

Testing for Dioxin and Furan Contamination in Triclosan

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Triclosan is a broad-spectrum antibacterial/antimicrobial agent first introduced commercially more than 30 years ago.¹ Because of its bacteriostatic properties against a wide range of both Gram-negative and Gram-positive bacteria, it has found popular and increased use in various personal care products such as toothpaste, deodorants, handsoaps, bodywashes, dishwashing liquids and cosmetics. It has also found uses as an additive for plastics and polymers and as a textile treatment that reportedly gives these materials bacteriostatic properties.

Triclosan is a diphenyl ether (bis-phenyl) derivative known as either 5-chloro-2-(2,4-dichlorophenoxy) phenol or 2,4,4'-trichloro-2'-hydroxydiphenyl ether. Structurally, it is related to several bis-phenyl polychlorinated and bis-phenyl chlorophenols.

As a consequence of the synthesis chemistry of polychloro diphenyl ethers and phenoxy phenols, which include triclosan, the potential exists for the formation of small quantities of unwanted trace and ultra trace byproducts that are of concern.^{2,7}

Research carried out over the past 25 years has revealed that phenoxy herbicides such as 2,4-dichlorophenoxy acetic acid and 2,4,5-trichlorophenoxy acetic acid,⁸⁻¹⁰ the bactericide hexachlorophene,¹¹⁻¹³ various chlorophenols (such as pentachlorophenol used in wood treatment¹⁴), certain polychlorophenoxy phenols,⁶ the polychlorodiphenyl ethers⁷ and the diphenyl ether herbicides¹⁵ can all contain varying levels of polychlorinated dibenzo-p-dioxins and dibenzofurans. Miller et al.¹⁶ identified hydroxy-chlorodiphenyl ethers that include triclosan as "pre- and iso predioxins."

Consequently, it is possible that several polychlorinated dibenzo-p-dioxins (dioxins) and polychlorinated dibenzofurans (furans) can be formed in low levels as synthesis impurities in triclosan.^{2,6,12}

Extensive studies have been performed on triclosan manufactured under the trade name Irgasan DP300 by Ciba Specialty Chemicals Company. This level of testing is principally due to the fact that Irgasan DP300 was the first

commercially available triclosan and, for many years, the only brand of triclosan manufactured and sold. Consequently, the chemistry of Irgasan DP300 in relation to dioxin and furan formation has been researched.^{2,5,15}

TCDD and TCDF

It is well established from the manufacture of hexachlorophene¹¹ that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) can be formed as unwanted contamination byproducts from the manufacturing process. Likewise, the manufacture of triclosan can also result in the formation of TCDD and TCDF as unwanted contamination byproducts. However, in high resolution gas chromatography - high resolution mass spectrometry (HRGC-HRMS) studies of Irgasan DP300, Beck et al.² reported finding no presence of TCDD or TCDF at a detection limit of 5 pg/g (ppt), which is one half of the 10 pg/g limit set by the Federal Republic of Germany and the EU.

Interest in determining the presence of TCDD and TCDF stems from their potential toxicity as well as the possibility of both compounds forming in triclosan during the manufacturing process. TCDD has itself earned the reputation, rightly or wrongly so, as one of the most toxic substances known.

While the toxicological effects and routes of action of these compounds continue to be researched and debated, it is generally agreed that human expo-

Key words

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Abstract

Each batch of triclosan should be analyzed for trace amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran, which can form as unwanted manufacturing by-products, as shown in triclosan from six commercial Asian sources.

sure to them should be significantly restricted. For example, this concern over TCDD levels led the Belgian government to order a mass slaughter and incineration of livestock in 1999.¹⁷

At the same time, triclosan is experiencing a substantially increased use in household, personal care product and consumer OTC formulations that make the drug claim of antimicrobial action. As a result, in 1996 the United States Pharmacopeia (USP) proposed a new monogram¹⁸ for the analysis of triclosan. The monogram included a Limit Test for both TCDD and TCDF. This proposed monogram underwent several revisions.^{19,20} The final monogram, in USP 24,²¹ set the limit for both TCDD and TCDF in triclosan at less than 1.0 pg/g (ppt), 5 times less than what Beck was able to measure and 10 times less than the EU standard.

The substantial increase in consumer demand for OTC products that employ triclosan as the antimicrobial active ingredient has prompted manufacturers to produce triclosan. There are now some 30 producers who manufacture triclosan. While the levels of TCDD and TCDF in Irgasan have been studied and characterized, "non-Irgasan" triclosan originating from these other manufacturers has not.

Evaluating Triclosan for TCDD and TCDF

Several sources of "non-Irgasan" triclosan were evaluated by our laboratory to determine the extent of the formation of TCDD and TCDF. Six different samples of triclosan were obtained from six different producers located in India and China. Each sample was prepared and analyzed according to the USP 24 Limit Test for both TCDD and TCDF.

Preparation: We dissolved 30 g of each sample in 1M NaOH. The solution of dissolved sample was then fortified with a known amount of internal standard solution containing ¹³C labeled TCDD and ¹³C labeled TCDF.

Subsequently, the sample solution was extracted with 30 ml of n-hexane (residue grade) four times. The hexane

extracts were combined and washed with 20 ml of reagent water. The 20 ml of wash water was also collected and extracted with 15 ml of n-hexane, and this hexane extract was added to the previously combined 120 ml of extract.

The 135 ml of hexane extract was dried over anhydrous sodium sulfate for 30 minutes and then filtered into an evaporative concentration apparatus. The extract was then concentrated down on a steam bath to a volume of 1 ml.

A chromatography column 10 mm x 250 mm was packed with 5.1 g of basic silica, 0.5 g of neutral silica, 6.2 g of sulfated silica and 3.2 g of anhydrous sodium sulfate. Once packed, the column was prepared for use by washing with n-hexane (residue grade).

A second column, 6 mm x 160 mm, was packed with 2.5 g of alumina and 2.5 g of anhydrous sodium sulfate. This column was also washed with n-hexane prior to use.

The 1 ml of sample extract was placed on the first column and eluted with n-hexane with the hexane eluant collected onto the second column. The eluant was then allowed to pass through the second column and was discarded. A mixture of n-hexane:methylene chloride (98:2) was then eluted through the second column and the eluant discarded. Finally, a mixture of n-hexane:methylene chloride (1:1) was eluted through the second column and collected into an evaporative concentration apparatus. The apparatus was placed on a steam bath and the extract eluant concentrated down to 1 ml.

The 1 ml concentrated extract was allowed to cool and then transferred to a 2 ml graduated screw cap conical vial. The extract was further concentrated down to dryness through a gentle stream of nitrogen. When dry, the sample was re-constituted to 10 µl with iso-octane (residue grade), and capped with an open-faced cap with teflon-lined septa.

HRGC-HRMS analysis: Each sample concentrate was analyzed by HRGC-HRMS using a GC/MS system^a with appropriate instrument control and data handling^b. The column^c was a 0.25 mm x 30 m, 0.25 micron DB-17MS (J&W) with helium as the carrier gas.

The temperature program employed was 80°C (held for 1 minute) to 180°C at 20°C per minute, then to 270°C at 4°C per minute, and held at 270°C for 5 minutes. The injector temperature was set at 280°C, as were both the MS interface and manifold temperatures.

The mass spectrometer was operated in the selective ion monitoring mode. Mass spectrometer resolution was adjusted to a minimum of 10,000. The instrument was calibrated by running a set of standards containing both unlabeled TCDD and TCDF at varying concentrations and ¹³C labeled TCDD and TCDF as internal standards.

Table 1. TCDD concentration (pg/g) in six different triclosan samples

Sample	Country of manufacturer	TCDD (pg/g)
1	India	17.2
2	China	95.4
3	India	111.8
4	India	41.5
5	India	1712.0
6	India	18.9

We injected 1 μ l of each standard and sample extract. Quantitation was achieved by comparing the peak response of the unlabeled TCDD at a mass-to-charge ratio (m/z) of 319.90 relative to the associated peak response of the ^{13}C -labeled TCDD internal standard at a m/z of 331.88, and that of the unlabeled TCDF at a m/z of 303.90 relative to the associated peak response of the ^{13}C -labeled TCDF internal standard at a m/z of 315.94.

Results: TCDD was found to be present in each of the samples from the six different producers (Table 1). All six samples exceeded the level set by USP 24 of less than 1.0 pg/g (ppt) and EU regulations of less than 10.0 pg/g (ppt), with the lowest concentration found in Sample 1 (India) at 17.2 pg/g (ppt) and the highest concentration found in Sample 5 (also from India) at 1711.9 pg/g.

Table 2. TCDF concentration (pg/g) in six different triclosan samples

Sample	Country of manufacturer	TCDF (pg/g)
1	India	0.70
2	China	7.13
3	India	3.43
4	India	8.51
5	India	0.43
6	India	207.30

With respect to TCDF, four of the samples exceeded the level set by USP 24 while the remaining two did not (Table 2). Of these four exceedence samples, one also exceeded the limit set by EU regulations of less than 10.0 pg/g. The highest concentration of TCDF was found in Sample 6 at 207.3 pg/g.

The detection limit for both TCDD and TCDF for all six samples was 0.33 pg/g.

Discussion

Triclosan is used in personal care, household care and cosmetic products, in amounts up to the low percent range.

Because these products come into contact with the skin, the presence of TCDD/TCDF above regulatory limits results in a product being adulterated and posing unwanted health risks to consumers. For example, triclosan exceeding the USP limits for TCDD or TCDF, assuming daily application of 1 g of soap to the skin, "would be in excess of the U. S. Environmental Protection Agency's one in a million cancer risk margin."¹⁷

Products containing adulterated triclosan - TCDD/TCDF above either the USP or EU regulatory limits - would be subject to product recall by the respective regulatory government agencies. From the U.S. perspective, the manufacturer of a personal care, household care or cosmetic product that contained adulterated triclosan would be subject to a product recall by the Food and Drug Administration as well as fines and adverse publicity.

Conclusion

TCDD and TCDF can and do form in triclosan as unwanted trace byproducts at ppt to ppb levels. The formation of these two compounds may be due to the quality or purity of the starting materials, the particular process used, or the inability to tightly control such physical parameters as reaction temperature and pressure.

Different brands of triclosan produced by different manufacturers can have significantly different concentration levels of TCDD and TCDF. When these compounds do form, they can adulterate triclosan rendering it unsuitable for use in personal care, household care and cosmetic formulations.

Furthermore, because of differing international regulatory levels (i.e., United States versus EU), manufacturers that use triclosan that meets EU but not USP requirements may run afoul of regulators if their products are imported into the U.S. market.

In selecting a source of triclosan, formulators should be aware that in addition to source-to-source variability, there can also be batch-to-batch variability in TCDD/TCDF levels. As a result, each batch of triclosan must be analyzed for the levels of TCDD/TCDF present using HRGC-HRMS.

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