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Chloe Anderson
Gregory O'Neil

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Synthesis of DDT-Related Pollutants tris(4-chlorophenyl) methane (TCPM) and tris(4-chlorophenyl)-methanol (TCPM-OH) for Investigations into Environmental Persistence

Chloe Anderson, Gregory O’Neil

Abstract:

This study focuses on the synthesis of radiolabeled tris(4-chlorophenyl) methane (TCPM) and tris(4-chlorophenyl)-methanol (TCPM-OH), compounds identified as environmental contaminants linked to DDT production. Given their persistence and bioaccumulation in marine and terrestrial ecosystems, understanding their environmental fate is critical. This research aims to provide a detailed methodology for the synthesis of carbon-14 labeled TCPM and TCPM-OH to facilitate further studies on their degradation and impact. TCPM-OH was synthesized via two distinct routes. The first method involved esterification of 4-chlorobenzoic acid followed by a double Grignard addition. The second method started with chlorobenzene, which was brominated using gold-catalyzed N-bromosuccinimide (NBS), followed by a lithium-halogen exchange and addition to a ketone. Purification was achieved through silica gel flash column chromatography, and product purity was confirmed using Thin Layer Chromatography (TLC) and Nuclear Magnetic Resonance (NMR) spectroscopy. TCPM was synthesized by reducing TCPM-OH using triethyl silane and trifluoroacetic acid. The reduction process was optimized to achieve high yields and purity, verified through TLC and NMR. The synthesized compounds were characterized, showing distinct Rf values and NMR spectra consistent with the desired products. The results demonstrate effective synthesis pathways for radiolabeled TCPM and TCPM-OH, setting the stage for environmental fate studies. These compounds will be utilized in future research to investigate their degradation in marine sediments, particularly in the San Pedro Basin, where significant contamination has been reported. The synthesized radiolabeled compounds will enable precise tracking and analysis using advanced mass spectrometry techniques, contributing to a deeper understanding of their persistence and ecological impact. This work provides a foundation for subsequent studies on TCPM and TCPM-OH, aiding in the development of strategies to mitigate their environmental and health risks.

Introduction:

The first molecules of DDT (dichlorodiphenyltrichloroethane) were synthesized by Othmar Zeidler at the University of Strasbourg by a reaction known as the Baeyer Condensation between chlorobenzene and chloral in the presence of sulfuric acid [1]. DDT was not used again for another 65 years until its insecticidal properties were investigated by Dr. Paul Muller and recognized to be astonishingly effective against a large variety of insects, including mosquitoes and potato beetles [2]. For this discovery he was awarded the Nobel Prize in 1948. Due to the emergence of World War II, there was an immediate interest in this cheap synthetic chemical which was “safe” to apply directly to human bodies. DDT was so persistent, it was capable of killing mosquitoes in treated houses months after application [3]. The effect of DDT’s use for public health has been estimated to have saved approximately 50 million human lives and averted more than 1 billion human illnesses [4]. In spite of this glowing success of DDT in public health, this was achieved with relatively small amounts of the compound. Much more DDT was used after 1945 for control of agricultural and forest pests. In the early 1960s, about 400,000 tons of DDT were used annually worldwide (70-80% of which was used for agriculture) [5]. Its use gave phenomenal results and yields in New York and Wisconsin crops increased from 56 to 58% over those previously obtained with the best
discovered from University California Santa Barbara (UCSB) of California. A waste disposal project was prevalent offshore Southern California between 1947 and 1961 along the San Pedro Basin. While DDT was being produced globally, DDT and its degradation products (referred to as DDX) are found everywhere in the global environment. DDXs are highly lipophilic, persist in the environment and biomagnifies through trophic levels, increasing roughly tenfold in concentration stored in lipids and in percentage of DDE at each trophic level [7]. Due to their lipophilicity, DDX products bioaccumulate in humans, taking 6 years for DDT and possibly up to 10 years for its primary metabolite DDE to leave a body [8,9]. This bioaccumulation poses risks to wildlife and human health, as exposure to DDT and its derivatives has been linked to adverse effects on reproductive health, immune function, and neurological development. DDX is known to have eggshell thinning effects in birds of prey which was the main factor attributed to the bald eagle endangerment [10]. In humans, manufacturing workers for the production of DDT (so having high rates of exposure to the compound) have a 7.4 times higher risk of pancreatic cancer and women exposed to DDT before the age of 14 are 5 times more likely to develop breast cancer [11]. The recognition of these environmental and health risks prompted the global ban on DDT usage under the Stockholm Convention on Persistent Organic Pollutants in 2001 [12].

While DDT was being produced globally, ocean waste disposal was prevalent offshore Southern California. An estimated 37 to 53 million liters of DDT-contaminated acid waste from the condensation reaction, containing 348-696 metric tons of DDT, was disposed at an ocean dump site 16 km northwest of Santa Catalina Island between 1947 and 1961 along the San Pedro Basin by Montrose Chemical Corporation of California [13]. Professor David Valentine from University California Santa Barbara (UCSB) discovered a large quantity of barrels of chemical waste in these areas with high concentrations of DDX compounds in the surrounding area. Among these compounds was a high concentration of the chemical TCPM (tris(4-chlorophenyl) methane) and its oxidized counterpart TCPM-OH (tris(4-chlorophenyl)-methanol) [14]. These findings were highly interesting because TCPM and TCPM-OH are not considered to be among the major byproducts of DDT degradation.

TCPM and TCPM-OH were first identified in animal tissues in 1992 and 1989 respectively [15]. There is a high correlation between appearances and concentrations of DDT and TCPM. This serves as evidence for the DDT manufacturing process as a source of TCPM, along with TCPM isomers being found at 150-180 ppm in manufactured DDT [16]. Like DDT, TCPM and TCPM-OH have been observed in animals and sediments globally and have a high bioaccumulation in fatty tissues. TCPM-OH concentrations are proven to increase in liver and adipose tissues of ringed seals as they mature [17]. TCPM has also been connected to adverse marine ecosystem impacts. Zebrafish survival percentages have been shown to decrease with age as TCPM-OH concentrations are increased [18]. These effects and prevalence led the UCSB group to pursue an incubation study of TCPM and TCPM-OH degradation in its currently found environment in the San Pedro Basin.

To complete their investigation, PhD candidate Jacob Schmidt and a UCSB team collected sediment samples from the San Pedro Basin which will be spiked with carbon-14 labeled TCPM and TCPM-OH. Aliquots of the spiked sediment samples will be taken every two weeks in triplicate and analyzed via sensitive accelerator mass spectrometry methods to monitor the evolution of the radioactive products and better understand the fate of TCPM and TCPM-OH in the environment. The purpose of this project was to synthesize the carbon-14 labeled TCPM and TCPM-OH.
Materials and Methods:

Purification – Products were purified using silica gel flash column chromatography with solutions of increasing polarity, starting with 10:1 Hexane: Ethyl Acetate, increasing to 4:1, 1:1, and then pure Ethyl Acetate flushing.

TLC Plates – Purity of products and column fractions was tested via Thin Layer Chromatography (TLC). Plates were developed using 4:1 Hexane: Ethyl Acetate developing solvent and stained with Cerium Molybdate.

TCPM-OH Synthesis from 4-Chlorobenzoic Acid – TCPM-OH was initially synthesized from 4-chlorobenzoic acid by undergoing an esterification in acidic conditions and a double Grignard addition. The 4-chlorobenzoic starting material was dissolved in 1 equiv. methanol and 1 equiv. sulfuric acid. The solution was stirred and heated to reflux for 6 hours then cooled to room temperature overnight. The volume was then reduced by half via rotary evaporation, then diluted to 100% volume using ethyl acetate. The reaction was washed with an equal volume of sodium bicarbonate then an equal amount of brine and dried over magnesium sulfate. Solvent was then removed by rotary evaporation and vacuum dried, leaving a white powder behind. This synthesized ester, methyl 4-chlorobenzoate, was then dissolved in 1 equiv. of THF and cooled to -78°C and stirred. 2.2 equiv. of (4-chlorophenyl) magnesium bromide was added in air free conditions and allowed to react overnight, after which the solution was quenched with ammonium chloride, dried over magnesium sulfate and subject to rotary evaporation. After purification, this Grignard addition formed the desired TCPM-OH, a viscous yellow oil with a TLC Rf value of 1.5 and a yellow staining spot when treated with the Cerium Molybdate TLC dye.

TMS-diazomethane Esterification – 4-chlorobenzoic acid was dissolved in Dichloromethane under nitrogen gas to a density of 0.05 g/ml. 0.5 equiv. of methanol was added as well as 1 equiv. of TMS-diazomethane dropwise. The reaction was stirred at room temperature for 15 minutes and then quenched with Acetic Acid and subjected to rotary evaporation, yielding methyl 4-chlorobenzoate.

n-BuLi Synthesis of TCPM-OH from Methyl 4-Chlorobenzoate – 3 equiv. of THF and 2.5 equiv. of n-BuLi were added in air free conditions and cooled to -78°C. 1 equiv. of 1-bromo-4-chlorobenzene was added and stirred for 30 minutes before adding 0.33 equiv. of the synthesized ester, methyl 4-chlorobenzoate, and allowed to react and warm overnight. The reaction was quenched with ammonium chloride and extracted with MTBE. Solvent was removed via rotary evaporation to yield TCPM-OH.

Synthesis of Grignard Reagent, (4-chlorophenyl) magnesium bromide – The Grignard reagent was synthesized from 1-bromo-4-chlorobenzene using a condensation reaction. 1.2 equiv. of solid Magnesium was added to a round bottom flask flame dried. Under the protection of nitrogen, 0.5 equiv. THF is added with a catalytic amount of crystalized iodine to the solid magnesium. A solution of 1 equiv. of 1-bromo-4-chlorobenzene to 0.5 equiv. THF is prepared and slowly added to the magnesium mixture and is heated to start the reaction. The 1-bromo-4-chlorobenzene THF solution is added continuously, with varying speed to maintain reaction temperature. After the completed addition, the reaction is allowed to cool and stir overnight. This yields the Grignard reagent, (4-chlorophenyl) magnesium bromide, which can be added to the synthesized ester, methyl 4-chlorobenzoate, to yield TCPM-OH.

Weinreb Amide Intermediate Synthesis of TCPM-OH – The formation of the Weinreb Amide was synthesized by dissolving 1 equiv. of 4-chlorobenzoic acid in Dichloromethane to a density of 0.02 g/ml. 1.2 equiv. of 1,1-carbonyldimidazole is added and allowed to stir 1 hour at room temperature. Then 1.2 equiv. of N, O-dimethyl hydroxylamine Hydrochloride and 2.5 equiv. Triethylamine are added at once and is allowed to stir and react overnight. The
reaction is quenched using 2M aqueous HCl until solids stop forming. This is stirred for 10 minutes and then separated using deionized water, saturated aqueous sodium bicarbonate and brine then dried over anhydrous magnesium sulfate. Solvent was removed via rotary evaporation. This synthesized Weinreb Amide was added to a dry and nitrogen gas filled shlenk flask and rinsed with 1 equiv. THF then cooled to -78°C and stirred. 1.2 equiv. of the Grignard reagent, (4-chlorophenyl) magnesium bromide, is added in air free conditions and allowed to react overnight before being quenched with Ammonium Chloride. This ketone, bis(4-chlorophenyl) methanone, was purified before being subjected to another round of the reaction with 1.2 equiv. of the Grignard reagent to yield TCPM-OH.

TCPM-OH Synthesis from Chlorobenzene – TCPM-OH could also be synthesized from chlorobenzene starting materials. 1 equiv. of N-Bromosuccinimide was added in dry conditions and under N₂ to 1% equiv. of gold trichloride. 6 equiv. of dichloroethane is added in succession with 1 equiv. of chlorobenzene to the NBS, gold mixture. This reaction is stirred for 12 hours at 80°C then rotary evaporated and checked via NMR for the success of the bromination and yield of 1-Bromo-4-Chlorobenzene. The 1-Bromo-4-chlorobenzene then undergoes a lithium-halogen exchange and addition to a ketone to yield TCPM-OH. 1-Bromo-4-Chlorobenzene is dissolved in 17.5 equiv. of Tetrahydrofuran and cooled to -78°C. 1.1 equiv. of n-Butyllithium in hexanes is added dropwise and stirred for 30 minutes. 4,4-dichlorophenone is then added in 1.2 equivalence and stirred for 30 minutes. The reaction is then quenched with ammonium chloride and dried with magnesium sulfate to yield impure TCPM-OH which can further be purified via flash column chromatography.

Reduction of TCPM-OH to TCPM – TCPM-OH was reduced to TCPM by dissolving TCPM-OH in 1 equiv. DCM at 0°C, adding 2 equiv. Triethyl Silane and then 4 equiv. Trifluoroacetic acid dropwise. This reacts and warms slightly for 1.5 hours then is quenched with sodium bicarbonate, extracted with ethyl acetate and dried with magnesium sulfate. After purification, the TCPM product is found to have a TLC Rf value of 0.8.

Boron Trifluoride Etherate Synthesis of TCPM – Synthesis of TCPM was attempted starting with an ozonolysis reaction using 1 equiv. of 4-chlorostyrene in a 0.5 M Dichloromethane solution, cooled to -78°C. The ozonolysis proceeds using an ozone generator until the reaction reaches completion as indicated by the clear solution turning a lavender color. The reaction is quenched with triphenyl phosphene and left to stir at room temperature for 1 hour before being isolated through rotary evaporation. This synthesized 4-chlorobenzaldehyde reacts under nitrogen with 1.2 equiv. of the (4-chlorophenyl) magnesium bromide Grignard reagent. The esterification step was checked via NMR for the success of the bromination and yield of 1-Bromo-4-Chlorobenzene. The 1-Bromo-4-Chlorobenzene is then added to 1.2 equiv. of BF₃-OEt₂ and 20 equiv. of chlorobenzene. The reaction is heated to 80°C for 2 hours before being quenched with sodium bicarbonate and extracted with ethyl acetate to yield TCPM.

NMR – NMR of samples were taken using Bruker Advance III HD operating spectrometer at magnetic field of 11.7 Tesla (500 MHz of 1H frequency). Scans were taken at 25°C at a spectral width of 14 ppm.

Results and Discussion:

Synthesis of TCPM-OH from 4-Chlorobenzoic Acid – The initially proposed synthesis of TCPM-OH involved the esterification of 4-chlorobenzoic acid using sulfuric acid and methanol followed by a double addition of a Grignard reagent. The esterification step was optimized by substituting the initial acidic conditions with TMS-diazomethane under nitrogen, which significantly reduced the
reaction time from 6 hours to 15 minutes and led to improved purity of the methyl 4-chlorobenzoate product as confirmed by NMR spectroscopy (Figure 1).

Initially, the Grignard addition to the ester worked but resulted in purification difficulties due to byproduct formation. An attempt to switch to n-Butyllithium did not resolve these issues, as evidenced by complex TLC patterns and NMR spectra (Figure 2).

**Figure 1.** Esterification of 4-chlorobenzoic acid. (A) reaction schema for esterification using sulfuric acid. (B) reaction schema for esterification using TMS-diazomethane. (C) 1D ¹H NMR spectra of the crude esterification product from (B).

**Figure 2.** Optimization of step 2 of the synthesis of TCPM-OH. (A) reaction schema for the Grignard addition of (4-chlorophenyl) magnesium bromide with methyl 4-chlorobenzoate to form TCPM-OH. (B) reaction schema of the n-BuLi addition of chlorobenzene with methyl 4-chlorobenzoate to form TCPM-OH. (C) 1D ¹H NMR spectra of methyl 4-chlorobenzoate starting material (bottom spectra), purified TCPM-OH product (second to bottom spectra), and two attempts at the n-BuLi addition B to form TCPM-OH (top two spectra). (D) TLC plate of n-BuLi addition B developed in 4:1 hexane: ethyl acetate and dyed with Cerium Molybdate.
To address the purification challenges, homemade Grignard reagents were synthesized and used, which were purer than commercial ones. This modification improved the overall yield and purity of TCPM-OH, confirmed by clearer NMR spectra (Figure 3).

Further optimization involved a Weinreb amide intermediate, which allowed for the controlled addition of the Grignard reagent. This approach yielded a purer product, simplifying the purification process (Figure 4).

The optimized synthesis route from 4-chlorobenzoic acid utilizing the Weinreb amide intermediate provided TCPM-OH with high purity and yield. However, due to the unavailability of C-14 labeled 4-chlorobenzoic acid, the focus shifted to an alternative synthesis pathway.

Figure 4. Weinreb Amide synthetic route to TCPM-OH. (A) reaction schema for the formation of the Weinreb Amide from 4-chlorobenzoic acid. (B) reaction schema for the Grignard addition of (4-chlorophenyl) magnesium bromide to the Weinreb Amide. (C) reaction schema for the Grignard addition of (4-chlorophenyl) magnesium bromide to bis(4-chlorophenyl) methanone from B. (D) 1D $^1$H NMR spectra of the crude bis(4-chlorophenyl) methanone product from B (bottom), purified bis(4-chlorophenyl) methanone product from B (second to bottom), crude TCPM-OH product from C (middle), resubjection of the middle spectra TCPM-OH with another addition of the Grignard reagent (second to top), and purified TCPM-OH product from C (top).
Synthesis of TCPM-OH from Chlorobenzene – Given the constraints with radiolabeled 4-chlorobenzoic acid, the synthesis route was adapted to use C-14 labeled chlorobenzene, which could be acquired. The initial attempt for synthesis (Figure 5) started with the ozonolysis of 4-chlorostyrene to yield 4-chlorobenzaldehyde. A subsequent Grignard reaction was performed at a lower equivalence to halt at the alcohol stage rather than forming a ketone (Figure 6).
The final chlorobenzene ring was added to the bis(4-chlorophenyl) methanol using a BF$_3$ arylation. The BF$_3$ arylation did not produce the desired product consistently, as evidenced by unexpected NMR peaks and complex TLC patterns (Figure 7).

An alternative approach involved the bromination of chlorobenzene using NBS catalyzed by gold trichloride, which successfully produced 1-Bromo-4-Chlorobenzene. This intermediate then underwent a lithium-halogen exchange followed by ketone addition, yielding TCPM-OH. The successful bromination and subsequent reactions provided a reliable synthesis route for TCPM-OH from chlorobenzene (Figure 8). The process was optimized to ensure high yield and purity, making it suitable for subsequent reduction to TCPM.

Reduction of TCPM-OH to TCPM – TCPM-OH was reduced to TCPM using triethyl silane and trifluoroacetic acid in dichloromethane at 0°C. This reaction was optimized to achieve high yield and purity, with TLC confirming an Rf value of 0.8 and NMR verifying the structure (Figure 9).

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**Figure 7.** The BF$_3$ arylation of bis(4-chlorophenyl) methanol. (A) 1D $^1$H NMR spectra of pure TCPM (bottom) and BF$_3$ arylation attempts (top three spectra). (B) TLC plate of BF$_3$ arylation attempts developed in 4:1 hexane: ethyl acetate and dyed with Cerium Molybdate.

**Figure 8.** Chlorobenzene synthesis of TCPM-OH. (A) reaction schema of gold catalyzed NBS bromination of chlorobenzene. (B) reaction schema of Butyllithium exchange and reaction with 4,4-dichlorophenone to yield TCPM-OH. (C) 1D $^1$H NMR spectra of synthesized TCPM-OH. (D) TLC plate of synthesized and purified TCPM-OH developed in 4:1 hexane: ethyl acetate and dyed with Cerium Molybdate.
Conclusion:

The synthesized C-14 labeled TCPM and TCPM-OH will be used in an incubation study by UCSB to investigate their degradation in marine sediments. This study aims to elucidate the environmental fate of TCPM and TCPM-OH, particularly in the contaminated San Pedro Basin. The ability to track these compounds through sediment samples will provide valuable insights into their persistence and degradation pathways. The research successfully demonstrated the synthesis and optimization of TCPM and TCPM-OH, overcoming several challenges to achieve high purity and yield. The development of these radiolabeled compounds is crucial for ongoing environmental studies, contributing to a better understanding of the impacts of persistent organic pollutants.

Figure 9. The reduction of TCPM-OH to TCPM. (A) reaction schema of TCPM-OH to TCPM using Triethyl Silane and Trifluoroacetic acid. (B) 1D $^1$H NMR spectra of TCPM-OH. (C) 1D $^1$H NMR spectra of TCPM.
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