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Decomposition of toxic and environmentally hazardous 2,3,7,8-tetra-chlorodibenzo-p-dioxin by gamma irradiation

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Summary. The decomposition of the toxic and environmentally hazardous 2378-TCDD by gamma irradiation was studied and successfully used to decontaminate laboratory wastes containing small quantities of this chemically and biologically stable compound. The method makes use of gamma irradiation from a commercial ^{60}cobalt facility at high dose levels (1000 kGy) to break down the compound into nontoxic products. Irradiation also decomposed 2378-TCDD in contaminated soil from the Seveso accident.
Key words. 2,3,7,8-Tetrachlorodibenzo-p-dioxin; hazardous waste; ionizing radiation; gamma irradiation; decomposition.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2378-TCDD) belongs to a group of toxic and environmentally hazardous compounds, the polychlorinated dibenzo-p-dioxins (PCDDs). There are 75 such compounds ranging from the mono- to the fully chlorinated octachloro species. The symmetrically substituted 2378-TCDD is apparently the most toxic congener of this group[1,2]. 2378-TCDD is an unwanted byproduct in the synthesis of certain industrial chemicals. It is one of the most toxic man-made chemicals and has no technical use or value. Several industrial accidents have occurred in which 2378-TCDD was formed and sometimes released into the environment, i.e. in the accident in Seveso, Italy, in 1976[3]. In all these incidents the synthesis of 2,4,5-trichlorophenol was involved. This industrial product is used for the production of herbicides, bactericides and wood preservatives. The safe disposal of residues and wastes from these productions poses a formidable toxicological and environmental problem. PCDDs have recently also been found as trace contaminants in emissions from incineration or combustion sources. In the case of municipal incinerators the main contaminants were not 2378-TCDD but other PCDDs[4].

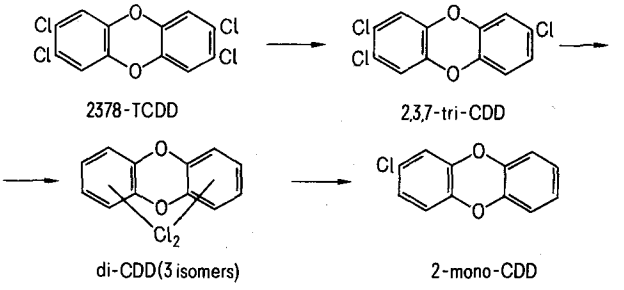
Incineration or deposition of wastes containing 2378-TCDD or other PCDDs may involve significant risks because of the possible emission of these hazardous compounds into the environment. Additionally, incineration or combustion is a problem in such cases where highly stable compounds are involved and toxic by-products can be formed. This is the case with the polychlorinated biphenyls (PCBs) which can be thermally converted into the polychlorinated dibenzofurans (PCDFs), an other group of toxic and environmentally hazardous compounds, closely related to the PCDDs[5].

Irradiation of these organochlorine compounds has been investigated previously. Photolysis using UV- or sunlight was found to dechlorinate 2378-TCDD and other PCDDs in pure solutions but was largely unsuccessful when these compounds where absorbed on soil or in the presence of large amounts of other materials[6,7]. Gamma irradiation has previously been applied for the decomposition of PCDDs, PCBs and other organochlorine compounds using solutions of the pure substances[8-12]. Considerable irradiation doses (up to 1000 kGy) were required, and the degree of success varied; practical applications were not documented. In this report we show the successful use of gamma irradiation for the decomposition of 2378-TCDD and other hazardous compounds in laboratory wastes and in small quantities of contaminated soil from Seveso.

Materials and methods. A commercial gamma irradiation facility (Sulzer, Winterthur, Switzerland) consisting of 14 ^{60}Co-rods with an activity of 10^{15} Bq was used. The rods were arranged in circles of 35 or 90 cm diameter. The incident dose was measured with Clear Perspex HX dosimeters, 3 mm (Gillette UK Ltd, Reading, England), based on Fe-sulfate dosimetry. The dose rates ranged from 1.6 to 8.0 × 10^3 Gy/h. Exposure periods were up to 280 h with doses up to 1148 kGy. Samples were exposed to different radiation doses by varying their distances from the ^{60}Co-rods.

Synthetic 2378-TCDD (96%, Givaudan Ltd, Dübendorf, Switzerland) was dissolved in n-hexane at a concentration of 0.5 µg/ml and portions of 1 ml exposed to gamma radiation (dose rate 1.6 kGy/h) in sealed borosilicate vials (3 ml volume, air not removed). The vials were placed inside an additional screw cap flask for safety reasons.

Laboratory waste solution containing 2378-TCDD, other PCDDs as well as additional components was carefully concentrated to about 200 ml and exposed to gamma radiation (dose rate 5 kGy/h) in a 250-ml Sovirel flask. This flask was placed in a plastic bag and a metal container filled with an absorbing mate-

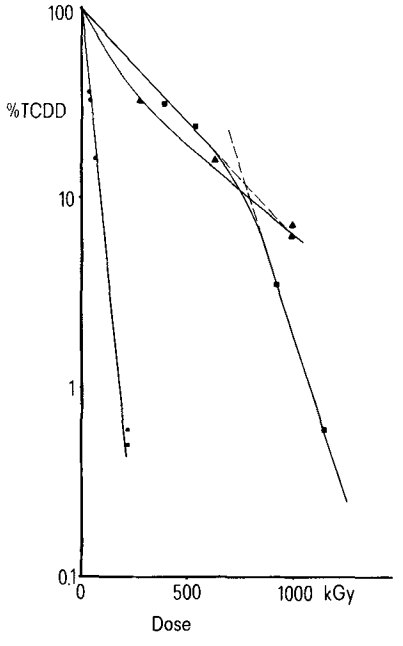


rial. Temperature and pH of the solution during irradiation treatment were periodically checked. No change in pH and only a minor temperature increase (~ 3 °C/24 h) were observed. The temperature was kept within 25–35 °C with occasional cooling by dipping the Sovirel flask into ice water. Periodically small aliquots of 0.1 ml of solution were removed and analyzed for 2378-TCDD and other components.

Experiments with contaminated soil from zone A at Seveso (removed in 1977, approximate contamination level 42 ppb 2378-TCDD) were carried out on 5-g portions in 50-ml Sovirel flasks. Previously, the soil was air-dried and debris removed. Known quantities of distilled water or iso-propanol (see table 3) were added to the soil in the flasks prior to gamma irradiation (dose rate 8 kGy/h).

The irradiated samples were analyzed by gas chromatography – mass spectrometry (Finnigan 4000 GC–MS, electron impact mode, 50 eV, 240 °C) using 25 m SE 54 and SP 2340 glass capillary columns (temperature programmed 80–180 °C, 20 °C/min, and to 250 °C at 2 °C/min). Multiple ion detection was used, m/z 320, 322 for native 2378-TCDD and m/z 332 for ^{13}C-2378-TCDD. Splitless injections were made with amounts in the range of 5 to 500 pg. Quantitations were carried out using ^{13}C-2378-TCDD (KOR Isotopes, Cambridge, Mass., USA) as the internal standard added to the samples in amounts of 7.5–30 ng prior to extraction and analysis. The results are reported in % relative to the amounts of 2378-TCDD present in the samples prior to irradiation. Sample aliquots of 2 µl were injected directly after suitable dilution of the samples except in the case of the contaminated soil. In the latter case the soil samples were extracted (n-hexane after iso-propanol addition) and the extract purified by silica gel chromatography.

Results and discussion. The results of the gamma irradiation of pure 2378-TCDD in solution are reported in table 1. Almost complete decomposition (99.9%) was achieved at the highest dose (446 kGy). The decomposition follows a pseudo first order process as indicated in the figure. GC-MS analysis of the irradiated samples indicated that most of the decomposed 2378-TCDD was present in the form of lower chlorinated dioxins (mono-, di- and tri-CDD). No isomerization of 2378-TCDD was observed. The major decomposition route is apparently via simple reductive dechlorination (see scheme).



Decomposition of 2378-TCDD by gamma irradiation. ● 2378-TCDD (0.5 ppm) in n-hexane. ■ 2378-TCDD (4.4 ppm) in laboratory waste solution. ▲ 2378-TCDD (42 ppm) in contaminated Seveso soil with addition of iso-propanol.

The results of the gamma irradiation of a laboratory waste solution are reported in table 2. This solution (about 200 ml) contained 2378-TCDD (4.4 ppm), various hexa- and octa-CDD and 3,4,3′,4′-tetrachloroazobenzene (TCAB, 400 ppm). The solvent was predominantly benzene and n-hexane. In an initial experiment small portions (0.1 ml) of this waste were irradiated; the results indicated a slower decomposition rate of the 2378-TCDD than with the pure substance. The remaining solution was therefore subjected to higher doses (390–1148 kGy). The results in table 2 indicate more than 99% decomposition of 2378-TCDD at the highest dose. A slower decomposition rate occurred during an initial phase of irradiation (up to ~ 500 kGy, see figure) followed by a faster rate during the next phase approaching that for the pure 2378-TCDD. This may be due to the presence of other components in the waste solution acting as radical scavangers during the initial phase of irradiation. TCAB was found to degrade significantly faster (> 99% at 390 kGy) than 2378-TCDD.

The results of the decomposition of 2378-TCDD (42 ppb) in contaminated soil from zone A at Seveso are reported in table 3. Preliminary experiments showed that irradiation doses of up to 840 kGy led to insignificant decomposition (< 3%) of 2378-TCDD in moist or wet soil. The addition of iso-propanol as a hydrogen donor to the air-dried soil however resulted in significant decomposition with more than 95% of the 2378-TCDD decomposed at the highest dose (1000 kGy). Decomposition was highest (95.3%) with the larger quantity of iso-propanol (6 g) added. Gamma irradiation of a water-soil slurry again gave insignificant decomposition. The decomposition rate with iso-propanol was similar to that of the chemical waste in the initial phase and significantly slower than that of pure 2378-TCDD.

Table 1. Gamma irradiation (dose rate 1.6 kGy/h) of pure 2378-TCDD (0.5 ppm in n-hexane)

Dose (kGy)	% 2378-TCDD
0 (control)	100
25	36
30	33
62	16.5
218	0.6
218	0.5
446	0.05

Table 2. Gamma irradiation (dose rate 5 kGy/h) of laboratory waste solution containing 2378-TCDD (4.4 ppm)

Dose (kGy)	% 2378-TCDD
0 (control)	100
390	31
510	24
920	3.5
1148	0.6

Table 3. Gamma irradiation (dose rate 8 kGy/h) of 5 g contaminated Seveso soil (42 ppb of 2378-TCDD)

Addition	g	Dose (kGy)	% 2378-TCDD
Iso-propanol	3	0 (control)	99.5
Iso-propanol	3	0 (control)	100.8
Iso-propanol	3	256	32
Iso-propanol	3	512	16.3
Iso-propanol	3	1000	7.8
Iso-propanol	3	1000	6.3
Iso-propanol	2	1000	8.4
Iso-propanol	6	1000	4.7
H_2O dist.	3	0 (control)	97
H_2O dist.	3	1000	100

No acceleration of 2378-TCDD decomposition in a later phase was observed. The major decomposition products found were again lower chlorinated dioxins indicating that the dechlorination follows the same route as with pure 2378-TCDD in hexane solution.

These studies document that gamma irradiation from a commercial ^{60}Co-facility can be successfully used for the decomposition of 2378-TCDD and other hazardous compounds. In our study we showed that decontamination of laboratory wastes and contaminated soil was achieved.

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Improved separation at low temperature of glycoproteins by Con A-Sepharose affinity chromatography in the presence of sodium dodecyl sulfate (SDS)[1]

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Summary. The Con A-Sepharose affinity chromatography of glycoproteins was even more effective at 4 °C than that at room temperature (26–28 °C) in the presence of sodium dodecyl sulfate (SDS). Application of this methodology to the separation of several glycoproteins from SDS-solubilized membrane proteins in rat cerebellum, including a glycoprotein characteristic of the Purkinje cells, was successful.
Key words. Con A-Sepharose; SDS; glycoproteins; cerebellar proteins; affinity chromatography; purification.

Affinity chromatography using immobilized lectins has been used for the fractionation and purification of glycoproteins in biological membranes. While membrane proteins can be readily solubilized by an anionic detergent, sodium dodecyl sulfate (SDS), the detergent has been reported to have a deleterious effect on the affinity for glycoproteins of lectin-Sepharose[2]. In the present study, we investigated effective conditions for purification of glycoproteins by Concanavalin A (Con A)-Sepharose in the presence of SDS. In addition, we tried to separate the GR-250 protein, a Con A-binding glycoprotein[3], with an apparent mol. wt of 250,000[4] from rat cerebellar membrane proteins using the improved method described here.

Materials and methods. Ovalbumin (Sigma, grade III), horseradish peroxidase (Toyobo, Japan) and fetuin from fetal calf serum (Sigma, type III) were used as test materials. Prior to the present study, the commercial preparations of three glycoproteins were checked for their adsorbability on a Con A-Sepharose (Pharmacia) column in the absence of SDS at room temperature. It was shown that peroxidase was completely adsorbed to the column, while ovalbumin and fetuin contained nonadsorbed fractions in their preparations. Thus, the latter two proteins adsorbed to the column were eluted with methyl-α-D-mannopyranoside, dialyzed against 0.1 M Tris-HCl buffer, pH 7.2 (buffer A), and served for later experiments as purified glycoproteins. Peroxidase was used without purification.

Cerebellar samples were prepared at 4 °C unless otherwise stated. Cerebella from 20-day-old Sprague-Dawley strain rats were homogenized in 9 volumes of 10 mM Tris-HCl buffer, pH 7.2. The homogenate was centrifuged at 105,000 × g for 60 min and the resultant precipitate was rehomogenized in one original volume of the same buffer, followed by the addition to 8 volumes of precooled 10 mM Tris-HCl buffer, pH 7.2, containing 0.44 M NaCl and 0.55% (w/v) Triton X-100. After being stirred for 60 min, the suspension was centrifuged again at 105,000 × g for 60 min. The pellet was suspended in the initial buffer and mixed with an equal volume of sample solubilizing solution consisting of 6% SDS; 20% glycerol, 2% 2-mercaptoethanol, and 0.125 M Tris-HCl buffer, pH 6.8, and then boiled for 3 min. The sample was dialyzed against buffer A containing 0.08% SDS and then subjected to Con A-Sepharose column chromatography.

Affinity chromatography of ovalbumin and peroxidase on Con A-Sepharose in the absence of SDS was carried out at room temperature (26–28 °C) or 4 °C. A Sepacol Mini column (Seikagaku Kogyo, Japan) packed with Con A-Sepharose (total volume, 2.5 ml; 0.75 × 5.7 cm) was washed with 5 bed volumes of 0.1 M Tris-HCl-0.02 M sodium borate buffer, pH 7.2 (buffer B), and then of buffer A. After 1 ml of each sample (2 mg/ml) was applied, the column was washed with 5 bed volumes of buffer A. The flow rate was 16 ml/h for ovalbumin and 4 ml/h for peroxidase. The proteins bound to Con A-Sepharose were eluted with buffer B containing 0.2 M methyl-α-D-mannopyranoside at a rate of 3 ml/h.

Affinity chromatography of three test glycoproteins in the presence of SDS was performed under the same conditions as were used in its absence, except for the following modifications. Con A-Sepharose columns were washed with 10 bed volumes of buffer B containing 0.08% SDS (SDS-buffer B) and then with the same volume of buffer A containing 0.08% SDS (SDS-buffer A) to remove loosely bound Con A. Further washing was carried out with 7.5 bed volumes of SDS-buffer A at flow rates