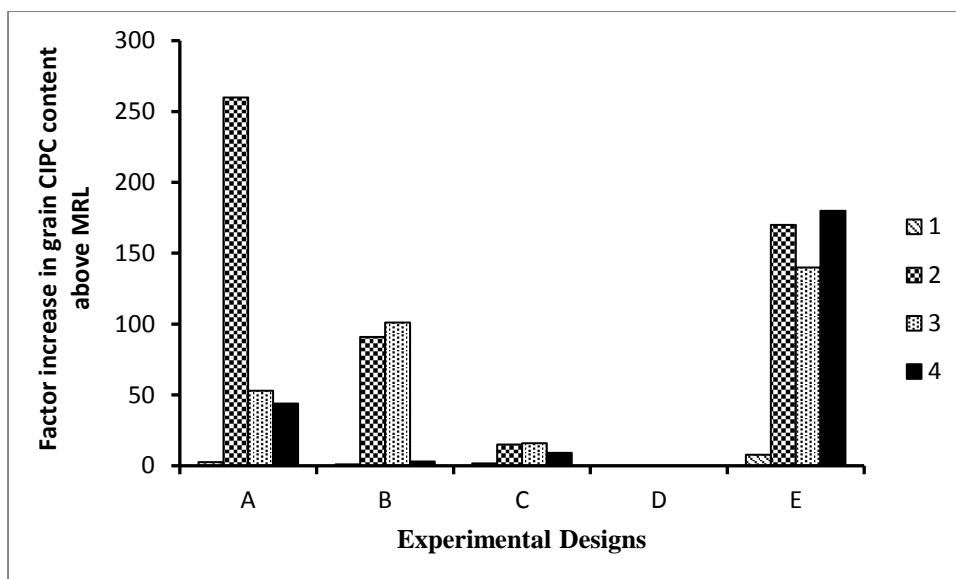
358 The contamination in the grains within the commercial store was calculated for experimental
359 designs A-E in relation to the increase in grain CIPC content above the MRL, for ease of
360 comparison of the factors that affected the route of cross contamination (Figure 6, Table 3).
361 Increase in grain CIPC content is calculated using the MRL, 0.01 mg kg^{-1} as the constant
362 factor i.e. CIPC concentration (mg kg^{-1}) of grain stored at Position 1 in experimental design
363 setup A is equal to $0.026 / 0.01 =$ factor of 2.6 increase whereas CIPC concentration (mg kg^{-1})
364 of grain stored at Position 2 in experimental design setup A is equal to $2.6 / 0.01 =$ factor of
365 260 increase. The results from this trial have shown that cross contamination of grain was
366 influenced by both direct and indirect contact with the concrete surface

367 Experimental design A illustrates how the headspace surrounding the grains (both from the
368 store headspace and the headspace above the contaminated concrete) is significantly
369 contributing to the contamination of the grains. Increase in CIPC content in grain ranged
370 from a factor of 2.6 – 260 times the MRL (Table 3). In an attempt to uncover which route of
371 atmospheric contamination dominated in this commercial store, experimental designs B and
372 C were deployed with results suggesting the headspace above the contaminated concrete is
373 responsible for the majority of the atmospheric transfer of CIPC to grains. The increase in
374 CIPC content in grain in B where only the concrete headspace interacted with the stored grain
375 ranged from a factor of 1.1 – 101 times the MRL whereas in C where only the store
376 headspace interacted with the stored grain, the range was much lower (1.7 – 16 times the
377 MRL, Table 3). A and B also confirm the risk of cross contamination even when grain is
378 stored at an elevated height above the floor. Experimental design D was sealed from both the
379 concrete flooring and the atmosphere and showed no evidence of contamination, confirming
380 the importance of these routes and the robustness of the experimental set up. E was sealed
381 from the atmosphere but was in direct contact with the concrete flooring and had the highest
382 concentration of CIPC in grain resulting in increases in grain CIPC content, ranging from 7.8

383 – 180 times the MRL (Table 3). This suggests a build-up of CIPC vapour in the confined
384 space in addition to direct contact with the contaminated concrete, leading to a relatively high
385 level of contamination. Based on these results, direct contact with the concrete is the most
386 important factor in the route of cross contamination of grain in this store. These results are
387 different to the initial study where exposure to the atmosphere brought about significant
388 enhancements in contamination compared to exposure to the concrete alone. There are
389 several factors that may have contributed to this including: 1. during the preliminary trial (7
390 months duration) there was either little air movement in the store leading to a build-up of
391 CIPC in the atmosphere, leading to increased contamination, 2. there was considerable air
392 movement (disturbance) in the store leading to increased CIPC-contaminated particles
393 eventually ending up on the grain, 3. the plastic boxes used in the initial study did not retain
394 the CIPC vapour or 4. natural environmental factors (temperature and humidity) in the store
395 were influencing the cross contamination process.

396 Overall, cross contamination of grain above the MRL for cereals was feasible irrespective of
397 the CIPC concentration levels in the concrete, indicating that remedial strategies are required
398 to decontaminate the entire structure of stores.

399



400

401 Figure 6.

402 3.4.3 Risk associated with physical storage of grains on contaminated concrete.

403

404 A proposed preliminary risk assessment calculation based on the CIPC levels in concrete can
 405 be used to determine the extent of cross contamination of commodities in contact with
 406 contaminated concrete (Douglas et al., 2018). The contamination risk assessment calculation
 407 using the CIPC level in the concrete at position 3 (126 mg kg^{-1}) gives a value of 0.29 mg kg^{-1} ;
 408 $29\times$ higher than the MRL of CIPC in wheat. The actual CIPC concentrations in the grain,
 409 in the vicinity of concrete at position 3, for the preliminary trial (section 2.4, 7 month
 410 duration) and for the full experimental trial (section 2.5, 3 month duration) suggested that: (I)
 411 the actual CIPC concentration found in the grain can be higher than expected from the
 412 calculated risk assessment value due to heterogeneity of CIPC distribution in the flooring and
 413 (II) the magnitude of contamination of grains is influenced by the total concentration of CIPC
 414 in the concrete flooring; with an increase in magnitude for higher concentrations ($10.5 - 304$
 415 mg kg^{-1} , positions 2-4) relative to position 1 with a lower concentration (0.58 mg kg^{-1} , Table
 416 3).

417 Table 3. CIPC concentrations (mg kg^{-1}) and factor increase in CIPC content above the MRL
 418 (in parentheses) in grains that were in the environment of a commercial store for 3 months.

419

Position	CIPC concentration in concrete flooring (mg kg^{-1})	CIPC Concentration (mg kg^{-1})				
		A	B	C	D	E
1	0.58	0.026 (2.6)	0.011 (1.1)	0.017 (1.7)	0.00 (0)	0.078 (7.8)
2	304	2.6 (260)	0.91(91)	0.15 (15)	0.00 (0)	1.7 (170)
3	216	0.53 (53)	1.02 (102)	0.16 (16)	0.00 (0)	1.4 (140)
4	10.5	0.44 (44)	0.030 (3)	0.092 (9.2)	0.00 (0)	1.8 (180)

420

421

422 4. Conclusion

423 Previously developed HPLC UV/VIS and GCMS methods for quantifying and confirming
 424 residues of chlorpropham in concrete were modified for grain and successfully used to assess
 425 the route of cross contamination in a commercial store. Our research has confirmed that the
 426 headspace above the concrete flooring and below the roof, dust particles and physical contact
 427 with the concrete flooring all contributed to the cross contamination of grains during storage.
 428 Contamination was possible irrespective of the actual CIPC levels in the concrete; with
 429 values of $0.011\text{-}2.6 \text{ mg kg}^{-1}$ all exceeding the MRL (0.01 mg kg^{-1}). This vital information,
 430 conducted in a real store environment, confirms the risk of contamination, even for grain held
 431 at elevated heights above the concrete. The information obtained from this study is
 432 invaluable to the farming industry because recommendations can be made with respect to the
 433 reuse of storage facilities with a history of CIPC use.

434

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505 Figure captions.

506 Figure 1. Floor plan of the commercial store.

507 Positions of the cores (black discs) and positions of the grain samples: mesh boxes (grey
508 rectangles) and plastic boxes (black squares).

509

510 Figure 2. Full experimental design to investigate the route of CIPC cross contamination in a
511 commercial potato store.

512 A) no direct contact with the contaminated concrete surfaces/both the headspace above the
513 concrete surface and the store headspace assessed; B) no direct contact with the contaminated
514 concrete surfaces/only headspace above the concrete surface assessed; C) no direct contact
515 with the contaminated concrete surfaces/only store headspace assessed; D) no direct contact
516 with the contaminated concrete surfaces/no interaction with headspace above the concrete
517 surface or the store headspace and E) only direct contact with the contaminated concrete
518 surfaces assessed as restricted from the store headspace.

519

520 Figure 3. Spatial arrangement of the five experimental designs around a previously analysed
521 core.

522

523 Figure 4. HPLC UV/VIS chromatograms for CIPC-free wheat extract and CIPC cross-
524 contaminated wheat extract.

525 (A) A CIPC-free wheat sample (scaled 0 to 2000 mV) and (B) A CIPC cross-contaminated
526 wheat sample (scaled 0 to 20000 mV). CIPC retention time : 7.2 mins.

527

528 Figure 5. GCMS chromatograms and mass spectra obtained for CIPC-free non-organic wheat
529 extract and CIPC cross-contaminated wheat extract.

530 Non-organic CIPC-free wheat sample (A & B) and a non-organic wheat sample exposed to a
531 store environment and cross contaminated with CIPC (C & D). CIPC retention time: 9.1
532 mins.

533

534 Figure 6. Increase in grain CIPC content above MRL within the five experimental designs at
535 four locations in a commercial store.

536 Increase in grain CIPC content is calculated using 0.01 mg kg^{-1} (MRL) as the constant factor
537 i.e. CIPC concentration (mg kg^{-1}) of grain stored at Position 1 in experimental design setup A
538 is equal to $0.026 / 0.01 =$ factor of 2.6 increase.