

# FORMATION OF THE PYRIDINE-ANALOGUE OF 2,3,7,8-TCDD BY THERMAL TREATMENT OF CHLORPYRIFOS, CHLORPYRIFOS-METHYL AND THEIR MAJOR DEGRADATION PRODUCT 3,5,6-TRICHLORO-2-PYRIDINOL

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## Introduction

Pesticide production, use and disposal have contributed significantly to polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) emissions in the past<sup>1-4</sup>. However, also in a recent monitoring program of current used pesticide formulations in Australia PCDD/F were detected in all assessed formulations with high concentrations found in particular in pentachloronitrobenzene/quintozone containing formulations<sup>4</sup> and formations of PCDD/F from sunlight exposure of selected pesticide formulations<sup>5</sup>. From a historic perspective the two active substance with the highest dioxin levels and release were 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and pentachlorophenol (PCP)<sup>1,6</sup>. The production and use of 2,4,5-T and 2,4,5-trichlorophenol – the precursor of 2,3,7,8-TCDD - resulted in large contaminated area during the Vietnam War (total release of 366 kg TEQ)<sup>7</sup>.

While today 2,4,5-T is not approved for use in the European Union' other high production volume pesticides used today – chlorpyrifos<sup>A</sup> and chlorpyrifos-methyl – have as chlorinated aromatic moiety the pyridine-analogue of 2,4,5-trichlorophenol (3,5,6-trichloro-2-pyridinol) (Figure 1). Therefore, these pesticides are potential direct precursors of the pyridine-analogues of 2,3,7,8-TCDD (2,3,7,8-TCDD-Py; Figure 1).

In thermal experiments between 200°C and 300°C, 2,3,7,8-TCDD-Py were not detected from pyrolysis of chlorpyrifos<sup>8</sup>. Therefore, the study was extended to include chlorpyrifos-methyl and the major degradation product of chlorpyrifos, 3,5,6-trichloro-2-pyridinol (TCPy)<sup>B</sup> at a temperature range between 300°C and 380°C.

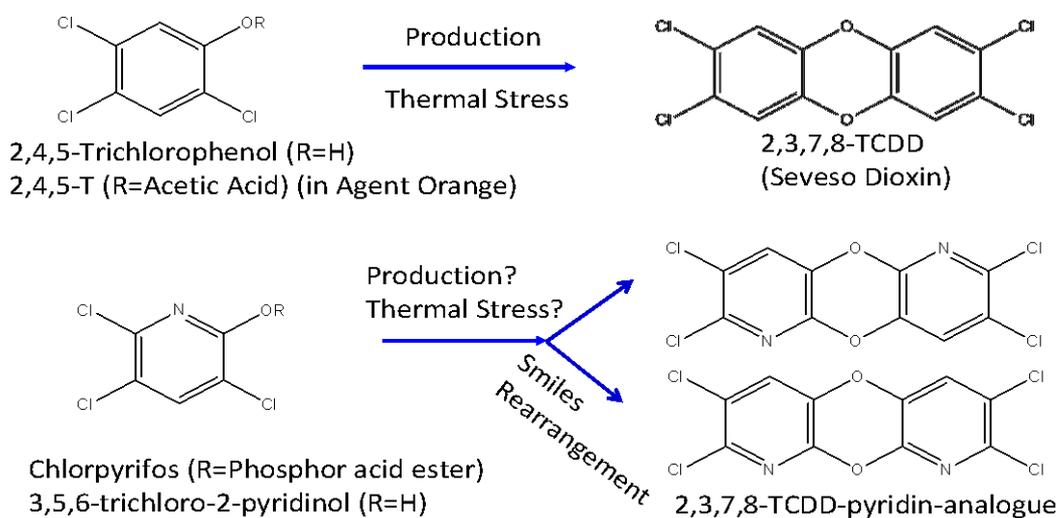


Figure 1: Formation of 2,3,7,8-TCDD from 2,4,5-T/2,4,5-TCP and potential formation of the 2,3,7,8-TCDD pyridine-analogue from chlorpyrifos or 3,5,6-trichloro-2-pyridinol (degradation product).

<sup>A</sup> Chlorpyrifos (O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) is an organophosphate insecticide that inhibits acetylcholinesterase and is used to control insect pests. Chronic exposure to chlorpyrifos has been linked to neurological effects, developmental disorders, and autoimmune disorders. (<http://en.wikipedia.org/wiki/Chlorpyrifos>)

<sup>B</sup> In soil, water, plants and animals, the major pathway of degradation of chlorpyrifos begins with cleavage of the phosphorus ester bond to yield TCPy<sup>9a</sup>. Similar findings were reported in USEPA studies conducting water treatment experiments<sup>9b</sup>.

## Materials and methods

**Chemicals and thermal treatment:** Chlorpyrifos and 3,5,6-trichloro-2-pyridinol were purchased as chemical standards from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and chlorpyrifos-methyl from Wako Pure Chemicals (Osaka Japan). All pyrolysis experiments were carried out in sealed brown glass ampoules (10 ml; air) with about 2 mg of respective chemical at temperatures between 300°C and 380°C in electric furnace (Eyela TMF-3200; Tokyo Rikakikai, Tokyo, Japan). After cooling to room temperature, ampoules were opened and reaction products extracted with toluene.

**GC/MS analysis:** A GC-TOF-MS:7890A (Agilent) with a 30 m DB-5MS (ID: 0.25 mm), a JMS-T100GC (JEOL), a GC-HRMS: 6890GC (Agilent) with a 60 m HT-8PCB (ID: 0.25 mm), an Autospec Ultima (micromass) were used for analysis. Temp. program DB-5MS: 130°C (1 min); 10°C/min to -320°C (10 min hold). HT-8PCB: 130°C (1 min); 30°C/min to 200°C; 20°C/min to 310°C (hold). The TOF/MS was operated at a resolution >5,000 (50% valley) and HRMS at a resolution >10,000. Further, a 450-GC/320-MS (Bruker) 320-MS was used with a 10 m Rapid-MS column for MS spectra and screening.

**DR CALUX<sup>TM</sup> bioanalysis<sup>10</sup>:** The DR CALUX<sup>TM</sup> by BDS bioassay was performed using a rat hepatoma H4IIE cell line stably transfected with an AhR-controlled luciferase reporter gene construct. Cells were exposed in triplicate on 96-well microtiterplates containing the standard 2,3,7,8-TCDD calibration range, the additional 2,3,7,8-TCDD calibration concentrations, a DMSO blank, an internal reference material and various samples extracts at multiple dilutions (e.g. sediment, foodstuffs, feeding stuffs). Following a 24-hour incubation period, cells were lysed, a luciferine containing solution was added and the luciferase activity was measured using a luminometer equipped with two dispensers.

## Results and discussion

### *Thermal treatment chlorpyrifos, chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol*

For assessment of the formation potentials of chlorpyrifos, chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol (TCPy major degradation product of chlorpyrifos in environment), all chemicals were heated under equal conditions (temperature, time) in separate experiments. Measurable concentrations of 2,3,7,8-TCDD-Py were formed from TCPy after 15 minutes at 300°C. Formations increased with increasing temperature by 60 times at 340°C and by 300 times at 380°C. Still after the experiment, 3,5,6-trichloro-2-pyridinol remained partly unreacted in the ampoules. For chlorpyrifos and chlorpyrifos-methyl no formation of 2,3,7,8-TCDD-Py was observed at 300°C and 340°C within 15 minutes reaction time (Table 1). However, at 380°C, from both chemicals 2,3,7,8-TCDD-Py was formed. The formation levels from chlorpyrifos-methyl were six times higher than formation from chlorpyrifos. Levels were approximately two orders of magnitude lower compared to TCPy. Therefore, the potential of chlorpyrifos and chlorpyrifos-methyl to form the 2,3,7,8-TCDD-Py was considerably lower compared to 3,5,6-trichloro-2-pyridinol (Table 1), indicating that the phosphoric acid ester reduces the dioxin-precursor potential compared to the phenol form.

In all experiments both isomers (cis and trans) of 2,3,7,8-TCDD-Py were formed. The cis-isomer was formed in higher concentrations at 340°C and 380°C (approximately 2:1 and 3:1), while at 300°C the cis- and trans-isomers were formed in approximately same concentrations (Table 1).

**Table 1:** Relative abundance of 2,3,7,8-TCDD-pyridine-analogues formed in thermal experiments (15 min)

	2378-TCDD-pyridine-analogue (1)*			2378-TCDD-pyridine-analogue (2)**		
	300°C	340°C	380°C	300°C	340°C	380°C
chlorpyrifos	0	0	1x10 <sup>5</sup>	0	0	3x10 <sup>5</sup>
chlorpyrifos-methyl	0	0	6x10 <sup>5</sup>	0	0	3x10 <sup>6</sup>
3,5,6-trichloro-2-pyridinol	1x10 <sup>5</sup>	6x10 <sup>6</sup>	3x10 <sup>7</sup>	1x10 <sup>5</sup>	1x10 <sup>7</sup>	1x10 <sup>8</sup>

\*trans-isomer of 2,3,7,8-TCDD-Py; \*\*cis-isomer of 2,3,7,8-TCDD-Py

### *Mass spectrum of 2,3,7,8-TCDD-Py*

The mass spectrum of 2,3,7,8-TCDD-Py is shown in Figure 2 along with other detected peaks (pentachloro hydroxybiphenyl derivative of pyridine or the pentachloro hydroxydiphenyl ether derivative) in this experiment

(300°C, 15 min). As for the PCDDs the molecular ion (M+) is stable and the most abundant signal in the mass spectrum of 2,3,7,8-TCDD-Py.

#### **GC-retention behaviour of 2,3,7,8-TCDD and 2,3,7,8-TCDD-Py**

The 2,3,7,8-TCDD-Py had a longer retention time compared to 2,3,7,8-TCDD and other TCDD isomers and did not elute within the time window of TCDD isomers (Figure 2). The reason for the longer retention time is the interaction of the free electron pair from N (aromatic base) with the column surface (e.g. pyridine has a higher McReynolds values compared to benzene). Furthermore, the two isomers of 2,3,7,8-TCDD-Py showed considerably different retention times (Figure 1), which can be explained by the higher polarity of the cis-isomer compared to the trans-isomer. Due to this retention behaviour 2,3,7,8-TCDD-Py do not elute in the normal GC time window of TCDD. Therefore, despite their similar mass (overlap of isotope cluster), they would not be discovered during routine analysis of PCDD/F. Also the 2,3,7,8-TCDD-Py – a basic heterocyclic compounds – might have a different behaviour in the clean-up compared to PCDDs. This might explain why these compounds have not been discovered e.g. in environmental matrices or biota up to now.

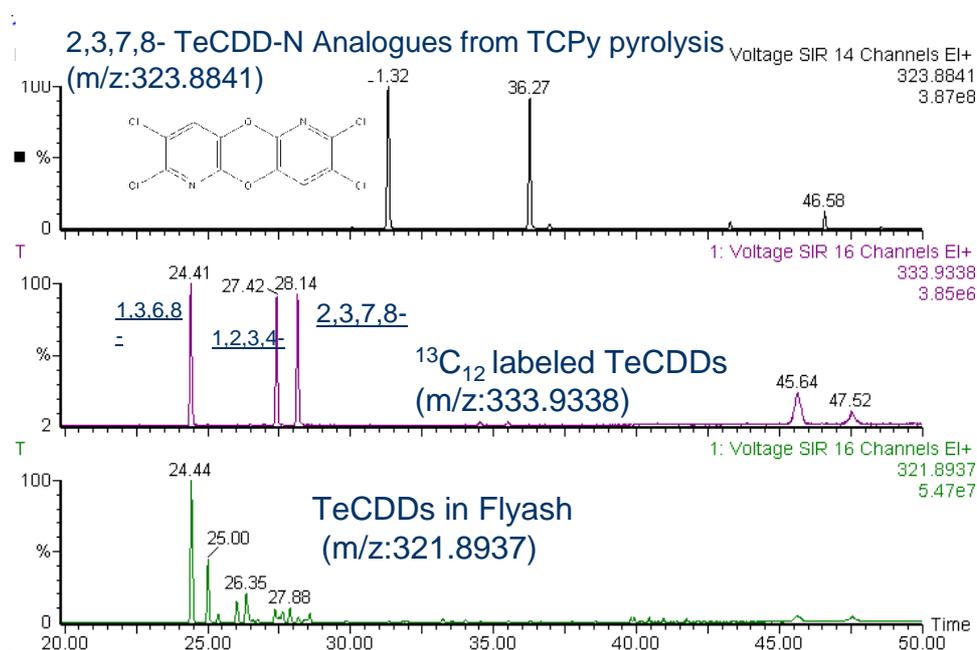


Figure 2: Retention time of N-analogues of 2,3,7,8-TCDD compared to 2,3,7,8-TCDD and TCDD isomers. (GC-HRMS; Column: HT-8PCB (60 m x 0.25 mm))

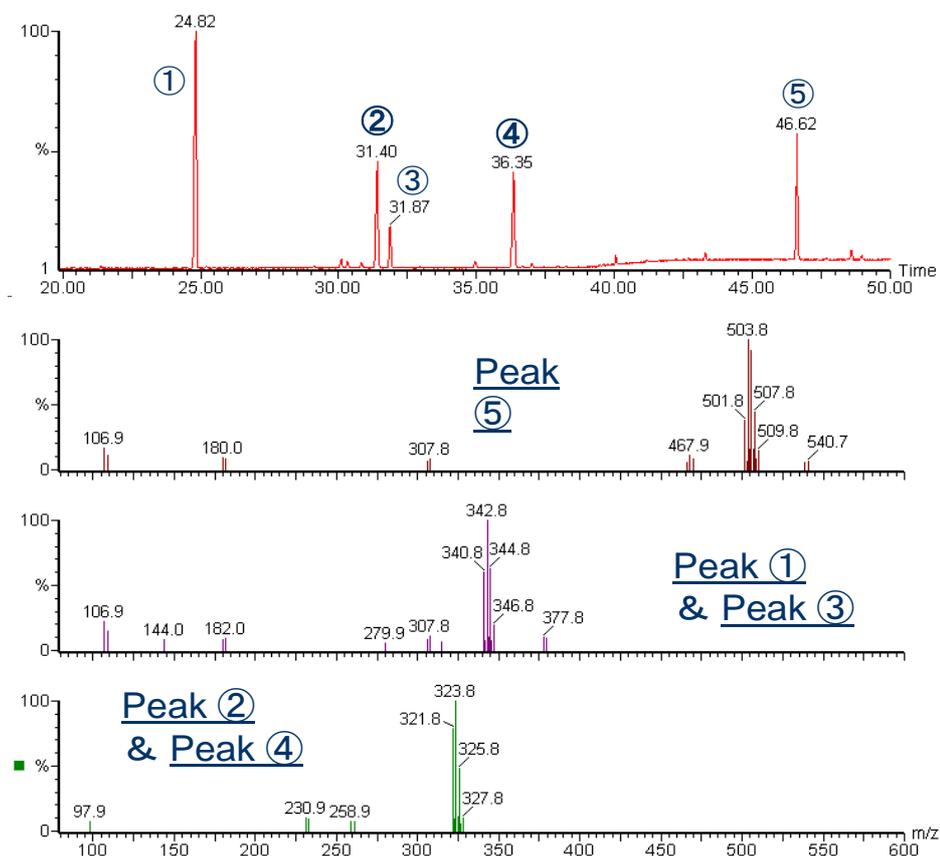
#### **DR CALUX measurements**

Dioxin-like toxicities were assessed with the DR-CALUX<sup>TM</sup> system<sup>10</sup>. For chlorpyrifos (purchased as standard), no dioxin-like toxicity was detected in the crude sample (note that the detection limit was 20 ppm due to the small amount dissolved). Extremely high dioxin-like toxicity was detected from the treatment at 380°C of TCPy (143000 ng 2,3,7,8-TCDD bio-TEQ/g treated TCPy). At 340°C, dioxin-like toxicity was about one order of magnitude lower compared to 380°C. For chlorpyrifos, high toxicity was detected at 380°C (1690 ng 2,3,7,8-TCDD bio-TEQ/g used chlorpyrifos) and lower toxicity at 300°C, each about two to three orders of magnitude lower compared to treated TCPy. The 48 h kinetic indicated that the dioxin-like active compounds were stable.

#### **Potential relevance of the findings**

The formation of the 2,3,7,8-TCDD-Py (having dioxin-like toxicity) formed from chlorpyrifos, chlorpyrifos-methyl and in particular their major degradation product (TCPy) highlights the necessity to investigate the formation of 2,3,7,8-TCDD-Py in real world scenarios like the combustion of post-harvest residues<sup>14</sup>, cigarette smoking or fires in pesticide production or storage<sup>11-13</sup>, in particular when considering that chlorpyrifos is a pesticide used in large amounts worldwide including tobacco cultivation<sup>15</sup>.

Furthermore, 2,3,7,8-TCDD-Py should be screened in chlorpyrifos pesticide formulations. Such assessments should include bio-assays for quantitative information on the extent of dioxin-like activity considering that also compounds other than 2,3,7,8-TCDD-Py seem to have dioxin-like activity in thermal treatment of chlorpyrifos<sup>8</sup>.



**Figure 3:** Chromatogram and mass spectra of the reaction mixture from thermal treatment of 3,5,6-trichloro-2-pyridinol (300°C, 15 min, on GC-TOF/MS; DB-5MS)

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