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4	Presence of the β -Triketone Herbicide Tefuryltrione in Drinking Water
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26 Abstract

27Triketone herbicides are becoming popular because of their herbicidal activity against sulfonylurea-resistant weeds. Among these herbicides, tefuryltrione (TFT) is the first 2829registered herbicide for rice farming, and recently its distribution has grown dramatically. 30 In this study, we developed analytical methods for TFT and its degradation product 2chloro-4-methylsulfonyl-3-[(tetrahydrofuran-2-yl-methoxy) 31methyl] benzoic acid (CMTBA). TFT was found frequently in surface waters in rice production areas at 32concentrations as high as 1.9 µg/L. The maximum observed concentration was lower than 33 but close to 2 µg/L, which is the Japanese reference concentration of ambient water 3435 quality for pesticides. However, TFT was not found in any drinking waters even though 36 the source waters were purified by conventional coagulation and filtration processes; this 37 was due to chlorination, which transforms TFT to CMTBA. The conversion rate of TFT 38to CMBA on chlorination was almost 100%, and CMTBA was stable in the presence of 39 chlorine. Moreover, CMTBA was found in drinking waters sampled from household water taps at a similar concentration to that of TFT in the source water of the water 40 41 purification plant. Although the acceptable daily intake and the reference concentration 42of CMTBA are unknown, the highest concentration in drinking water exceeded 0.1 µg/L, which is the maximum allowable concentration for any individual pesticide and its 43relevant metabolites in the European Union Drinking Directive. 44

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46 Keywords: pesticide, HPPD inhibitor, river water, chlorination by-products, tap water

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49 **1. Introduction**

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The annual global consumption of pesticides is estimated to be 24 million tons as active 51ingredients, and these ingredients represent approximately 900 different chemicals 5253(USEPA, 2011). This intentional application of such a huge amount of pesticides to open surface areas may have a strong impact on both the environment and human health. Due 54to high public concern regarding residual pesticides in drinking water and food, 5556governments restrict authorized concentration in drinking waters through each standard or directive. In drinking water quality standards or directives, however, the degradation 57products of pesticides are seldom considered. However, some survey studies have 58reported higher concentrations of degradation products than of original forms in river 59waters (Hladik et al., 2008; Botta et al., 2009; Iwafune et al., 2010; McMahen et al., 2016). 60

Rice-farming herbicides easily enter natural river waters at higher rates than other 61 pesticides used in upland farming because they are applied to paddy fields where a large 62amount of water is irrigated, ponded, and drained during the rice-farming seasons (Narita 63 64 et al., 2014). According to a national survey of drinking and source water quality (Matsui, 2016), rice-farming herbicides such as molinate and pretilachlor are detected more 65 frequently at high concentrations than other pesticides. The ADI (acceptable daily intake) 66 67 values of pesticides, which span a broad range (more than five orders of magnitude), affect the significance of measured concentrations relative to the toxicities of pesticides, 68 69 because the individual guideline values of pesticides are derived from their ADI values.

Given this background information, we focused on a new rice-farming herbicide, tefuryltrione (TFT) (Table 1), and its degradation product. TFT is a β -triketone herbicide, which causes weed whitening and death by inhibiting HPPD (hydroxyphenylpyruvate

73 dioxygenase) activity. Triketone herbicides including TFT are becoming popular, replacing sulfonylurea herbicides, because of their herbicidal activity against resistant 74 weeds (Itoh et al., 1999; Uchino et al., 2000). In Japan, TFT was registered in 2007, and 75since then has been increasingly used in rice farming. TFT was also registered in 2015 in 76 77Korea (Park et al., 2012), and other triketone herbicides (mesotrione, sulcotrione, and 78tembotrione) are registered in many other countries, including the USA and several European countries (USEPA, 2007, 2009a; ECHA, 2011). Environmental monitoring 79 80 information about triketone herbicides is gradually accumulating, as is information about 81 their behaviour during water purification processes (Freitas et al., 2008; Patil et al., 2016; Dumas et al., 2017). However, information is still limited regarding their degradation 82 products (Freitas et al., 2004; Durand et al., 2010; Bensalah et al., 2011; Murati et al., 83 2012; Jović et al., 2013; Barchanska et al., 2016), in particular those found in drinking 84 85 water.

In an assessment conducted by the Food Safety Commission of Japan (FSC), the ADI of 86 TFT was set at 0.8 µg/kg/day (FSC, 2009), and a prospective reference concentration of 87 $2 \mu g/L$ can be derived from this ADI value multiplied by standard body weight (50 kg) 88 and allocation factor (10%), divided by drinking water consumption (2 L). This value is 89 90 the lowest among the herbicides registered in Japan. Yet, TFT is not included in the list of motoring candidates of the Japanese Drinking Water Quality Guideline (JDWQG) 91(MHLWJ, 2003), and it is not listed in any of the national drinking water quality standards 92 or guidelines. The low ADI value of TFT, coupled with the recent increase in the quantity 93 of TFT that is shipped annually, raises concern that TFT should be monitored in drinking 9495and source waters.

Accordingly, the aim of this research was to develop a method to analyse TFT in water, to reveal its presence in drinking and source waters, and to investigate its behaviour during the water purification process. Importantly, we identified a degradation product of 99 TFT during the chlorination process, the presence and behaviour of which are also100 described.

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102 **2. Materials and Methods**

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104 2.1. Sampling points of river waters

Twelve rivers that flow through Kanagawa prefecture were selected for water sampling. 105Sampling at the Tsurumi River [Fig. S1 in Supplementary Information (SI)] was 106 conducted first and with the highest frequency of every two weeks from April to 107 108 September 2013 and then April to September 2014. Sampling at the other rivers was conducted every month from April to July 2014. Because high concentrations were 109 detected in the Shibuta River, sampling was conducted every week from May to June 110 111 2015. Samples were transported to the laboratory at Kanto Gakuin University for TFT 112concentration analysis according to the procedure described in the section 2.2. In addition 113to this sampling and analysis, in 2014 and 2015, sampling and analysis were also 114conducted in 8 prefectures by waterworks authorities (Fig. S2 and Table S1 in SI).

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116 2.2. Analytical method

Because no analytical method for TFT concentration in environmental and drinking waters has yet been reported, we developed one (Fig. S3 in SI) in this study after referring to the analytical method for neonicotinoid pesticides because of their similar low Log120 Kow and high water solubility (Kawasaki et al., 2012). After filtrating the sample water 121through a glass fiber filter with nominal pore size of 1.0 µm (GA-100, Advantec Toyo Kaisha, Ltd.) and adjusting the filtrate to pH 3.0 by adding formic acid (the pH adjustment 122is less than necessary: it was implemented for the simultaneous analysis of other 123124pesticides), a specified volume (250 mL) of the resultant sample water was passed through an Oasis HLB cartridge (250 mg, Waters Corporation, USA) at a 5 mL/min flow 125rate by using a concentrator; the Oasis HLB was previously rinsed and conditioned with 12610 mL of MeOH and 20 mL of ultrapure water (LC/MS analytical grade, Wako Pure 127128Chemical Industries, Ltd., Japan) (Kawasaki et al., 2012). The Oasis HLB was then dried 129with N₂ gas for 5 minutes, and then it was eluted with 10 mL of MeOH. The volume of the eluate was reduced under an N_2 gas stream to 0.1 mL. After adding 100 μ L of a 1% 130 formic acid aqueous solution, that mixture was made up to 1 mL with the ultrapure water 131and filtrated through a 0.22-µm filter (PTFE, RephiLe Bioscience, Ltd.). The final 132133concentration factor was, therefore, 250 fold. The pretreatment using the Oasis HLB was 134not carried out with the samples obtained in the laboratory experiment of TFT chlorination because of their high concentrations. Recovery tests for the pretreatment 135were conducted using distilled water and river water spiked with reagent-grade TFT 136(Wako Pure Chemical Industries, Ltd. JAPAN) to obtain a concentration of 2 µg/L, which 137 138is equivalent to the prospective reference concentration in JDWQG. Each test was conducted five times, and the average recovery rate was determined. 139

The liquid chromatography tandem-mass spectrometry (LC-MS/MS) (ACQUITY UPLC System and Quattro Premier XE, Waters Corporation, USA) analytical conditions used are shown in Table S1. The monitor ion and confirmation ion were set to m/z=340.8 and m/z=262.0, respectively. After creating a calibration curve, the detection limit was fixed. The same analytical procedure was applied to the quantification of a degradation product. The monitor and the confirmation ions for the degradation product were set at m/z = 247.0 and 85.0, respectively. The TFT analyses of the samples taken at the 8 prefectures were
conducted at each local waterworks authority according to the above-described method
with some modifications. Further details are provided in Table S2 (SI).

The quantification limit of TFT was 0.02 μ g/L (Table S2). The coefficients of variance (CVs) for multiple analyses (n = 5) at 0.02 μ g/L were 12.0% and 19.6% for distilled water and the river water, respectively. The recovery ratios, by solid phase extraction and elution, were fairly good; they were 118% (n = 5) and 116% (n = 5) for distilled water and the river water, respectively, both at 0.02 μ g/L TFT (this is 1/100 of the prospective reference concentration of drinking water in Japan, 2 μ g/L), when the concentration factor was 250 fold after extraction and elution.

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157 2.3. Identification of the chlorination degradation product of TFT

158To detect and identify degradation product(s), we conducted chlorination experiments in glass bottles containing 25 mL of TFT solution. Reagent-grade TFT was added to a 159160 phosphate buffer solution of pH 7 to bring the concentration to 0.4 µmol/L. Sodium 161hypochlorite solution was then added to bring the free residual chlorine concentration to 1621 mg/L as the chlorination reaction proceeded for 2 hours after the addition of the sodium 163 hypochlorite solution. After the chlorination reaction was allowed to proceed for 2 hours, 164the residual chlorine was quenched to terminate the chlorination reaction by adding 7.5 165 μ L of ascorbic acid solution (0.28 mol/L).

166 The chlorination degradation product formed in these chlorination experiments was first 167 analyzed by liquid chromatography time-of-flight mass spectrometry (LC/TOF-MS) 168 (Xevo G2-XS Tof, Waters Corporation). The elemental composition of the degradation product was determined from its accurate mass obtained by LC/TOF-MS analysis. The structural formula of the degradation product was estimated from its elemental composition and the structural formula of the parent compound (TFT) by using a chemical structure estimation software (Elemental Composition, Waters Corporation, USA).

The structural formula was further confirmed by using orbitrap-tandem-mass 173174spectrometry (Orbitrap-MS) (Q-Exactive Quadrupole-Orbitrap mass spectrometer equipped with a high-resolution accurate-mass OrbitrapTM detector, Thermo Fisher 175Scientific, USA). The injection volume was 5 µL and an Acclaim C18 column (150×2.2 176mm, 2.6 μ m) was used in gradient mode with a flow rate of 0.4 μ L·min⁻¹. Thirty seconds 177after the sample injection, the gradient was started at 2% MeOH/98% water (10 mM 178179ammonium acetate solution), and then the ratio linearly increased to reach 98% MeOH at 180 15 min. Ionization was performed in the electrospray positive mode. The flow rates of the auxiliary and sheath gases were 15 (arbitrary units) and 50 (arbitrary units), respectively. 181 182A spray voltage of 3 kV and S-less TF level of 50 were applied. The temperature of the ion transfer capillary was 350 °C, and the heart temperature was 250 °C. The resolution 183 of the MS analysis was 70,000. The AGC target was 1×10^6 , and the maximum time of 184 injection was 200 ms. For all samples, a 60-900-amu mass range was applied. For 185186 MS/MS analysis, the resolution was set to 17,500, with an isolation window of 5 amu. The collision energy was normalized between 10 and 60%, and the AGC target was 2×10^5 . 187

To confirm the chemical structural formula of the degradation product and to quantify it, a reagent-grade version of the degradation product was obtained from Hayashi Pure Chemical Ind., Ltd. (Osaka, Japan). The analytical method for the degradation product was developed with the LC-MS/MS. After determining the monitor ion and creating a calibration curve, the minimum determination limit was fixed (Table S1). Pretreatment for the extraction and elution using the Oasis HLB cartridge was not conducted because the initial and final concentrations of the samples were sufficiently high without anypretreatment.

To observe changes in TFT and the degradation product, a long-term chlorination experiment was conducted. The chlorination condition are the same as that described above except that the chlorination reaction proceeded for 24 hours after the addition of the sodium hypochlorite solution. The analytical condition for the determination of TFT and the degradation product are shown in Table S2 (SI).

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202 2.4. Sampling points of tap waters

203To determine the level of TFT and the degradation product in drinking water from 204household water taps, we sampled tap water in Niigata prefecture because TFT sales was 205high this region compared with others (see the section 3.1). Tap water samples (25 mL) 206 were collected approximately every day from June to August 2015. Immediately after 207 sampling, residual chlorine was removed by adding an ascorbic acid solution of 0.28 mol/L at a ratio of 7.5 µL per 25 mL, and the samples were shipped to the laboratory at 208209Kanto Gakuin University for analysis. Samples were also corrected at the water treatment 210plant that treats and supplies the drinking water to the household water taps. The source 211water (37.93124°N, 139.11807°E) and the process water were sampled and analyzed for 212TFT concentration at the laboratory of the water treatment plant in Niigata prefecture.

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214 **3. Results and Discussion**

216 *3.1. Distribution and use of TFT*

217TFT is included in 21 commercial pesticide products, all of which are mostly used in rice 218farming. Of these 21 products, 3 contain TFT as the single active ingredient (FAMIC, 2192016). The 18 products are multi-component mixtures containing TFT and other active 220ingredients: 6 products contain oxaziclomefone, 3 contain pyraclonil, 3 contain pyraclonil 221and metazosulfuron, 3 contain fentrazamide, and 3 contain mefenacet. By examining the 222TFT contents and the annual sales of these pesticide products, we calculated the total TFT 223annual sales for each prefecture in Japan (Japan Plant Protection Association, 2010-2015). The quantity of TFT being shipped began to increase in 2009 in Japan, and 44.4 tons were 224225shipped in 2014 (Fig. S4). Yamagata and Niigata prefectures received the largest 226quantities (Fig. S5). The fact that Kanagawa, the site selected for river water sampling, 227 had high TFT sales relative to the area devoted to rice farming (MAFFJ, 2011) suggests 228that there was intensive application of TFT in rice farming in this prefecture.

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230 3.2. TFT concentrations in river waters and drinking waters

231TFT concentrations in the Tsurumi River (the main test river) and the other rivers in 232Kanagawa prefecture are shown in Fig. 1. The maximum detected concentration was 0.19 233µg/L, and the detection rate was 33% (49 samples out of 146) in 2013. In 2014, the maximum detected concentration increased to 1.14 µg/L and the detection rate was 39% 234235(84 samples out of 213). In 2015, the maximum detected concentration increased further 236to 1.85 μ g/L, and the detection rate was 43% (12 samples out of 28). Kanagawa is not a 237prefecture where a large quantity of TFT was sold, although the TFT quantity divided by the rice-farming field area was moderate among other prefectures (Fig. S5). Therefore, 238the TFT detection in Kanagawa suggested that contamination with TFT likely also 239

occurred in other prefectures, particularly Niigata and Yamagata, into which a largeamount of TFT was shipped, as described in the previous section.

242Nationwide monitoring of TFT concentrations in rivers, including drinking water sources, was conducted at waterworks authorities in 7 prefectures, including Niigata in 2014 and 2432442015. The results are shown in Fig. 2. TFT was detected in all of the river waters of the 245prefectures tested. In Niigata and Fukuoka, the river waters used as source waters for drinking water production exhibited the maximum TFT concentration of 0.81 µg/L. From 246247the end of May to the middle of June both 2014 and 2015, the primary period when TFT 248was applied to rice fields, the concentrations were higher. The high concentrations were observed in small rivers whose catchment areas were used mainly for rice farming. The 249250maximum observed concentration was 1.9 μ g/L, which was near 2 μ g/L: the prospective 251reference concentration of the DWQG and the predicted environmental concentration of 252environmental risk assessment for ambient water (MOEJ, 2009).

253However, TFT was not detected in any of the finished waters in the water treatment plants 254of the water authorities (Table S4), indicating that TFT might have been removed or 255transformed during the water treatment processes. Some water treatment plants use 256conventional water treatment processes such as coagulation, sedimentation, filtration, and chlorination. It was unlikely for the TFT to be removed by coagulation, sedimentation, or 257258filtration because its water solubility (64.2 g/L at pH 7) is high and its Log-Kow (1.05) is 259relatively low. In fact, we observed no TFT removal by coagulation and membrane 260filtration in our laboratory experiments (Fig. S6). In the water purification plants, TFT was still detectable in the process water after coagulation-sedimentation at the same level 261262as that in the influent water (Fig. S7). However, TFT disappeared after sand filtration and 263chlorination. It is highly probable that the TFT reacted with the chlorine and degraded. 264We therefore hypothesized that degradation products were formed from the TFT during 265chlorination rather than the TFT being completely mineralized.

268Orbitrap-MS chromatograms were obtained in the positive mode before and after 269chlorination of the TFT-containing solution (Fig. 3). No peak corresponding to TFT was 270observed for the sample after chlorination, but a single peak was observed, suggesting the 271production of a main degradation product. The MS spectra for the peak showed a 272molecular ion at 349.052, which indicated a compound consisting of C₁₄H₁₇ClO₆S. From 273these data, we estimated the structure and proposed that the main product was probably 2742-chloro-4-methylsulfonyl-3-[(tetrahydrofuran-2-yl-methoxy) methyl] benzoic acid 275(CMTBA).

276The MS and MS/MS characteristics, including the theoretical and observed m/z values 277and the molecular structure, are shown in Table 1. The chemical structure of CMTBA was 278in agreement with the molecular formula obtained from the exact mass of the molecular 279ion. The MS spectra of CMTBA showed an NH₄-adapted molecular ion at 366 Da and a protonated molecular ion at 349 Da. For this product, an isotopic distribution typical of 280281the presence of one chlorine atom was observed. A mass loss of 94 was observed for 282CMTBA to the mass of its parent TFT. Moreover, no carboxylic acid function loss was observed in the MS/MS analyses. Therefore, CMTBA would be formed by the cleavage 283284of the ether bond of the chemical side chain of TFT. The formation of CMTBA was finally 285confirmed by observing that the ratio of the two major fragments for the chlorinated TFT 286solution was the same as that for a reagent-grade version of CMTBA.

Quantification of the TFT and CMTBA concentrations before and after chlorination are
shown in Fig. 4. The CMTBA concentration after chlorination was almost the same as the
TFT concentration before chlorination. TFT was not detected after chlorination.

290Therefore, TFT was entirely transformed to CMTBA: the transformation percentage of 291TFT to CMTBA was 94-103%. When a solution containing only CMTBA was subjected 292to chlorination, the CMTBA concentration did not change, even after 24 hours. These 293data indicate that CMTBA, formed by chlorination, is stable in the presence of chlorine, 294and no further degradation of CMTBA occurs. According to the agrochemical evaluation report (FSC, 2009), CMTBA is a degradation product in plant fate tests, soil fate tests, 295and aquatic photo-degradation tests. However, in these tests the transformation 296percentage of TFT to CMTBA was very low (less than 10%) (FSC, 2009). Therefore, any 297 special reaction condition in the presence of chlorine would transform TFT to its stable 298299degradation product CMTBA. The almost 100% transformation percentage of TFT to 300 CMTBA and the stability of CMTBA in the presence of chlorine suggest that CMTBA 301 may be present in drinking water from household water taps if the water undergoes 302 chlorination at the water treatment facility.

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304 *3.5. Presence of CMTBA in drinking water and its source water*

TFT and its degradation product CMTBA were measured in tap waters in Niigata 305 306 prefecture (Fig. 5). Niigata was selected because of the large amount of TFT purchased 307 and the high level of TFT detection in the river water (the source water of the drinking 308 water). TFT was not detected in any of the tap water samples, but CMTBA was detected 309 from late May to the middle of July 2015. The highest observed concentration was 0.35 310 μ g/L. Although this was low compared with the TFT reference concentration of Japan (2 311µg/L), it exceeded the 0.1 µg/L of European Union (EU) Drinking Water Directive (European Union, 1998). Of the 50 samples tested, 36 (72%) contained CMTBA at 312313concentrations > 0.1 μ g/L. TFT concentrations in the source waters of the water 314 purification plant of Niigata showed a similar trend to that of CMTBA concentrations in tap waters. CMTBA concentrations in tap waters were sometimes higher than TFT concentrations in the source waters, but this could have been due 1) to the presence of CMTBA in the source waters, 2) to the limited number of samplings and analyses conducted for raw water at the water purification plant, and/or 3) to a time lag in water distribution from purification at the plant to retrieval from the household water tap.

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321 3.6. Implications

322In drinking water quality guidelines and standards, active ingredients of pesticides are 323usually listed, but their degradation products are seldom considered (WHO, 2011) 324(USEPA, 2009b). The EU directive refers to degradation compounds, but the compounds 325are not specifically named (European Union, 1998). Oxon, sulfone, and sulfoxide, which 326 are by-products formed from organophosphorus pesticides, are listed in the JDWOG 327(MHLWJ, 2003). These products are formed by chlorination process for the water 328purification as well as environmental processes, and they are generally no less toxic than 329their parent compounds (Chambers and Carr, 1993). Their concentrations in drinking 330 waters are monitored by water authorities, and the detection of these degradation products 331is reported in the Water Supply Statistics (JWWA, 2009–2012). In this context, our data clearly call for attention to be paid to the degradation product (CMTBA) of TFT. 332333 Moreover, our findings highlight the need to also consider the degradation products of 334the other triketone herbicides. Actually, the degradation of other triketone herbicides 335 follows the same pattern as TFT (Dumas et al., 2017).

The results of acute toxicity tests in rats indicate that the acute toxicities are low ($LD_{50} > 2500 \text{ (mg/kg)}$ for TFT and > 2500 (mg/kg) for CMTBA) (FSC, 2009). By using the EPI Suite (USEPA, 2012), we compared CMTBA and TFT for their acute toxicity against

339 aquatic organisms, and we found that CMTBA has less toxic than TFT: in fish, the 96-h 340 LD₅₀ for TFT was 31 mg/L, whereas that for CMTBA was 20.4 g/L, and in daphnia, the 48-h LD₅₀ for TFT was 102 mg/L, whereas that for CMTBA was 10.6 g/L. No 341mutagenicity would be seen for CMTBA according to a reverse mutation test with S. 342343typhimurium (TA98, TA100, TA1535, TA1537) with and without S9 mix (FSC, 2009). The ADI for TFT was derived from a two-year chronic toxicity test with rats that 344 confirmed that corneal opacity and keratitis could occur at the lowest dose (FSC, 2009). 345The mechanism behind this corneal effect in rats is HPPD inhibition: triketone-based 346 347compounds inhibit HPPD in the liver, increasing the blood tyrosine concentration, and 348 uptake of tyrosine crystals by corneal epithelial cells (FSC, 2009). In plants, TFT inhibits 349 HPPD, thereby blocking the formation of homogetisate, which leads to foliage bleaching. In an *in vitro* study using the HPPD of *arabidopsis thaliana*, a popular model plant, the 350inhibitory activity was lower for CMTBA than for TFT(MHLWJ, 2016). In mammals, 351352therefore, the inhibitory activity of CMTBA may be similarly lower than that of TFT, 353because the basic structure of triketone is degraded in CMTBA. Mesotrione, which is 354another triketone herbicide, forms two major degradation products [MNBA (2-355methylsulfonyl-4-nitrobenzoic acid) and AMBA (2-amino-4-methanesulfonyl benzoic acid)] (FSC, 2009), possibly through reaction similar to the degradation of TFT. In an in 356 357 vitro test, MNBA weakly inhibits HPPD, but the inhibitory activity is lower than the parent, mesotrione (FSC, 2009). AMBA might be less toxic because it is more degraded. 358359 Compared with TFT, overall, CMTBA is considered to have no higher chronic toxicity 360 through the HPPD inhibition, which facilitates the establishment of the ADI of TFT. However, given the high concentrations of CMTBA found in drinking waters, further 361362 study of its toxicity might be needed to confirm that CMTBA is indeed lower in the 363 chronic toxicity than TFT.

366 4. Conclusion

1) An intensive survey investigating rivers in Kanagawa prefecture revealed TFT contamination. TFT was detected in 37% of 387 samples analysed; concentrations ranged from 0.02 to 1.85 μ g/L. In subsequent examinations of river waters used as drinking water sources in other prefectures with high rice-farming activity, TFT was detected at concentrations up to 0.81 μ g/L during the period when TFT was applied to the ricefarming paddy.

2) TFT was not removed by coagulation, but it was 100% transformed by chlorination to
CMTBA. CMTBA was stable in the presence of chlorine.

375 3) CMTBA was found in drinking water sampled from a household water tap. The 376 concentrations of CMTBA in drinking waters were similar to those of TFT in the source 377 waters of the drinking waters. The highest observed concentration was 0.35 μ g/L. The 378 reference concentration of CMTBA was not derived because there is no defined ADI 379 value for CMTBA, but the concentration of CMTBA exceeded 0.1 μ g/L, the maximum 380 allowable concentration according to the EU directive.

381

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394 References

- 395 Barchanska, H., Kluza, A., Krajczewska, K., Maj, J., 2016. Degradation study of mesotrione
- and other triketone herbicides on soils and sediments. Journal of Soils and Sediments 16,125-133.
- 398 Bensalah, N., Khodary, A., Abdel-Wahab, A., 2011. Kinetic and mechanistic investigations of
- mesotrione degradation in aqueous medium by Fenton process. Journal of HazardousMaterials 189, 479-485.
- 401 Botta, F., Lavison, G., Couturier, G., Alliot, F., Moreau-Guigon, E., Fauchon, N., Guery, B.,
- 402 Chevreuil, M., Blanchoud, H., 2009. Transfer of glyphosate and its degradate AMPA to
- 403 surface waters through urban sewerage systems. Chemosphere 77, 133-139.
- 404 Chambers, J.E., Carr, R.L., 1993. Inhibition patterns of brain acetylcholinesterase and
- 405 hepatic and plasma aliesterases following exposures to three phosphorothionate
- 406 insecticides and their oxons in rats. Toxicological Sciences 21, 111-119.
- 407 Dumas, E., Giraudo, M., Goujon, E., Halma, M., Knhili, E., Stauffert, M., Batisson, I.,
- 408 Besse-Hoggan, P., Bohatier, J., Bouchard, P., Celle-Jeanton, H., Costa Gomes, M., Delbac,
- 409 F., Forano, C., Goupil, P., Guix, N., Husson, P., Ledoigt, G., Mallet, C., Mousty, C., Prévot,
- 410 V., Richard, C., Sarraute, S., 2017. Fate and ecotoxicological impact of new generation
- 411 herbicides from the triketone family: An overview to assess the environmental risks.
- 412 Journal of Hazardous Materials 325, 136-156.
- 413 Durand, S., Sancelme, M., Besse-Hoggan, P., Combourieu, B., 2010. Biodegradation
- 414 pathway of mesotrione: Complementarities of NMR, LC-NMR and LC-MS for qualitative
- 415 and quantitative metabolic profiling. Chemosphere 81, 372-380.
- 416 ECHA, 2011, Committee for Risk Assessment RAC Opinion Proposing Harmonised
- 417 Classification and Labelling at Community Level of Sulcotrione,
- 418 <u>http://echa.europa.eu/documents/10162/4028e39a-b0ce-47e7-8007-f670e803b0f9</u>, accessed

- 419 on 28/11, 2016.
- 420 European Union, 1998, The European Union Drinking Water Directive, <u>http://eur-</u>
- 421 <u>lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31998L0083</u>, accessed on 28/11 2016.
- 422 FAMIC, 2016, Agrochemicals Registration Information System, Food and Agricultural
- 423 Materials Inspection Center, <u>http://www.acis.famic.go.jp/searchF/vtllm001.html</u>, accessed
- 424 on 28/11 2016, in Japanese.
- 425 Freitas, L.G., Götz, C.W., Ruff, M., Singer, H.P., Müller, S.R., 2004. Quantification of the
- 426 new triketone herbicides, sulcotrione and mesotrione, and other important herbicides and
- 427 metabolites, at the ng/l level in surface waters using liquid chromatography-tandem mass
- 428 spectrometry. Journal of Chromatography A 1028, 277-286.
- 429 Freitas, L.G., Singer, H., Müller, S.R., Schwarzenbach, R.P., Stamm, C., 2008. Source area
- 430 effects on herbicide losses to surface waters a case study in the Swiss Plateau. Agriculture,
- 431 Ecosystems & Environment 128, 177-184.
- 432 FSC, 2009, Risk Assessment Reports of Pesticides; TFT, Food Safety Commission
- 433 Government of Japan, <u>https://www.env.go.jp/council/10dojo/y104-16/ref04.pdf</u>, accessed on
- 434 28/11 2016, in Japanese.
- 435 Hladik, M.L., Bouwer, E.J., Roberts, A.L., 2008. Neutral chloroacetamide herbicide
- 436 degradates and related compounds in Midwestern United States drinking water sources.
- 437 Science of The Total Environment 390, 155-165.
- Itoh, K., Wang, G.X., Ohba, S., 1999. Sulfonylurea resistance in Lindernia micrantha, an
 annual paddy weed in Japan. Weed Reseach -Oxford- 39, 413-423.
- 440 Iwafune, T., Inao, K., Horio, T., Iwasaki, N., Yokoyama, A., Nagai, T., 2010. Behavior of
- 441 paddy pesticides and major metabolites in the Sakura River, Ibaraki, Japan. Journal of
- 442 Pesticide Science 35, 114-123.
- 443 Japan Plant Protection Association, 2010-2015. Agrochemical Handbook. Japan Plant
- 444 Protection Association, , Tokyo, Japan, in Japanese.
- 445 Jović, M., Manojlović, D., Stanković, D., Dojčinović, B., Obradović, B., Gašić, U., Roglić, G.,
- 446 2013. Degradation of triketone herbicides, mesotrione and sulcotrione, using advanced
- 447 oxidation processes. Journal of Hazardous Materials 260, 1092-1099.
- 448 JWWA, 2009–2012. Statistics on Water Supply 2007–2010. Japan Water Works Association,
- 449 Tokyo, Japan, in Japanese.
- 450 Kawasaki, E., Kajita, K., Nakata, Y., Sudo, M., Shouhei, H., Motoyuki, K., 2012,
- 451 Development and application of analytical method for neonicotinoid pesticides in drinking
- 452 water. Proc. the 46th Annual Conference of Japan Society on Water Environment, p.501,
- 453 Tokyo, Japan, in Japanese.
- 454 MAFFJ, 2011, Crop Statistics, Ministry of Agriculture, Forestry and Fisheries, Government

- 455 of Japan, <u>http://www.maff.go.jp/j/tokei/kouhyou/sakumotu/menseki/,2011</u>, accessed on 28/11
- 456 2016, in Japanese.
- 457 Matsui, Y.,2016, The FY2015 Report of Health and Labour Sciences Research Grant
- 458 (Research on Health Security Control): Multidisciplinary Research on Risk Assessment and
- 459 Control for Drinking-Water Quality. Ministry of Health, Labour and Welfare, Government
- 460 of Japan, in Japanese.
- 461 McMahen, R.L., Strynar, M.J., McMillan, L., DeRose, E., Lindstrom, A.B., 2016.
- 462 Comparison of fipronil sources in North Carolina surface water and identification of a novel
- 463 fipronil transformation product in recycled wastewater. Science of The Total Environment464 569–570, 880-887.
- 465 MHLWJ, 2003, Revision of Drinking Water Quality Standard. The 4th Meeting of Health
- 466 Sciences Council, Document 3-III, Ministry of Health, Labour and Welfare, Government of
- 467 Japan, Tokyo, Japan, http://www.nilim.go.jp/lab/bcg/siryou/tnn/tnn0264pdf/ks0264011.pdf,
- 468 accessed on 28/11 2016, in Japanese.
- 469 MHLWJ, 2016, High concetration pesticide in source of drinking water, Ministry of Health,
- 470 Labour and Welfare, Government of Japan, Tokyo, Japan, <u>http://www.mhlw.go.jp/file/05-</u>
- 471 Shingikai-10601000-Daijinkanboukouseikagakuka-Kouseikagakuka/0000112645.pdf,
- 472 accessed on 23/02 2017, in Japanese.
- 473 MOEJ, 2009, Standards to Withhold Agricultural Chemicals Registration, Ministry of the
- 474 Environment and Government of Japan,
- 475 <u>https://www.env.go.jp/water/dojo/noyaku/odaku_kijun/rv/t01_tefuryltrione.pdf</u>, accessed on
- 476 28/11 2016, in Japanese.
- 477 Murati, M., Oturan, N., Aaron, J.-J., Dirany, A., Tassin, B., Zdravkovski, Z., Oturan, M.A.,
- 478 2012. Degradation and mineralization of sulcotrione and mesotrione in aqueous medium by
- 479 the electro-Fenton process: a kinetic study. Environmental Science and Pollution Research
- 480 19, 1563-1573.
- 481 Narita, K., Matsui, Y., Iwao, K., Kamata, M., Matsushita, T., Shirasaki, N., 2014. Selecting
- 482 pesticides for inclusion in drinking water quality guidelines on the basis of detection
- 483 probability and ranking. Environment International 63, 114-120.
- 484 Park, M.S., Kim, S.M., Park, Y.S., Lee, K.S., Jung, W., 2012. Herbicidal activity of newly
- 485 rice herbicide TFT mixture against sulfonylurea resistant weeds in Korea. Korean Journal
- 486 of Weed Science 32, 133-138 Korean.
- 487 Patil, C., Calvayrac, C., Zhou, Y., Romdhane, S., Salvia, M.-V., Cooper, J.-F., Dayan, F.E.,
- 488 Bertrand, C., 2016. Environmental Metabolic Footprinting: A novel application to study the
- 489 impact of a natural and a synthetic β-triketone herbicide in soil. Science of The Total
- 490 Environment 566–567, 552-558.

- Uchino, A., Itoh, K., GuangXi, W., Tachibana, M., 2000. Sulfonylurea resistant biotypes of
 Lindernia species in the Tohoku region and their response to several herbicides. Journal of
 Weed Science and Technology 45, 13-20.
- 494USEPA, 2007, EFED Risk Assessment for The Registration of The New Chemical495Tembotrione,EnvironmentalProtectionAgency,
- 496 https://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-012801_26-Feb-
- 497 <u>07 a.pdf</u>, accessed on 28/11 2016.
- USEPA, 2009a, Extension of the Protection Period for Mesotrione Exclusive Use Data
 Environmental Protection Agency, <u>https://www.epa.gov/sites/production/files/2014-</u>
 <u>04/documents/mesotrione-petition.pdf</u>, accessed on 28/11 2016.
- 501 USEPA, 2009b, National Primary Drinking Water Regulations, Environmental Protection
- 502 Agency, <u>http://water.epa.gov/drink/contaminants/index.cfm</u>, accessed on 28/11 2016.
- 503 USEPA, 2011, Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates,
- 504 <u>http://www.epa.gov/sites/production/files/2015-10/documents/market_estimates2007.pdf</u>,
- 505 accessed on 28/11 2016.
- 506USEPA, 2012, EPISuiteVersion4.11,EnvironmentalProtectionAgency,507http://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-
- 508 <u>interface-v411</u>, accessed on 28/11 2016.
- 509 WHO, 2011, Guidelines for Drinking-water Quality 4th ed,
- $510 \qquad \underline{http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/, \ accessed and \ accessed and \ accessed and \ accessed \ acces$
- 511 on 28/11 2016.
- 512



9 Fig. 1. Changes in TFT concentrations in the Tsurumi River and other rivers, including
10 the Shibuta and Suzu Rivers, in Kanagawa Prefecture.
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Fig. 2. Changes in TFT concentrations in river waters used as source waters for drinking
 waters.



Fig. 3. Orbitrap-MS chromatographs of samples obtained (Panel A) before and (Panel B)
after chlorination of a TFT-containing solution. The small panels in Panels A and
B are the MS spectra corresponding to the chromatogram peaks at 8.39 min (TFT)
and 7.02 min (CMTBA), respectively.



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Fig. 4. Changes in TFT and CMTBA concentrations due to chlorination (the error bars show SD for triplicate samples).

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Fig. 6. Changes in TFT and CMTBA concentrations in water sampled from household tap water, source water of drinking water, and treated water at the water purification plant.

	Composition	Theoretical m/z [M-NH4] ⁺	Observed m/z [M-NH4] ⁺	Δ ppm	MS	MS2	Structure
TFT	C20H27O7NCIS	460.1191	460.1194	0.593	460.1194	262.0392 85.0648 305.0479 341.0242	
СМТВА	C14H21O6NCIS	366.0773	366.0772	-0.17	366.0774	349.0507 85.0647 246.9823	

Table 1. Mass spectrum characteristics of TFT and its degradation product (CMTBA).

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2	Supplementary Information
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5	Frequent Presence of the β -Triketone Herbicide Tefuryltrione in Drinking
6	Water Sources and its Degradation Product in Drinking Waters
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 Fig. S1. Sampling points in Kanagawa Prefecture, Japan (the blue lines are rivers, and the dashed red lines show the prefecture border).

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Fig. S2. Prefectures investigated in this research (the dashed red lines show the prefecture
 borders).

Sample water of 250 mL





Fig. S5. Total TFT sales, TFT sales per land area, and TFT sales per rice-field area for
 each prefecture.



Before coagulation-filtration Before coagulation-filtration

Fig. S6. Changes in TFT concentration after coagulation, settling and membrane filtration. Coagulant (poly-aluminum chloride) dose: 20 mg-Al/L. Rapid mixing: 150 rpm. Slow mixing: 40 rpm. Settling: 10 min, Pore size of membrane filter: 0.22 µm. pH: 6.5. Temperature: 20 °C. Analytical condition of TFT: Table 1S. The error bars show SD for triplicate samples.



Fig. S7. Changes in TFT concentration during water treatment processes (May to June,

76 2015, the Waterworks Bureau of the City of Niigata).

Table S1. Analytical methods applied at Kanto Gakuin University and at the water supply facilities in the 8 prefectures.

	Laboratory of Kanto Gakuin University (river and tap water samples)	Laboratory of Kanto Gakuin University (coagulation and chlorination experiments)	Nigata	Kanagawa	Aomori	Hiroshima	Miyagi	Fukuoka	Chiba
Pretreatment	SPE: Oasis HLB (Waters)	Filtration	SPE: Oasis HLB (Waters)	SPE:Sep-Pak PS- 2 and Oasis HLB (Waters)	Filtration	Filtration	Filtration	SPE:Sep-Pak PS2 (waters)	Filtration
LC	AQUITY UPLC (Waters)	UltiMate3000 (Thermo Fisher Scientific)	Quattro Micro API (Waters)	Acquity UPLC (Waters)	AQUITY UPLC (Waters)	NexeraX2 (Shimadzu)	6410B (Agilent)	UltiMate3000 (Thermo Fisher Scientific)	AQUITY UPLC (Waters)
Column	ACQUITY UPLC HSS T3, 2. 1× 100 mm, 1.8 µm (Waters)	Acclaim RSLC C18, 2.1 × 100 mm, 2.2 μm (Thermo Fisher Scientific)	Atlantis dC18, 2.1 \times 150 mm, 3 μ m (Waters)	ACQUITY UPLC HSS T3, 2.1 × 100 mm, 1.8 µm (Waters)	ACQUITY UPLC HSS T3, 2.1 × 100 mm, 1.8 µm (Waters)	L-column2 ODS, 2.1 × 75 mm, 2 µm (CERI)	ACQUITY UPLC HSS T3, 2.1 × 100 mm, 1.8 µm (Waters)	Mightysil RP-18 PA, 2.0×100 mm, 3 μ m (Kanto Chemical)	ACQUITY UPLC HSS T3, 2. 1× 100 mm, 1.8 µm (Waters)
Gradient condition	A: 90 % \rightarrow (14.0 min) \rightarrow 5 % \rightarrow (2.5 min) \rightarrow 5 % \rightarrow (0.1 min) \rightarrow 90 % \rightarrow (3.4 min))	A: 98 % \rightarrow (1.5 min) \rightarrow 60 % \rightarrow (1.5 min) \rightarrow 40 % \rightarrow (1 min) \rightarrow 2 % \rightarrow (1.0 min) \rightarrow 98 % \rightarrow (0.5 min)	A: 90 % \rightarrow (2.0 min) \rightarrow 55 % \rightarrow (15.0 min) \rightarrow 0 % \rightarrow (20.0 min) \rightarrow 90 %, C: 1 %	A: 100 % \rightarrow (5.0 min) \rightarrow 50 % \rightarrow (3.0 min) \rightarrow 5 %	A: 99 % \rightarrow (0.5 min) \rightarrow 60 % \rightarrow (4.0 min) \rightarrow 25 % \rightarrow (8.0 min) \rightarrow 1 % \rightarrow (10.0 min) \rightarrow 99 %	A: 1% \rightarrow (9.8 min) \rightarrow 98% \rightarrow (7.7 min) \rightarrow 98% \rightarrow (0.1 min) \rightarrow 1% \rightarrow (7.5 min) \rightarrow 1%	A: 80% \rightarrow (1.0 min) \rightarrow 60 % \rightarrow (9.5 min) \rightarrow 5%	A: 99 % \rightarrow (1.0 min) \rightarrow 99 % \rightarrow (3.0 min) \rightarrow 40 % \rightarrow (7.0 min) \rightarrow 10 % \rightarrow (8.5 min) \rightarrow 10 % \rightarrow (8.5 1 min) \rightarrow 2 % \rightarrow (9.5 min) \rightarrow 2 % \rightarrow (9.51 min) \rightarrow 99 % \rightarrow (15.0 min) \rightarrow 99 %	A: 98 % \rightarrow (1.5 min) \rightarrow 30 % \rightarrow (1.5 min) \rightarrow 5 % \rightarrow (3.5 min) \rightarrow 5 % \rightarrow (7.0 min) \rightarrow 98 % \rightarrow (5.0 min)
Mobile phase	A: 0.05% formic acid in 2 mM ammonium acetate, B: MeOH in 2 mM ammonium acetate A: 0.05% formic acid in 2 mM ammonium acetate, B: MeOH in 2 mM ammonium acetate	A:10 mM ammonium acetate, B:MeOH	A:Water, B:MeOH, C:1 % formic acid	A: 0.2 % Formic acid : Acetonitrile = 95:5, B: Acetonitrile	A: 0.05 % formic acid, B:MeOH	A: 0.05% formic acid + 0.1% acetate acid, B:MeOH	A:5 mM ammonium acetate, B:MeOH	A:0.1 % formic acid, B:0.1 % formic acid MeOH	A: 0.05 % formic acid, B:MeOH
Injection volume	5 μL	5 μL	50 µL	5 µL	30 µL	30 µL	30 µL	2 μL	40 µL
Flow rate	0. mL min ⁻¹	0.3 mL min ⁻¹	0.2 mL min ⁻¹	0.35 mL min ⁻¹	0.4 mL min ⁻¹	0.2 mL min ⁻¹	0.3 mL min ⁻¹	0.3 mL min ⁻¹	0.4 mL min ⁻¹
Column temperature	40 °C	40 °C	40 °C	45 °C	40 °C	40 °C	40 °C	40 °C	40 °C
MS	Xevo TQ-S (Waters)	Q-Exactive Focus (Thermo Fisher Scientific)	Quattro micro API(Waters)	Xevo TQ MS (Waters)	Xevo TQ-S (Waters)	LC-8040 (Shimadzu)	1200SL(Agilent)	TSQ Quantiva (Thermo Fisher Scientific)	Xevo TQ MS (Waters)
Ionization	ESI positive	ESI positive	ESI positive	ESI positive	ESI positive	ESI positive	ESI Positive	ESI Positive	ESI positive
m/z	443.10, 341.05	460.1194 366.0774	443.10, 261.9	443.10, 341.05	443.05, 341.03	443.10, 341.05	460.2, 341.1	459.9, 443	443.10, 261.9

Detection Limit (µg/L)	0.0004	0.01	0.01	0.002	0.02	0.04	0.02	0.01	0.02
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os fable 52. Il fallu Civit DA fectivel y fales allu Civ	83	Table S2. TFT	and CMTBA	recovery rates and CV
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Target	Water	Recover rate (%)	CV (%)	n	Concentration (µg/L)	Concentration method
TFT	Distillated water	116	12.0	5	0.02	solid-phase extraction
IFI	River water	118	19.6	5	0.02	solid-phase extraction

Target	Water	Recover rate (%)	CV (%)	n	Concentration (µg/L)	Concentration method
СМТВА	Distillated water	97	9	5	0.1	No concentration

Table S3. Detection of TFT in source waters and finished waters at water treatment plants.

Site of water	Source	e water	Finished water		
treatment plant	Number of samples	Number of detected samples	Number of samples	Number of detected samples	
Kanagawa	43	1	43	0	
Miyagi	8	1	8	0	
Chiba	8	3	24	0	
Niigata	51	22	85	0	
Hiroshima	25	3	25	0	
Fukuoka	14	14	14	0	