Short Communication

Measurement of Chlorinated Dioxins and Furans in Cigarette Mainstream Smoke *

by

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SUMMARY

Chlorinated dioxins and furans ('dioxins') are not ordinarily detected in cigarette smoke at levels likely to exceed the recommended 'safe' limits recognised by health and environmental protection agencies. By convention they are measured in terms of their total toxic equivalence (TEQ). The method developed by this group utilises high sensitivity gas chromatography-tandem mass spectrometry (GC-MS/MS). The limit of detection (LOD) was determined to be 4.3 pg TEQ/cig; under ISO 3308 smoking conditions. Dioxins are not subject to any routine regulatory control in cigarette smoke. However, certain dioxin congeners are highly toxic and they have been cited by the United States Food and Drug Administration (FDA) as harmful and potentially harmful constituents (HPHC) of tobacco smoke. This raises the issue of how to monitor and measure uncertainty when the analyte(s) of interest are not normally present in the matrix of interest. In the absence of a reference cigarette containing levels of dioxins above the detection limit; we prepared test pieces containing known quantities of 17 dioxin congeners, regarded by the World Health Organisation (WHO) as significantly toxic. This paper provides data for the transfer rates performed under ISO 3308 (ISO) and Health Canada Intense (HCI) machine-smoking conditions; and proposes a means to estimate the uncertainty (*U*) based on TEQ. [Beitr. Tabakforsch. Int. 26 (2015) 214–218]

ZUSAMMENFASSUNG

Chlorierte Dioxine und Furane („Dioxine“) werden in Zigarettenrauch gewöhnlich nicht in Mengen nachgewiesen, die die empfohlenen von Gesundheits- und

Umweltschutzbehörden anerkannten „sicheren“ Grenzen überschreiten könnten. Konventionsgemäß werden sie mit ihrer gesamten Toxizitätsäquivalenz (TEQ) gemessen. Die von dieser Gruppe entwickelte Methode verwendet hochempfindliche Gaschromatographie-Tandem-Massenspektrometrie (GC-MS/MS). Die Nachweisgrenze wurde unter ISO 3308-Rauchbedingungen mit 4,3 pg TEQ/Zigarette ermittelt. Dioxine unterliegen keiner routinemäßigen regulatorischen Kontrolle. Allerdings sind bestimmte Dioxin-Kongenere hochtoxisch und wurden von der US-amerikanischen Food and Drug Administration (FDA) als schädliche und potenziell schädliche Inhaltsstoffe (HPHC) von Tabakrauch aufgeführt. Dies wirft die Frage auf, wie man die Unsicherheit überwachen und messen kann, wenn die betreffenden Analyte in der untersuchten Matrix normalerweise nicht vorhanden sind. Mangels einer Referenzzigarette mit Dioxinmengen oberhalb der Nachweisgrenze haben die Autoren Proben hergestellt, die bekannte Mengen von 17 Dioxin-Kongeneren enthalten, welche von der Weltgesundheitsorganisation (WHO) als signifikant toxisch angesehen werden. Die vorliegende Arbeit liefert Daten für die Transferraten unter ISO 3308 (ISO) und Health Canada Intense (HCI)-Maschinenrauchbedingungen und schlägt eine Möglichkeit zur Schätzung der Unsicherheit (*U*) basierend auf TEQ vor. [Beitr. Tabakforsch. Int. 26 (2015) 214–218]

RESUME

D'ordinaire, les furanes et les dioxines chlorés (ci-après dénommées 'les dioxines') ne sont pas détectés dans la fumée de cigarette à des niveaux susceptibles de dépasser les limites dites de sécurité recommandées et tracées par les

agences de protection de l'environnement et de la santé. Par convention, ces substances sont mesurées selon leur équivalence toxique totale (TEQ). La méthode mise au point par le présent groupe combine la chromatographie en phase gazeuse ultrasensible et la spectrométrie de masse en tandem (GC-MS/MS). Le seuil de détection (LOD) a été posé à 4,3 pg TEQ/cigarette, à savoir en-dessous de la norme ISO 3308 relative au tabac et produits du tabac. Les dioxines présentes dans la fumée de cigarette ne sont pas soumises à un quelconque contrôle réglementaire de routine. Cependant, certains congénères de la dioxine sont hautement toxiques et ont été qualifiés par la United States Food and Drug Administration (FDA) de constituants nocifs et potentiellement nocifs (HPHC) de la fumée de cigarette. Par conséquent se pose la question des modalités de suivi et de mesure de l'incertitude lorsque la (les) substance(s) à analyser n'apparaissent normalement pas dans la matrice concernée. En l'absence d'une cigarette de référence contenant des niveaux de dioxine supérieurs au seuil de détection, nous avons préparé des éprouvettes contenant des quantités connues de 17 congénères de la dioxine considérés par l'Organisation mondiale de la Santé (OMS) comme présentant une toxicité significative. Le présent article apporte des données qui concernent les taux de transfert et ont été relevées à l'aide d'une machine à fumer utilisée dans des conditions conformes aux recommandations de Health Canada Intense (HCI) et à la norme ISO 3308 (ISO). Par ailleurs, il propose un moyen d'estimer l'incertitude (*U*) en se fondant sur la valeur TEQ. [Beitr. Tabakforsch. Int. 26 (2015) 214–218]

Legislation in the United States in 2009 gave regulatory authority over the tobacco industry to the Food and Drug Administration (FDA) (1). A list of 96 (later reduced to 93) harmful and potentially harmful constituents (HPHC) were published in the Federal Register on August 12, 2011 (2, 3). Chlorinated dioxins and furans ('dioxins') were included in the HPHC list. It is the only constituent on the list that is not a single compound, rather it refers to a group of structural congeners. Mandatory testing of 18 HPHCs in cigarette smoke was introduced in 2012 (4); however, regulatory implementation of the other constituents including dioxins has currently not been specified by the FDA and any discernible action is likely to be subject to critical review.

Dioxins are ubiquitous in the environment due to anthropogenic sources such as waste incineration and chemical

processing of substrates containing dioxin pre-cursors (5, 6). The World Health Organisation (WHO) and other agencies regard polychlorinated dibenzo-dioxins (PCDD) and polychlorinated dibenzo-furans (PCDF), containing lateral chlorine atoms, that is, at the 2,3,7,8-positions (Figure 1), as having significant toxicity (7). The toxic equivalence factor (TEF) expresses the toxicity of individual congeners in terms of the most toxic form of dioxin (2,3,7,8-tetrachlorodibenzo-dioxin; 2378-TCDD). The total toxic equivalence (TEQ) is a single figure which expresses the product of the smoke concentration and TEF of individual congeners (given in Table 1) as a yield per cigarette. The United States Environmental Protection Agency (EPA) has set a threshold for 'safe' dioxin exposure at 0.7 pg TEQ/kg body weight per day (8).

Previous studies have found certain dioxin congeners in the cigarette mainstream smoke (MSS) of certain brands (9, 10) albeit not the most toxic species such as 2378-TCDD and, in terms of toxic equivalence, well below concentrations that could potentially expose the smoker to levels above 'safe' limits. Studies by this group and presented to the CORESTA Smoke Science and Product Technology Joint Meeting, in 2013, have determined that dioxins are not ordinarily produced during the combustion and pyrolysis processes in cigarette smoke at detectable levels (11). Three types of reference cigarettes were sampled under ISO 3308 (ISO) (12) and Health Canada Intense (HCI) (13) machine-smoking conditions. The limits of detection (LOD) were 4.3 pg TEQ/cig (ISO) and 8.6 pg TEQ/cig (HCI). It is reasonable to assume that certain dioxin congeners could be found in tobacco smoke but based on these findings at insufficient levels to realistically contribute to any toxic or carcinogenic properties of smoke. Any dioxins detected in smoke are likely to have been transferred from the tobacco. This could be due to contamination during growing or during packaging / shipment by pre-cursors such as polyvinyl chloride (PVC) and chlorophenol.

The issue that this author feels should be addressed concerns how to monitor and estimate uncertainty of measurement of smoke analytes where no 'natural' levels occur. Batch-to-batch reproducibility is vitally important and forms the basis of any accredited method under ISO/IEC 17025:2005. The approach presented in this paper involves the preparation and analysis of monitor test-pieces 'spiked' with known quantities of the 17 congeners recognised as having significant toxicity.

The test-pieces were prepared by injecting 1 µL stock solution (0.5 µg/mL each; toluene) through the lit end of

Figure 1. Tetrachlorodibenzo-dioxin (TCDD) and Tetrachlorodibenzo-furan (TCDF) with chlorines at lateral 2,3,7,8 positions.

Table 1. Toxic Equivalence Factors (TEF) of polychlorinated dibenzo-dioxins (PCDD) and dibenzo-furans (PCDF) defined as significantly toxic by the World Health Organisation (WHO, revised in 2005). There are 17 dioxin congeners (tetra- through -octa chlorinated).

Dioxin congener	TEF Values (WHO, 2005)
2378-TCDF	0.1
2378-TCDD	1
12378-PCDF	0.03
23478-PCDF	0.3
12378-PCDD	1
123478-HxCDF	0.1
123678-HxCDF	0.1
234678-HxCDF	0.1
123478-HxCDD	0.1
123678-HxCDD	0.1
123789-HxCDD	0.1
123789-HxCDF	0.01
1234678-HpCDF	0.01
1234678-HpCDD	0.01
1234789-HpCDF	0.01
OCDD	0.0003
OCDF	0.0003

Kentucky reference (3R4F) monitor cigarettes to a depth of 20–30 mm. The smoke condensate from 20 cigarettes (ISO) or 10 (HCl) was collected on Cambridge filter (CF) pads and 10 replicate tests performed for each regime. The total particulate matter (TPM) was determined in accordance with ISO 4387 (14). TPM values were consistent with unspiked cigarettes and within in-house control limits and inter-laboratory collaborative trials (15) (Figure 2). A further 10 replicates of 3R4F cigarettes were smoked and the dioxins spiked directly onto the smoked CF pad. A clean-up method developed by MAEOKA and co-workers for analysis of industrial emissions (16) was adapted for the extraction of dioxins from the smoke condensate. This comprised of a two-stage procedure in which the extract was passed through a multi-layer activated silica gel column followed by a dual-layer activated charcoal column. Analysis was performed using gas chromatography-tandem mass spectrometry (GC-MS/MS) (11). The 3R4F tobacco rods were spiked with 500 pg of each congener, equivalent of 1535.3 pg TEQ/cig. Measured mean values were obtained for each individual congener. The mean values expressed in terms of toxic equivalence were 146.5 pg TEQ/cig (ISO) and 367.0 pg TEQ/cig (HCl) (Table 2). The overall transfer rates were 9.5% (ISO) and 23.9% (HCl). The estimate of uncertainty (U (%)), derived from the standard deviation (SD) multiplied by a coverage factor ($k = 2$), ranged between 15.0% and 32.5% (ISO), and 8.4% to 48.6% (HCl). It would appear to make sense to represent U (%) in terms of TEQ to keep it consistent with the reporting of concentration. The overall estimate of uncertainty (U TEQ (%)) is calculated from the sum of the 17 individual U (%) values multiplied by each respective TEF value. Therefore, U TEQ (%) for this test piece would be reported as 64.4% TEQ (ISO) and 61.5% TEQ (HCl). The recovery from the pad was evaluated. The CF pads were theoretically spiked with 50 pg/cig (ISO) or 100 pg/cig (HCl) of each congener, equivalent of 153.5 pg

TEQ/cig (ISO) and 307.1 pg TEQ/cig (HCl). The measured mean values were 144.8 pg TEQ/cig (ISO) and 296.4 pg TEQ/cig (HCl) (Table 3). The U TEQ (%) values were 27.2% TEQ (ISO) and 38.6% TEQ (HCl). The relative recoveries were 94.4% (ISO) and 96.5% (HCl).

As well as obvious toxicological reasons for reporting in terms of TEQ; it also provides a useful means of simplifying otherwise unwieldy sets of data. By applying TEQ to uncertainty a concise measurement was made which reflects the upper and lower extremes of the matrix. Although generally thermally stable, some thermal decomposition will occur during transfer from the tobacco to the smoke at the temperatures produced in the cigarette coal (~600–900 °C). Higher variability is to be expected from cigarettes in which the analytes of interest might be present in a small ‘localised’ area of the tobacco rod (during artificial rod spiking) compared with ‘natural’ abundances which have far greater homogeneity. Losses due to decomposition and sidestream smoke aerosol will vary due to the positioning of the material inside the rod. This should be offset by smoking multiple cigarettes (10 or 20 cigarettes) per replicate test. However, it would be very difficult to exactly replicate and would also be very time-consuming for use as a routine control monitor. The results serve to demonstrate that low levels of dioxins theoretically contained within the spiked tobacco filler can be trapped and analysed in MSS. The U TEQ values represent the upper extreme of the method variability.

Figure 2. Comparison of TPM data from (a) ISO and (b) HCl.

Table 2. Mean values (n = 10) and estimates of uncertainty (U (%)) for 17 dioxin congeners obtained under ISO and HCl machine-smoking conditions from 3R4F tobacco rods spiked with 500 pg of each congener, equivalent of 1535.3 pg TEQ/cig. The mean concentration in terms of TEQ (pg TEQ/cig) is used to derive the overall transfer rate. Estimate of Uncertainty (U TEQ (%)) is the sum of each individual toxicity weighted U (%). Samples were collected and ran over a 14-day period at unspecified times.

Dioxin congener	ISO mean (pg/cig)	ISO U (k = 2) (%)	HCl mean (pg/cig)	HCl U (k = 2) (%)
TCDF	53.0	15.0	136.0	9.4
TCDD	55.5	22.3	135.7	14.0
12378-PCDF	51.6	16.0	128.2	11.0
23478-PCDF	25.5	25.9	61.4	48.6
12378-PCDD	51.3	18.3	131.5	17.1
123478-HxCDF	45.1	17.3	117.6	8.4
123678-HxCDF	47.6	28.4	129.6	34.1
234678-HxCDF	19.3	21.2	46.0	38.0
123478-HxCDD	45.7	16.5	116.0	19.3
123678-HxCDD	44.8	17.2	110.1	16.0
123789-HxCDD	33.7	32.1	82.3	20.3
123789-HxCDF	23.8	17.9	61.5	21.8
1234678-HpCDF	44.2	16.2	111.5	18.9
1234678-HpCDD	43.3	21.6	109.8	13.7
1234789-HpCDF	35.9	22.1	90.7	36.7
OCDD	45.8	32.5	110.2	24.6
OCDF	35.8	23.5	91.8	20.6
Mean (pg TEQ/cig; n = 10)	146.5		367.0	
Overall Transfer Rate	9.5%		23.9%	
U TEQ (%) = $\sum U(\%) \times \text{TEF (C1-C17)}$		64.4		61.5

U TEQ (%): Estimate of Uncertainty TEQ;

$\sum U(\%) \times \text{TEF (C1-C17)}$: sum of U (%) multiplied by each corresponding TEF value.

Table 3. Mean values (n = 10) and estimates of uncertainty (U (%)) from pad extracts spiked with 50 pg (ISO) or 100 pg (HCl), equivalent of 153.5 pg TEQ/cig (ISO) and 307.1 pg TEQ/cig (HCl). The relative recovery is derived from the mean concentration (pg TEQ/cig) divided by the theoretical. Samples were collected and ran over a 14-day period at unspecified times.

Dioxin congener	ISO mean (pg/cig)	ISO U (k = 2) (%)	HCl mean (pg/cig)	HCl U (k = 2) (%)
TCDF	48.3	4.6	97.4	7.6
TCDD	49.0	5.2	99.5	9.6
12378-PCDF	47.9	7.7	96.6	9.6
23478-PCDF	43.3	14.4	87.3	18.0
12378-PCDD	46.6	9.4	98.4	11.7
123478-HxCDF	47.8	11.5	94.7	15.5
123678-HxCDF	46.5	14.3	96.4	21.0
234678-HxCDF	39.7	15.4	80.8	23.2
123478-HxCDD	49.7	6.7	94.9	10.3
123678-HxCDD	48.1	8.1	96.9	14.5
123789-HxCDD	48.3	15.0	95.0	17.9
123789-HxCDF	43.6	13.5	84.6	16.8
1234678-HpCDF	47.2	8.7	97.1	15.8
1234678-HpCDD	47.8	13.0	96.4	13.2
1234789-HpCDF	56.9	9.5	104.1	21.5
OCDD	52.7	9.2	103.2	18.8
OCDF	46.6	11.9	96.9	18.4
Mean (pg TEQ/cig; n = 10)	144.8		296.4	
Relative Recovery	94.4%		96.5%	
U TEQ (%) = $\sum U(\%) \times \text{TEF (C1-C17)}$		27.2		38.6

U TEQ (%): Estimate of Uncertainty TEQ;

$\sum U(\%) \times \text{TEF (C1-C17)}$: sum of U (%) multiplied by each corresponding TEF value.

Spiking directly onto a smoked pad assumes that the 'transfer rate' onto the CF pad is consistent for different cigarettes and U is only affected by the extraction efficiencies. As expected, U TEQ values were much lower than for the spiked test-pieces. This approach represents the lower extreme of the method variability. The 'true' uncertainty of measurement probably sits somewhere between these two extremes and is academic for the purposes of routine analysis. Dioxins are found in the 'semi-volatile' fraction of the smoke aerosol and would be expected to be filtered out in similar proportion to overall "tar". Therefore, provided that the studied cigarettes give consistent TPM yields collected on the CF pad then it is reasonable to assume any transfer of dioxins would be reproducible from batch-to-batch.

In conclusion, effective monitoring of dioxins in cigarette MSS can be achieved by pad spiking of a smoked 3R4F reference cigarette within specified TPM control limits. The U TEQ (%) determined from the prepared test-pieces provides a more realistic estimate of uncertainty.

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