γ Irradiation-Induced Degradation of Organochlorinated Pollutants in Fatty Acid Esters and in Cod

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The γ irradiation-induced degradation of 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT), 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (DDD), and 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (DDE) dissolved in methyl myristate and methyl oleate was studied. DDT and DDE produced DDD and 2,2-bis(4-chlorophenyl)chloroethylene (DDMU) respectively, in agreement with a previous study performed with aliphatic solvents. The degradation of these two former compounds was larger in methyl myristate than in methyl oleate and addition products between methyl myristate and the organochlorines were found. While DDD, DDE, and many PCB congeners in a cod sample were not measurably degraded at 15 kGy, DDT underwent 30% degradation.

Keywords: *Organochlorines; DDT; γ irradiation; fatty acid; dechlorination*

INTRODUCTION

In a previous publication, we reported the effects of γ irradiation on 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT) and its metabolites 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (DDD) and 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (DDE) in cyclohexane, cyclohexene, and 2-propanol (Lépine et al., 1994). The objective of the present work is to study, in a food irradiation perspective, the γ irradiation-induced degradation of these organochlorinated substances in the methyl esters of myristic and oleic acid, which are a saturated and an unsaturated fatty acids respectively, and in raw fish. Because organochlorinated pesticides are mostly found in the lipids of living organisms, fatty acid ester solutions of these compounds should be a good model in which to study lipid–organochlorine interactions in food undergoing irradiation. In this report, we compare the degradation yields and the nature of the dechlorination products of these organochlorinated substances in fatty acid esters and in organic solvents. In addition, we investigated the nature of the addition products between fatty acids esters and the organochlorines in the manner of the cyclohexane and 2-propanol study. A similar comparative study has been previously performed with aldrin in organic solvents and in a lipidic matrix but the various degradation products were not identified (Carp et al., 1972ab).

MATERIALS AND METHODS

Chemicals. DDT, DDD, DDE, and other chemicals were purchased from Aldrich Chemicals (Milwaukee, WI), and their purity was checked by GC/MS .

Gas Chromatography/Mass Spectrometry. The instrumentation and analytical conditions have been described previously (Lépine et al., 1994).

Sample Preparation and Irradiation. The degradation yields of the organochlorines in the two fatty acid esters were obtained using 1.0 mL of a 100 ppm solution in a 1.4 mL glass vial closed with a Teflon-lined screw cap. Irradiations were performed in triplicate with a Gammacell 220 (Nordion Int., Kanata, ON) using a ^{60}Co source delivering 12.9 kGy/min. The radiation dose was 10 kGy. Quantification of the degradation yields was performed by HPLC, with hexachlorobenzene as internal standard at a final concentration of 60 ppm. The HPLC system was a Varian Vista 5500 equipped with a reverse-phase C-18 Supelcosil SPLC-18 column (10 × 250 mm) (Supelco, Oakville, ON) with UV detection (234 nm). A methanol–water gradient was used. Since degradation products generated by irradiation in methyl oleate coeluted with DDE, the degradation yield of DDE in this solvent could not be determined.

Isolation of Addition Products. In order to get sufficient amounts of addition products, a larger concentration of each organochlorine (50 mg in 5 mL of solvent (10000 ppm)) and a larger radiation dose (80 kGy) were used. Fractions obtained after two HPLC purifications were evaporated and analyzed by GC/MS and NMR. The NMR spectra were obtained with a Varian Gemini 300 MHz in deuteriochloroform.

Irradiation of Cod. A fresh 80 cm long cod captured in the North Atlantic ocean was cut into eight sections of 125 g. Four of these sections were wrapped in aluminum foil and irradiated at 15 kGy using an underwater irradiator equipped with a ^{60}Co source delivering 484 kGy/min. Irradiated and nonirradiated samples were blended separately and 25 g aliquots of the homogenates were taken. Decachlorobiphenyl was added as internal standard and the paste was Soxhlet extracted (Bowes and Lewis, 1974). The solvent was evaporated and the residue purified by gel exclusion chromatography using 200–400 mesh S-X3 gel (Bio-Rad, Mississauga, ON) (Norstrom et al., 1986). The concentrated residue was further purified by chromatography on Florisil, and the fractions containing the organochlorinated compounds were pooled, concentrated, and analyzed by GC using an electron capture detector.

RESULTS

Irradiation in Fatty Acid Esters. The degradation yields of DDT and its metabolites after a 10 kGy irradiation in the two fatty acid esters are presented in Table 1. DDT is more extensively degraded than DDD and DDE in either fatty acid ester. The degradation yields of DDT and its metabolites are also larger in methyl myristate than in methyl oleate.

In methyl myristate, the only dechlorination product of DDT observed by HPLC was DDD. The amounts of

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Table 1. Degradation Yields of DDT, DDD, and DDE in Two Fatty Acid Esters

	DDT	DDD	DDE
methyl myristate	63.0 (1.6)[a]	20.5 (1.0)	25.5 (3.0)
methyl oleate	39.5 (3.7)	10.8 (1.2)	NA

[a] Numbers in parentheses are the standard deviations for triplicate analyses.

DDD obtained correspond to 13.9% of the initial DDT. In the same solvent, the sole degradation product of DDE observed was 2,2-bis(4-chlorophenyl)-1-chloroethylene (DDMU) in amounts corresponding to 22.0% of the starting material. In methyl oleate, DDD represented 27.0% of the initial DDT. No degradation products of DDD could be observed in either solvent at the wavelength used (234 nm).

Upon irradiation of DDT in methyl myristate, another peak appeared in reverse-phase HPLC at retention times longer than the starting material, while the dechlorinated products normally appeared at shorter retention times. This addition product had an integrated area that was 5.0% of the area of the DDT starting material. At this wavelength and with this concentration no other addition products could be observed in HPLC for DDD and DDE in either solvent. In order to analyze this addition product, along with the others potentially formed with DDE and DDD, concentrated solutions of the organochlorines were irradiated with a larger radiation dose and the addition products isolated by HPLC and analyzed by GC/MS and NMR. From each of the methyl myristate solution of DDT, DDE, and DDD, 5, 3, and <1 mg, respectively, of a single different addition product were isolated. No addition product could be isolated in methyl oleate because of the large number of HPLC peaks generated by the fatty acid ester itself, especially with the large radiation dose used.

The addition product obtained with DDT presents in negative chemical ionization (NCI) mass spectrometry a molecular ion at *m/z* 522 and fragments at *m/z* 488, 429, and 235. This corresponds to a DDT molecule having lost two chlorine atoms, addition of a methyl myristate moiety, and formation of an additional double bond. The *m/z* 488 fragment corresponds to the loss of a chlorine on the protonated molecule. Loss of 34 Da from the molecular ion in NCI mass spectrometry has been reported with many organochlorinated substances (Stemmler and Hites, 1985). The *m/z* 429 fragment corresponds to the further loss of the carboxymethyl function. The *m/z* 235 fragment is characteristic of a bis(4-chlorophenyl)methane cation. This indicates that the methyl myristate group was bound to the carbon bearing initially the three chlorine atoms (Figure 1a). The NMR of that compound shows the aromatic protons as two multiplets at 7.5–7.3 ppm and the methine proton as a singlet at 4.77 ppm. This implies that there is no proton on the carbon adjacent to the methine proton and that the double bond has to be located at this position, the other substituent on that carbon being the *remaining* chlorine atom. The methyl ester group appears as a singlet at 3.67 ppm and the methylene group adjacent to the methyl ester function appears as a multiplet at 2.30 ppm. This indicates that the myristate moiety is not attached to DDT at the methylene adjacent to the methyl ester function (Figure 1a). The aliphatic part of the molecule appears as a series of multiplets around 1.3 ppm.

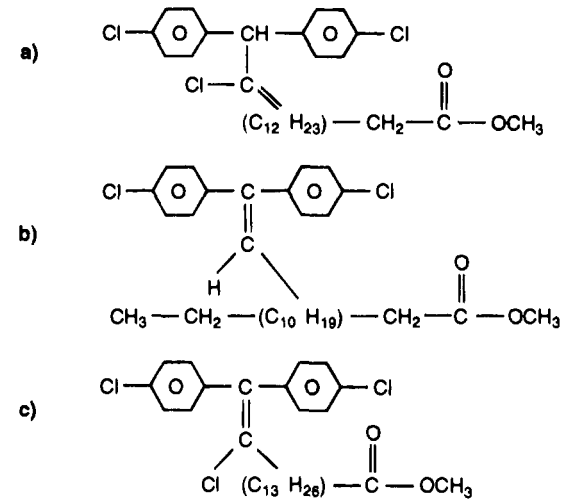

Figure 1. Proposed structures of the methyl myristate addition products of (a) DDT, (b) DDE, and (c) DDD.

The addition product obtained from irradiated DDE shows a molecular ion in NCI at *m/z* 488 and a fragment at *m/z* 453. The molecular ion corresponds to the addition of a methyl myristate moiety to DDE with the loss of two chlorines. The *m/z* 453 fragment corresponds to the loss of a chlorine from the molecular ion. The NMR shows the aromatic protons as a multiplet at 7.2 ppm and the proton on the sp^2 carbon at 6.0 ppm as a doublet. This doublet is coupled to a doublet of triplets at 3.1 ppm. These latter represent a methine proton of the myristate to which the DDE part of the molecule is attached (Figure 1b). Because this proton is coupled to two methylenes, the attachment of the DDE moiety cannot be located at the carbon adjacent to the methyl ester function or at the two terminal carbons of the alkyl chain.

Upon irradiation of DDD in methyl myristate, only a small amount (<1 mg) of an addition product could be obtained, probably because of the small reactivity of DDD toward irradiation (Table 1). It was not possible to get a useful NMR spectrum from such small amounts, but the EI mass spectrum shows a molecular ion at *m/z* 522 and a fragment at *m/z* 463. No fragment was observed at *m/z* 235. The molecular ion corresponds to DDD to which is added a methyl myristate moiety with the loss of one chlorine and formation of a double bond. The absence of a *m/z* 235 fragment indicates that the double bond has to be located between the α and β carbon of DDD. This also implies that the chlorine loss occurred at the β carbon (Figure 1c).

Irradiation of Cod. Irradiation of cod was also performed in order to see to what extent the various organochlorinated compounds contained in it were affected by irradiation. These results are presented in Table 2. Because of the low levels of organochlorinated pesticides in cod, these analyses were performed with an electron capture detector. The results presented in Table 2 show that the levels of DDD, DDE, and of all the PCB congeners were not significantly affected by irradiation. However, the DDT concentrations after this treatment were significantly smaller than in the untreated cod. Because of the cleanup procedures, which involve removal of the fatty acids, analysis of the fatty acid–organochlorine addition products could not be performed.

Table 2. Degradation of Various Organochlorinated Compounds Found in Cod after a 15 kGy Treatment[a]

chlorinated compounds[b]	relative peak area before treatment	relative peak area after treatment
80–86[c]	0.185 (0.012)[d]	0.200 (0.025)
101	0.265 (0.015)	0.293 (0.028)
99	0.417 (0.037)	0.398 (0.07)
DDE	0.327 (0.036)	0.336 (0.002)
97	0.096 (0.007)	0.106 (0.004)
87	0.344 (0.05)	0.389 (0.016)
136–112[c]	1.05 (0.091)	1.16 (0.060)
110	0.344 (0.005)	0.320 (0.062)
118	0.504 (0.007)	0.564 (0.030)
DDD	0.409 (0.052)	0.442 (0.029)
146	0.079 (0.006)	0.106 (0.009)
132	0.469 (0.048)	0.522 (034)
DDT	0.259 (0.032)	0.177 (0.002)
138–115[c]	0.604 (0.026)	0.651 (0.030)
187	0.144 (0.004)	0.151 (0.01)
156	0.143 (0.013)	0.159 (0.007)
180	0.510 (0.008)	0.551 (0.04)
MIREX	0.123 (0.18)	0.105 (0.021)
190	0.084 (0.001)	0.087 (0.005)

[a] The peak area of each compound is expressed relative to the internal standard (decachlorobiphenyl). [b] **PCB** congeners are numbered according to IUPAC nomenclature. [c] Chromatographically unresolved congeners. [d] Numbers in parentheses are the standard deviation of the triplicate.

DISCUSSION

Solvated electrons generated by the interactions of highly energetic photons with solvent molecules are responsible for the reductive dechlorination reactions observed in γ-irradiated solutions of organochlorinated substances. The degradation yields of DDT, DDD, and DDE in the two fatty acid esters are somewhat smaller than those observed a previous study in cyclohexane and cyclohexene (Lépine et al., 1994). This reduction is probably due to the electrophilic character of the ester group which acts as a solvated electron scavenger. However, all these degradation yields are of the same order of magnitude, which indicates that simple hydrocarbon solvents such as cyclohexane can mimic to a certain extent a more complex fatty acid ester matrix for irradiation studies. In either solvent system (i.e. aliphatic solvent versus fatty acid ester) DDT is degraded more than its two other metabolites, presumably because of its higher chlorine content which increases its affinity for solvated electrons. It is also interesting to note that in both the ester and hydrocarbon solvent systems, degradation yields are larger in the saturated solvent than in the unsaturated ones.

The dechlorinated products of DDT and DDE observed in methyl myristate are also similar to those observed in cyclohexane and cyclohexene. In both solvent systems, DDT generates DDD as the most abundant degradation product and DDE produced predominantly DDMU. However, in cyclohexane large amounts of 4,4′-dichlorobenzophenone were observed upon irradiation of DDT, DDD, and DDE while in cyclohexene this degradation product was generated in much smaller amounts. Because 4,4′-dichlorobenzophenone was not detected in fatty acid esters and because of the smaller degradation yields observed in cyclohexene versus those obtained in cyclohexane, cyclohexene appears to be a better model than cyclohexane to mimic the degradation of these organochlorinated compounds in fatty acid esters.

Solvent addition products could be isolated from irradiation experiments in fatty acid esters and from aliphatic solvents. The addition product observed in the myristate solution of DDE corresponds to replacement of one of the β chlorines of DDE by a myristate moiety (Figure 1b). The same type of adduct was also observed as one of the solvent addition products in cyclohexane. In the myristate solution of DDD, the addition product obtained corresponds to DDD with one of its β chlorine substituted by a myristate molecule with formation of a double bond between the α and the β carbon (Figure 1c). A similar type of compound was also observed with DDD irradiated in cyclohexane.

In the myristate solution of DDT, the amount of addition product obtained was sufficiently large to be observed directly by HPLC with the 100 ppm solution irradiated at 10 kGy. This reflects the larger reactivity of DDT toward irradiation compared to its two other metabolites as seen in Table 1. This addition product corresponds to DDT having two of its β chlorine replaced by a myristate molecule with the formation of a double bond at the junction of the two molecules (Figure 1a). No such compounds were observed in irradiated cyclohexane or in cyclohexene. Although upon irradiation of DDT in cyclohexane some addition products contained a double bond, it was located between the α and β carbon of DDT, and not at the junction between the β carbon and the cyclohexane ring.

The position of the attachment of the various organochlorines on the aliphatic chain of methyl myristate could not be elucidated as it would be very difficult by NMR to discriminate between all the almost equivalent carbons of the chain. The only position which could be significantly chemically different from the others should be the methylene adjacent to the carbonyl. A radical formed by hydrogen abstraction at this position should be stabilized by the carbonyl function and addition at this position should be favored. However the NMR clearly shows that, for DDT and DDE, addition to the myristate does not occur at this position.

The relative areas of the peaks of the organochlorinated substances found in cod before and after a 15 kGy treatment clearly shows that DDD and DDE are not significantly degraded. However, the relative amount of DDT is reduced by 32% by irradiation. A nonsignificant increase in the relative amounts of DDD, the dechlorinated byproduct of DDT in every solvent studied, was observed, but the relative amount of initial DDT in the unirradiated sample is relatively small in comparison with the existing amounts of DDD, and dechlorination of DDT could not significantly increase the amount of DDD after irradiation. The smaller degradation yield of DDT, DDD, and DDE in an irradiated biological matrix versus pure aliphatic solvents or fatty acid esters was to be expected as many other compounds are present in such a complex matrix and they can act as electron scavengers and inhibit dechlorination. Nevertheless, at 15 kGy DDT is significantly degraded. The maximum radiation dose recommended by a joint FAO/IAEA/WHO expert committee for food irradiation is 10 kGy.

There were other organochlorinated compounds detected in cod and their relative abundances before and after a 15 kGy treatment are presented in Table 2. Contrary to the observation of Cichy and co-workers, no significant degradation of the PCB congeners listed was observed. These authors reported a 38% degradation of the total PCB concentration in lake trout irradiated at 10 kGy (Cichy et al., 1979). They observed a reduction in the proportions of the lower chlorinated

PCB congeners which was attributed to the formation of water-soluble derivatives of these congeners which would be lost during the subsequent purification procedures. This is in contrast with our own observations (Lépine et al., 1990) and those of Sawai and coworkers (Sawai and Shinozaki, 1972) which show that irradiation of a commercial PCB mixture, like Arochlor 1260 or Kanechlor 300 in cyclohexane or 2-propanol increases the proportion of lesser chlorinated congeners in the mixture, owing to the dechlorination of the more reactive higher chlorinated congeners. Cichy and co-workers used packed column gas chromatography in their analyses and it is difficult with this technique to achieve sufficient resolution of the various PCB congeners in a complex mixture to allow reliable quantification.

CONCLUSION

The degradation yields and the nature of the dechlorinated products of DDT, DDD, and DDE irradiated in fatty acid esters were very similar to those obtained in aliphatic solvents like cyclohexane or cyclohexene. Solvent addition products were also observed in fatty acid esters as in organic solvents. In irradiated cod, DDD and DDE and various PCB congeners were not significantly degraded even with a 15 kGy irradiation dose. Under these conditions DDT was 30% degraded.

LITERATURE CITED

Bowes, G. W.; Lewis, J. A. Extraction of polychlorinated biphenyls. Evaluation of a column technique applied to polar bear and seal tissue. *J. Assoc. Off. Anal. Chem.* **1974**, *57*, 138–144.

Carp, A. E.; Liska, B. J.; Ziemer, P. L. Decomposition of Aldrin by γ Radiation I. In Organic Solvents. *Bull. Environ. Contam. Toxicol.* **1972a**, *7* (6), 321–330.

Carp, A. E.; Liska, B. J.; Ziemer, P. L. Decomposition of Aldrin by γ Radiation II. In Lipid Solutions. *Bull. Environ. Contam. Toxicol.* **1972b**, *7* (6), 331–337.

Cichy, R.; Zabik, M.E.; Weaver, C.M. Polychlorinated biphenyl reduction in lake trout by irradiation and broiling. *Bull. Environ. Contam. Toxicol.* **1979**, *22* (6), 807–812.

Lépine, F.; Massé, R. Effect of γ irradiation on a PCB mixture in organic solvent. *Bull. Environ. Contam. Toxicol.* **1990**, *44*, 549–554.

Lépine, F.; Brochu, F.; Milot, S.; Mamer, O. A.; Pépin, Y. γ Irradiation Induced Degradation of DDT and its Metabolites in Organic Solvents. *J. Agric. Food Chem.* **1994**, *42* (9), 2012–2018.

Norstrom, R. J.; Simon, M.; Mulvihill, M. J. A gel- permeation/column chromatography cleanup procedure for the determination of CDDs in animal tissue. *Int. J. Environ. Anal. Chem.* **1986**, *23* (4), 267–287.

Sawai, T.; Shinozaki, Y. Radiolytic Dechlorination of Polychlorinated Biphenyls (PCB) in Organic Solutions. *Chem. Lett.* **1972**, *10*, 865–866.

Stemmler, E. A.; Hites, R. A. Methane enhanced negative ion mass spectra of hexachlorocyclopentadiene derivatives. *Anal. Chem.* **1985**, *57* (3), 684–692.

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