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TOXAPHENE METHODOLOGY VALIDATION

by

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MANAGEMENT PERSPECTIVE

This interlaboratory comparison for toxaphene analysis was requested by D.O.E., Ontario Region to validate that results originating from various laboratories could be compared. For the laboratories reporting, the results show that comparisons can be made and trends of results from one laboratory are similar to those of the others. These observations are encouraging as analysis from the contributing laboratories can be used for comparisons.

PERSPECTIVE GESTION

Le ministère de l'Environnement, Région de l'Ontario, nous a demandé d'effectuer une étude comparative interlaboratoires pour prouver qu'il était possible de comparer les résultats de dépistage du toxaphène provenant de divers laboratoires. À la lumière des résultats qui ont été communiqués par les laboratoires ayant participé à l'étude, il semble possible d'établir des comparaisons. De plus, les tendances indiquées par les différents laboratoires concordent. Ces observations sont encourageantes dans la mesure où elles permettent de conclure que les résultats envoyés par les laboratoires participants peuvent être utilisées aux fins de comparaison.

ABSTRACT

This report contains the results of an interlaboratory comparison of the measurement of toxaphene in fish tissue. Included are the results for standard solutions and spiked standard solutions which provide an estimate of the laboratories' quantitation capabilities and the results from homogenized fish pastes. These results, from a fewer number of laboratories than originally enlisted, show that there is some variation between laboratories, but the results are of the same order of magnitude and usually within a factor of two. The coefficient of variation ranges between 8 and 36% at concentrations between 200 and 1500 ng/g. This implies that the methodologies can be used for comparisons and trends from one lab should be the same as from the others.

SOMMAIRE

La présente étude renferme les résultats d'une étude comparative interlaboratoires sur le dépistage du toxaphène dans les tissus des poissons. Certains résultats correspondent à l'analyse de solutions types tandis que d'autres se rapportent à des échantillons enrichis. Les résultats ainsi obtenus à partir de l'analyse de pâtes de poisson homogénéisées permettent de déterminer l'exactitude des méthodes de quantification des laboratoires participants. Bien que les résultats transmis par ceux-ci (compte tenu du fait que certains laboratoires n'ont pas mené à bien l'étude) varient quelque peu d'un laboratoire à l'autre, les résultats se maintiennent dans un même intervalle de grandeur et s'écartent au plus les uns des autres d'un facteur de deux. Les coefficients de variation s'échelonnent de 8 à 36 p. 100 pour des concentrations variant entre 200 et 1 500 ng/g. Par conséquent, il semble que les méthodologies des différents laboratoires soient compatibles aux fins de l'étude comparative et que les différents laboratoires soient susceptibles d'enregistrer les mêmes tendances.

INTRODUCTION

Toxaphene, a pesticide which is generally produced from the chlorination of camphene, has been found in environmental samples collected from areas not treated with this chemical. Unlike many pesticides, such as methoxychlor, toxaphene is characterized by a G.C. trace of over a hundred contributors to the formulation, most of which are polyisomers. Accordingly, using regular gas chromatographic techniques, minimal detectable amounts are much higher than for other organo-chlorine pesticides. The usual minimal detectable amount is 20 ng/mL (Onuska et al, 1980).

A number of laboratories are analysing for toxaphene, and a number of methods with minor variations are being utilized. To determine if results differ between laboratories, a validation study was initiated. Originally, five different laboratories were involved, but at the time the samples were distributed, only three took the samples and, of these, two responded. To augment these results, prepared samples were given to a colleague for analysis by GC-MS selective ion monitoring. This provided analysis of the fish tissue from three separate laboratories and analysis of the prepared samples by five analysts. Although this is not what was originally conceived, it was the best that could be done under the circumstances.

METHODS

Two separate sets of samples were provided to each of the participating laboratories. The first set contained 15 vials containing a blank, standards, treated standards, standards with interferences and extracted fish samples. The blank was isooctane, the standards were toxaphene obtained from the National Bureau of Standards, Washington, D.C., at concentrations of 200 ng/mL, 1000 ng/mL and 10000 ng/mL. One segment of the treated samples was standards passed through GPC and silica gel (Ryan and Scott, 1985)) and another segment contained organochlorines listed in Table 1. The concentrations were set at the level for each individual chemical at the maximum concentration found in fish taken from the Great Lakes area. Samples from homogenized fish pastes were prepared as previously described (Ryan and Scott, 1985). Another series from the fish pastes was spiked with 1 ug/g of toxaphene prior to the cleanup, and the extracts prepared for distribution. The complete sets of samples were placed in 1 mL amber "Reacti-vials" (Pierce Chem. Co. Rockford, Ill.) for distribution.

The other set of samples contained three separate fish homogenates. Each original homogenate was stored at -28°C. The homogenates were from fish from Lake Opeongo, Lake Ontario and Lake Superior. A 5 g aliquot of each fish was delivered under dry-ice conditions to each participating laboratory. These were to be

extracted and analysed, with a copy of the results, an aliquot of the extract and the analytical method used returned to the originating laboratory for analysis by the originating laboratory.

RESULTS

The sample types and results for the liquid samples are listed in Table 2. These show that not one of the participating laboratories are consistently high or low. With only one exception, the results from any particular sample are within a factor of two of the others, the exception being at the 200 ng/mL concentration level. Results for the 200 and 1000 ng/mL level are generally about the intended values, but at 10 ug/mL, the results are consistently higher than the amount added. When toxaphene is subjected to the cleanup procedures, there is little change relative to the standards at the three concentrations. The addition of the interferences do not affect results at the 1000 ng/mL level, but there is an increase at the 200 ng/mL level relative to the standard or the cleaned up standard. This may arise from an increase in the unresolved component of the chromatogram. The results from the worked up fish homogenates are also included in Table 2. These values are all within a factor of two of each other. Again, there is no reporting laboratory that is consistently high or low. There is a trend with the fish samples

from Lake Superior having more toxaphene than the fish from Lake Ontario, which in turn have more than those from Lake Opeongo. The results show the greatest variance at 200 ng/mL level followed by the results from the fish from Lake Superior as well as its spiked sample.

In Table 3 are the results from the participating laboratories for the fish homogenate samples. These are all of the same order of magnitude and for a particular fish are within a factor of three. Except for one fish analysis, all three laboratories have the same relative ranking of the fish, with the fish from Lake Opeongo having the least amount and the fish from Lake Superior having the most.

As part of the validation study, each laboratory was to return extracts of the fish for analysis by the originating laboratory. Usually, the cleanup requires passage of the sample through a silica gel column using hexane and then benzene as eluates. The benzene fraction is retained for analysis. One laboratory returned each fraction separately and the other returned the combined silica gel eluates. Table 4 lists the results of the samples contained only in the benzene eluate along with analysis of those samples in the originating laboratory. These are also analysed using an aliquot of the standard used from the laboratory providing the benzene eluate. The values for the fish from Lake Opeongo are lower by a factor of two, while the values from the other two fish are also lower but by a

lesser amount. On reanalysing the fish homogenates, with no special precautions other than normally taken, the participating laboratory calculated the results in brackets in Table 4.

DISCUSSION

The quantification of toxaphene in environmental samples is difficult. This is based on the observations that toxaphene is a multicomponent pesticide and its quantification must involve more than one of its components; that the components respond to treatment of the cleanup and detection in the same manner; and not all laboratories use the same peaks in their quantification. Some peaks may be interfered with by other compounds in the samples which are not removed by the cleanup procedures.

The methodology has three major aspects: extraction, cleanup and analysis. From the results listed here and in other work, the extraction techniques used are adequate. The cleanup stage may produce problems on the scale or size used as denoted in other works (Onuska et al, 1980). The recoveries are not quantitative below 10^{-9} g. The actual analysis or quantification of the formulation is the area of concern. Use of capillary column gas chromatography results in over 80 peaks in the chromatogram, but only a fraction of these are used in the analysis. As all interferences can not be removed, those

in the cleaned-up sample can cause a shift in a peak of interest or mask it producing a false concentration. Accordingly, the selection of peaks to be used in the chromatogram of toxaphene is critical and is dependent on the gas chromatograph's operating conditions. Complicating this is the fact that, if an organism does take up some toxaphene, certain of the components may be broken down or eliminated in preference to others. This necessitates considering a fingerprint of the compound rather than an individual peak. If only one peak could be used to quantify toxaphene, its identity would be established and analysis would be simple. However, several peaks are used but their structures are unknown, and accordingly authentic samples cannot be used for calibration, thus complicating the analysis.

The results presented in Table 2 show that the largest coefficient of variation is, at the 200 ng/mL level followed by the results from the fish from Lake Superior, ~28% then 25% respectively. In Table 2, only the analytical procedures of the analyst and his method of quantitation are being checked. The lowest coefficient of variation is 8%. In Table 3, the aspects of cleanup and analysis are being compared. The lowest coefficient of variation is 31%. This is 13% greater than the 18% found for the fish and spiked fish samples contained in Table 2. The degree of difficulty in analysing toxaphene is shown in Table 4, where the same aliquots were analysed three times. Using the results from the same analyst in this table, there

is a precision of less than 5% error for the fish from Lakes Ontario and Superior. This increases to 23% for the results from the fish from Lake Opeongo. When the same aliquot is analysed by two analysts, the coefficient of variation increases to 14% for fish from Lakes Ontario and Superior. A consideration of all the fish extract values from Tables 3 and 4 indicates that the 876 ng/g value may include an artifact as evidenced by the reanalysis of this sample.

The precision of the results for a fish sample from the various laboratories is greater than 30%. However, the trends from all analysts are in the same order and the results are of the same order of magnitude. This indicates that comparisons can be made but with some care if statements involving equivalent amounts are to be made.

The results listed in column 4 of Table 2 are encouraging as they were obtained using selective-ion-monitoring on a GC-MS instrument. In this method, only the masses at 159 and 162 were monitored, and these contain the breakdown product of many congeners of toxaphene. The results lie within the range reported by the other laboratories.

RECOMMENDATIONS

1. The toxaphene methodology practised in each of the participating laboratories is adequate and comparisons of results can be made at a general level. The values for a given sample were always of the same order of magnitude and generally differed by less than a factor of two.

2. To obtain a higher degree of comparison, the structures of many of the components of toxaphene need to be elucidated. The time and cost of such an exercise must be weighed against the advantages obtained from having a closer fit.

3. A mechanism for encouraging commercial laboratories to cooperate in future validation studies must be secured, especially when the laboratory states that it has expertise for the analysis.

4. Samples should be run in duplicate or at least split through two columns and their detectors to provide greater confidence in the analytical result.

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Table 1. Levels of Interferences in Standard Solutions

Organochlorine	Concentration (ug/L) OC in Standard Solution
Total PCB's	4.0
Mirex	0.2
Total DDT	2.2
p,p-DDE	1.3
DDT	0.9
Dieldrin	0.1
Total Chlordane	0.35

Table 2. Concentration of Toxaphene as Reported by Participating Analysts (ng/mL)

Sample Contents	Lab 1A	Lab 2	Lab 3	Lab 4	Lab 1B	Average with Standard Deviation
Blank	-	-	-	6.9	-	
200 ng/mL	185	260	127	198	198	193±47
200 ng/mL+cleanup	195	270	105	130	216	183±66
200 ng/mL+inteferences	254	390	416	374	235	333±83
1000 ng/mL	975	950	1105	1302	1020	1066±143
1000 ng/mL+cleanup	1006	1070	929	919	1096	1004±80
1000 ng/mL+interferences	1031	940	1358	1024	1016	1073±163
10,000 ng/mL	16146	12000	17597	15350	13680	14955±2174
10,000 ng/mL+cleanup	16980	14800	15684	13770	14282	15103±1265
Lake Opeongo fish	323	260	168	268	309	266±61
Lake Opeongo fish + 1000 mg/mL spike	1296	1500	1174	1086	1308	1272±157
Lake Ontario fish	491	510	360	428	469	452±60
Lake Ontario fish + 1000 ng/mL spike	1470	1640	1606	1011	1745	1494±88
Lake Superior fish	755	410	455	546	773	588±168
Lake Superior fish + 1000 ng/mL spike	1718	1200	1388	1906	1922	1626±321

Table 3. Toxaphene Concentrations in Fish Tissue Reported for Validation Study (ng/g).

Sample	Lab 1A	Lab 3	Lab 4	Average with Standard Deviation
Lake Opeongo	323	297	876	499±327
Lake Ontario	491	513	828	611±189
Lake Superior	755	943	1381	1026±321

Table 4. Toxaphene Concentrations Determined by Re-evaluation of Interlaboratory Samples (ng/g).

Sample	Lab 4(a)	Lab 4(b)	Lab 1A	Average of Lab 4	Average with Standard Deviation
Lake Opeongo	876	(547)	458	712±23%	499±327
Lake Ontario	828	(910)	713	869±5%	611±189
Lake Superior	1381	(1281)	1030	1331±4%	1230.67±181