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SYNTHESIS OF ^{14}C-LABELLED HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX), 2,4,6-TRINITROTOLUENE (TNT), NITROCELLULOSE (NC) AND GLYCIDYL AZIDE POLYMER (GAP) FOR USE IN ASSESSING THE BIODEGRADATION POTENTIAL OF THESE ENERGETIC COMPOUNDS

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SUMMARY

Within the framework of an R & D project on bioremediation of soils contaminated with energetic compounds, the biodegradation of energetic products such as hexogen (RDX), trinitrotoluene (TNT), nitrocellulose (NC) and glycidyl azide polymer (GAP) is under study. Microcosm assays must be performed with radioactive carbon-14 labelled products in order to follow the biodegradation process. ^{14}C-RDX was prepared by nitration of hexamethylenetetramine (HMTA) according to the Hale process. ^{14}C-ring and methyl labelled TNTs were synthesized according to the Dorey and Carper procedure. ^{14}C-cellulose was synthesized from ^{14}C-glucose by *Acetobacter xylinum.* Nitration of the ^{14}C-cellulose yielded ^{14}C-nitrocellulose. ^{14}C-glycidyl

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azide polymer was obtained by polymerization and azidation of ^{14}C-epichlorohydrin (ECH) which was synthesized from ^{14}C-glycerol. Hydrochlorination of ^{14}C-glycerol and epoxidation of the resulting ^{14}C-1,3-dichloro 2-propanol yielded ^{14}C-ECH. The syntheses of these ^{14}C-labelled explosives are described in this paper.

KEYWORDS: ^{14}C-hexahydro-1,3,5-trinitro-1,3,5-triazine; ^{14}C-benzene-2-methyl-1,3,5-trinitro; ^{14}C-nitrocellulose; ^{14}C-glycidyl azide polymer; ^{14}C-RDX; ^{14}C-TNT.

INTRODUCTION

During this decade, many needs have already emerged related to identification, quantification, delimitation and elimination of contaminants and pollutants dispersed by munitions or present in explosives dumps, trials or destruction fields, firing areas and laboratories and their chemical sewage systems. Possible solutions for site decontamination include recycling, chemical treatment, incineration, or open burning. However, this last option will soon be no longer possible since open burning and open detonation of ammunition will not be allowed in the near future. Bioremediation is presently being considered as a viable treatment option. This technology uses microorganisms to degrade organic contaminants into less hazardous compounds. Bioremediation has the advantage of being less expensive than incineration and acceptable to the public. Within this context, a feasibility study was initiated on the biodegradation of selected energetic materials by microorganisms enriched from contaminated soils.

To identify microorganisms capable of biodegrading or mineralizing explosives, laboratory-scale microcosm studies must be performed with ^{14}C-labelled explosives so that mineralization could be followed by monitoring the ^{14}C-carbon dioxide evolution. Since radioactive explosives are not generally available commercially, it was necessary to synthesize ^{14}C-labelled RDX, TNT, NC and GAP for such a study. In order to achieve this, synthetic routes to these substances were required. To our knowledge, this is the first report of the preparation of ^{14}C-nitrocellulose and ^{14}C-glycidyl azide polymer. Moreover, although ^{14}C-RDX and ^{14}C-TNTs were previously synthesized (Refs.1-4), comparable activity results for ^{14}C-RDX and better yields for the synthesis of the ^{14}C-TNTs were obtained in our syntheses. This paper described the syntheses of ^{14}C-labelled RDX, TNT, NC and GAP.

RESULTS AND DISCUSSION

Macro amounts of carbon-14 labelled RDX, TNT, NC and GAP were prepared by mixing the available labelled starting materials with their unlabelled homologues in sufficient quantities to obtain 2 g of the desired end-products. Therefore, the radioactive carbons could be found statistically in all RDX rings which are not fully ^{14}C-labelled, fully ^{14}C-labelled TNTs are found mixed with unlabelled TNTs, and in the case of the polymers (NC and GAP), fully ^{14}C-labelled monomers could be found statistically all along the chains.

Synthesis of ^{14}C-RDX (3)

Most of the energetic compounds targeted for our study are produced industrially in many countries. For example, review articles describing industrial processes (Refs.5-7) and patents describing syntheses for RDX or its precursor can be found (Refs.8-10). The easiest and oldest process consists of introducing hexamethylenetetramine (HMTA) into concentrated nitric acid at 25-30°C. Hale noted that the nitration must be done in a 4 to 8 fold excess of nitric acid (Ref.11). At the end of the reaction, RDX is isolated by precipitation in cold water. This reaction is illustrated in figure 1 where the possible locations of carbon-14 are indicated by dots. During the nitration of HMTA, three methylenes are cleaved and oxidized to formaldehyde (Refs.12,13). This loss of carbon contributes to a decrease in the total radioactivity of the RDX end-product.

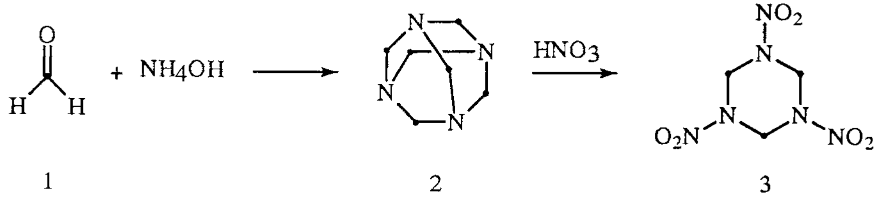

Figure 1: Synthesis of ^{14}C-RDX

To avoid this, the Knöffler process (Ref.7), involving the addition of ammonium nitrate, could be used to compensate for the missing nitrogen in HMTA. The reaction is illustrated in equation 1.

$$\underset{\text{HMTA}}{C_6H_{12}N_4} + 4\ HNO_3 + 2\ NH_4NO_3 \longrightarrow \underset{2\ \text{x RDX}}{2\,(CH_2NNO_2)_3} + 6\ H_2O$$

Equation 1

Since, there is no loss of carbon in this process, a better yield in RDX can be obtained with no loss of radioactivity. Although, the reaction is said to proceed without risk of an explosion only at elevated temperatures (80°C), in our hands, all trials in the laboratory resulted in a violent or at least a very vigourous reaction at 75°C. For this reason, the Hale process was preferred over the Knöffler process for our syntheses. RDX could also be obtained using dinitrogen pentoxide chemistry with yields similar to those obtained using classical methods (Ref.14). In addition, nitrolysis of HMTA with acetic anhydride and ammonium nitrate-nitric acid was used by Bachmann et al. (Ref.15) in their process to obtain a mixture of RDX and octogen (HMX). Castorina and co-workers modified the original Bachmann process to obtain also mixtures of RDX/HMX (Ref.2). More recently, Horváth and Alworth obtained ^{14}C-RDX by nitrolysis of hexahydro-1,3,5-tripropionyl-1,3,5-triazine (Ref.3). These authors obtained an ^{14}C-RDX sample with a total activity of 0.358 mCi corresponding to a 36% yield for their overall process .

In the present ^{14}C-RDX synthesis, ^{14}C-formaldehyde (1) (1 mCi,) was mixed with unlabelled aqueous formaldehyde (37%) and reacted with concentrated ammonium hydroxide to yield ^{14}C-HMTA (2) quantitatively. Nitration of this product yielded ^{14}C-hexogen (3) (60%) (mp: 201-208°C, lit. 202°C, ref.14 lit. 204°C, ref.6) which was phlegmatized with ethanol (8%). The infrared and NMR spectroscopies of the product was identical to those of a commercial sample. The yields for the two steps of the synthesis were similar to those found in the literature (Ref.7).

Taking into account that the first step of the synthesis is quantitative and also considering that the yield of the second step is 60 %, a 600 μCi should theoretically be obtained. However, from figure 1, it is seen that half of the methylenes are lost during the second step of the synthesis. Consequently, 1 mCi of initial radioactivity should give a 300 μCi total activity at the end of the reaction and thus a 30% theoretical yield for the overall process. This is comparable to the activity obtained by Horváth (Ref.3).

Liquid scintillation counting revealed a total activity of 120 μCi for our ^{14}C-RDX sample meaning that the original quantity of radioactivity of the starting material was not 1 mCi. Therefore, it appears that we received 400 μCi of activity instead of 1 mCi. Nevertheless, the ^{14}C-RDX, even with a total activity of 120 μCi, has a level of radioactivity high enough to be used in the biodegradation study.

Synthesis of ^{14}C-TNTs (5 and 7)

The syntheses of trinitrotoluene (TNT) and its isomers are well known and are generally realized by a nitration in three steps with increasing nitric acid concentration (Ref.16). Fuming sulfuric acid is added to ensure a dry medium which favors the formation of the nitrating species ($^{+}NO_2$). Syntheses for labelled TNTs with carbon-14 and nitrogen-15 are also known (Refs. 4,17). Toluene radiolabelled either on the ring or on the methyl group, are available as starting materials for TNT's syntheses. For the ^{14}C-TNTs syntheses, both types of ^{14}C-toluene were used as starting materials in order to be able to verify if microorganisms attack the ring or the methyl or both. The Dorey and Carper procedure was used to synthesize ^{14}C-TNTs (Ref.17). Radioactive toluene was mixed with toluene and the nitration was done as illustrated in figure 2.

The syntheses were realized by slightly modifying the literature procedure resulting in a doubling of the yields. ^{14}C-TNT-ring (5) was isolated in 61% yield and ^{14}C-TNT-methyl (7) in 47% yield compared with 26% yield found in the literature (Ref.17). In these syntheses, fuming sulfuric acid (20% oleum) was used instead of a 15% oleum concentration as described by Dorey *et al.* At this concentration of sulfur trioxide (20%), sulfuric acid can dehydrate more efficiently thereby resulting in a better nitration. Numerous washings were done and recrystallization under favorable conditions led to increased yields. Nitration of the ^{14}C-toluene-ring (4) (1 mCi) mixed with unlabelled toluene gave solid crude ^{14}C-TNT which, upon recrystallization, yielded pure ^{14}C-TNT-ring (5) (49%) (mp:80.6-81.2°C, lit. 80.8°C, Ref.6). Further work on the filtrate yielded an additional quantity of ^{14}C-TNT-ring (mp: 80.1-80.8°C) to give a total reaction yield of 61%. The same procedure was repeated with ^{14}C-toluene-methyl (6) (1 mCi) and after recrystallization, ^{14}C-TNT-methyl (7) was isolated (47% yield, mp: 80.8-81.1°C). The IR and NMR spectroscopic analyses for the ^{14}C-TNT-ring and ^{14}C-TNT-methyl were identical, and corresponded to those of the industrially produced material. The melting points revealed that ^{14}C-TNTs were of military grade.

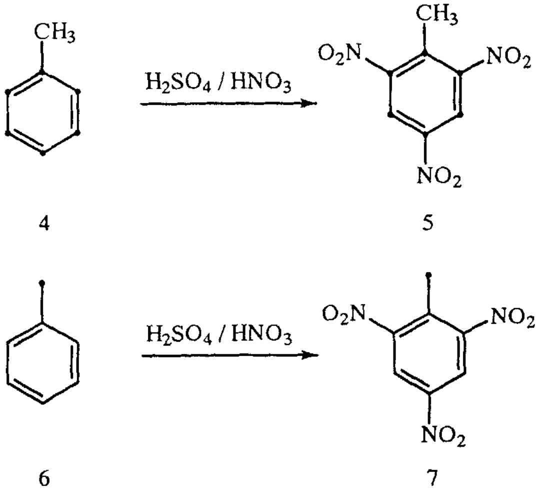

Figure 2: Synthesis of ^{14}C-TNT's

Liquid scintillation counting revealed that the ^{14}C-TNT-ring had a specific activity of 527 μCi/g, while ^{14}C-TNT-methyl had a specific activity of 170 μCi/g. Considering the yields for the syntheses of TNT-ring and TNT-methyl, the total activities for these products were 1.581 mCi and 395 μCi, respectively.

Synthesis of ^{14}C-Nitrocellulose (10).

Usually, the cellulose sources for nitrocellulose production come from well-cleaned cotton linters or from wood pulp. Nitrocellulose is prepared by the nitration of cellulose into a nitric-sulfuric acid mixture. During this process, the hydroxyl groups of the cellulose are transformed into nitrate esters. The kinetics of the nitration reaction and the effect of the nitrating mixture composition on the power of the nitration have been thoroughly studied (Refs.18-20). By varying the concentration of the nitrating medium and the experimental conditions, two grades of nitrocellulose, characterized by their nitrogen contents, are obtained. If all hydroxyl groups were transformed into nitrate esters, nitrocellulose would have a 14.14% nitrogen content. However, this amount is never reached because cellulose is a fibrous material and it is very difficult for the nitrating species to migrate inside the fiber thus resulting in an incomplete reaction. For this reason, two grades of nitrocellulose are encountered, they are pyrocellulose (12.6% nitrogen) and military guncotton grade (13.4% nitrogen).

^{14}C-cellulose (9) is not available commercially and thus must be prepared in order to synthesize ^{14}C-nitrocellulose (10). Within the framework of this study, bacteriological ^{14}C-cellulose was prepared from a mixture of ^{14}C-glucose (8) (1 mCi) and unlabelled glucose. For this biosynthesis the microorganism "*Acetobacter xylinum*" was selected and cultivated on the medium of Masaoka *et al.* (Ref.21). This microorganism is capable of linking glucose units together by creating β 1-4 osidic bonds. By feeding the microorganisms with a mixture of ^{14}C-glucose and glucose, a ^{14}C-cellulose was obtained and isolated (11% yield). The synthesis is illustrated in figure 3.

It should be noted that the ^{14}C-cellulose obtained was only partially labelled since a mixture of glucoses was used to feed the microorganism. The nitration of this ^{14}C-cellulose was done in accordance with Olsen and Greene (Ref.22) and yielded ^{14}C-nitrocellulose (91%) which was phlegmatized with ethanol. Elemental analysis revealed that the ^{14}C-nitrocellulose was a pyrocellulose and liquid scintillation counting showed its activity was 140 μCi. Since the microbial synthesis of ^{14}C-cellulose was difficult and yielded cellulose with an 11% yield, and

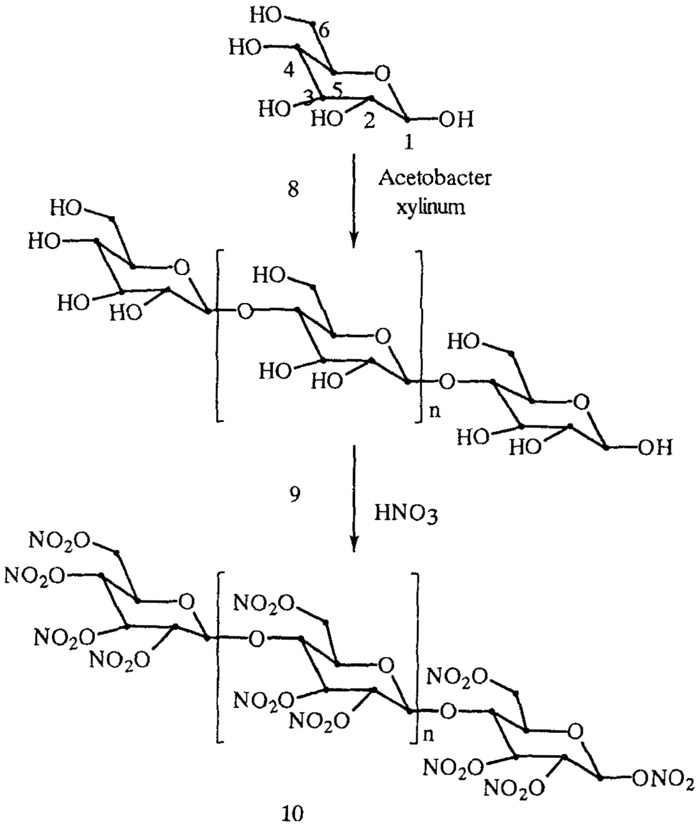

Figure 3: Synthesis of ^{14}C-Nitrocellulose

considering the yield of the nitration, a theoretical total activity of 100 μCi was expected for the ^{14}C-nitrocellulose. This is in agreement with the experimental value. ^{14}C-nitrocellulose with an activity of 0.140 mCi is considered suitable for the biodegradation study.

Synthesis of ^{14}C-Glycidyl Azide Polymer (14)

The synthesis of glycidyl azide polymer was a greater challenge than the other syntheses. Generally, GAP is obtained by the azidation of polyepichlorohydrin (PECH) which is obtained by the polymerization of epichlorohydrin (ECH) (Refs.23,24). According to Frankel, by varying the experimental conditions, one could synthesize PECH with molecular weights varying from 500 to 5000. The synthesis of ^{14}C-GAP (14) involves the preparation of ^{14}C-epichlorohydrin (13), its polymerization, followed by the azidation of the resulting polymer.

Frankel's two-step process consists, firstly, of polymerizing the ECH, and secondly, performing the azidation of the resulting PECH to obtain glycidyl azide polymer. Because it is obvious that each step of a synthesis implies a loss of material, the GAP one-step process developed by Ahad (Ref.25) appeared preferable and was used for this synthesis. This process consists of initiating the polymerization of ECH with sodium azide and performing the azidation simultaneously. With this process the resulting glycidyl azide polymer has a molecular weight of 500 which is suitable for the biodegradation study.

The ^{14}C-epichlorohydrin used to synthesize ^{14}C-GAP was prepared from ^{14}C-glycerol (11). Glycerol is transformed into 1,3-dichloro 2-propanol which is epoxidized under basic conditions to epichlorohydrin. Modern syntheses of epichlorohydrin are based on allyl chloride epoxidation but this radioactive chemical is not available commercially. Berthelot did the first syntheses of 1,3-dichloro 2-propanol from glycerol in 1854 (Refs.26,27) but the reaction times were quite long. Later, it was found that acetic acid could serve as a catalyst for this reaction (Refs.28-30). We thus decided to modify the Hill and Fischer method (Ref.29) to synthesize the ^{14}C-1,3-dichloro 2-propanol (12) which was subsequently epoxidized by sodium hydroxide in diethyl ether. The resulting ^{14}C-ECH was polymerized and azidized in accordance with the Ahad process (Ref.25) as illustrated in figure 4.

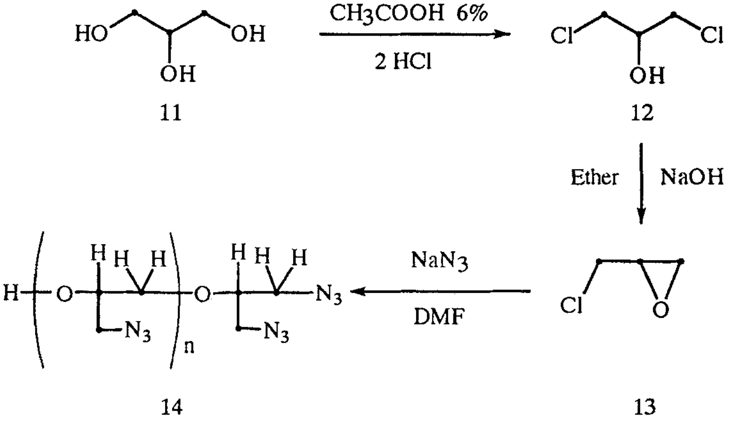

Figure 4: Synthesis of ^{14}C-Glycidyl Azide Polymer

Therefore, the total synthesis involved three steps. Firstly, hydrochlorination of a dried mixture of unlabelled glycerol, ^{14}C-glycerol (4 x 250 μCi) and acetic acid yielded, after purification, ^{14}C-1,3-dichloro 2-propanol (83%). This first step has three parameters which must be controlled to obtain a good yield and reproducibility. These include the temperature of the reaction, the amount of gaseous hydrochloric acid which is passed through glycerol, and the duration of the reaction. Many reaction conditions were found in the literature, such as the duration of the reaction (24, 36 hrs) at different temperatures of the mixture (100, 105, 110°C) (Refs.26-31). Novelli mentioned that the temperature should not be above 100°C and the duration of the reaction should be 36 hours (Ref.28). In our synthesis, a low gas output was used for 48 hours and this resulted in an excellent yield. The temperature of the reaction was adjusted to 90°C and the purification procedure was modified to remove the water produced by the reaction before the distillation of the product. In this way, distillation of the ^{14}C-1,3-dichloro 2-propanol was easier without any loss and a pure product was isolated. The IR and NMR spectroscopies of this product were compared and were identical to the ones of a pure sample from Aldrich.

The second step of the synthesis was the epoxidation of this ^{14}C-1,3-dichloro 2-propanol under basic conditions to yield quantitatively ^{14}C-epichlorohydrin. The epoxidation took place in diethyl ether in the presence of sodium hydroxide. When the reation was complete, the solids were removed by filtration and the diethyl ether was distilled. The ^{14}C-epichlorohydrin produced was directly used in the following step without further purification. No distillation was done since ^{14}C-epichlorohydrin is highly volatile and thus it is very difficult to avoid loss of material during epichlorohydrin distillation even with a dry ice-acetone trap. A quantitative yield was assumed for the ^{14}C-epichlorohydrin formation and this was confirmed by the 84% yield obtained for the formation of glycidyl azide polymer with this ^{14}C-epichlorohydrin. This experimental value is comparable to the yield usually observed for the azidation and polymerization of a pure sample of epichlorohydrin.

Finally, the last step in the GAP synthesis consisted of the polymerization and azidation of the previously isolated ^{14}C-epichlorohydrin in N,N-dimethyl formamide (DMF) to give GAP in a single step. A ratio for ECH:DMF;NaN$_3$ of 1:1:1 was used for the polymerization and azidation. Glycidyl azide polymer was isolated, characterized (84% yield, M_N= 368 g/mole, M_W= 859 g/mole, polydispersity = 2.34), and determined suitable for the biodegradation study. Liquid scintillation counting revealed that the ^{14}C-GAP synthesized had a specific activity of 226 μCi/g, and therefore a total activity of 626 μCi. This experimental activity is in good agreement with the theoretical activity (697 μCi).

CONCLUSION

The syntheses of carbon-14 labelled energetic compounds were performed successfully. In all cases, the IR and NMR spectroscopies of the ^{14}C-products were identical to the spectroscopies of known samples. ^{14}C-RDX was synthesized according to the Hale process and the ^{14}C-RDX sample had a total activity of 120 μCi. The procedure modifications used in the TNTs syntheses resulted in increased total yields, the ^{14}C-TNT-ring had a total activity of 1.581 mCi while that of ^{14}C-TNT-methyl was 395 μCi. ^{14}C-glucose was linked by *Acetobacter xylinum* to yield ^{14}C-cellulose which was nitrated to ^{14}C-nitrocellulose with a total activity of 140 μCi. ^{14}C-glycerol was used to synthesize the ^{14}C-glycidyl azide polymer which had a total activity of 626 μCi. Considering the syntheses of NC and GAP, the experimental activities are in good agreement with the theoretical values. The purity of all the products isolated in the syntheses was monitored by a rigourous control of the IR and NMR spectroscopies.

EXPERIMENTAL

All solvents were purchased from Fisher Scientific Co. except for the anhydrous ethanol which was obtained from "Les alcools de commerce limitée". Chemicals and starting materials such as glycerol, formaldehyde and cellulose were purchased from Aldrich Chemical Co. The Aldrich cellulose (20 μm) was used to adjust the synthesis parameters which were subsequently used with the bacterial cellulose in order to compare both nitrocelluloses. The acids and bases were purchased from Baker except fuming nitric acid and fuming sulfuric acid (20% oleum) which were purchased respectively from Aldrich and ACP Chem. Co, respectively. Toluene was purchased from Caledon and purified by distillation before use. Gaseous hydrochloric acid was obtained from Matheson Gas Products. Hexogen (RDX), TNT and nitrocellulose respectively come from Expro Chemical Products Inc., C.I.L. and Radfort Army Ammunition Plant. Finally, ^{14}C-glycerol (9.6 mCi/mmol) and ^{14}C-glucose (4.1 mCi/mmol) were purchased from Dupont Canada Inc. while ^{14}C-formaldehyde (43.6 mCi/mmol), ^{14}C-toluene-ring (10.2 mCi/mmol) and ^{14}C-toluene-methyl (9.5 mCi/mmol) were obtained from Sigma Chemical. Co. All radioactive starting materials were fully ^{14}C-labelled.

Infrared absorption spectra were registered with a Perkin-Elmer IR spectrophotometer (model 580) or with a spectrophotometer Digilab FTS-40 combined with a DTGS detector. the infrared spectra for the solids were recorded with solid suspension in KBr or in acetone or in methylene chloride solutions. The infrared spectrum of nitrocellulose was obtained from a film which was made by evaporating an acetone solution of NC and placing it in a support. The infrared spectra for liquids were recorded by using neat samples between sodium chloride cells.

Nuclear magnetic resonance spectra for ^{1}H and ^{13}C were obtained from a Varian Gemini spectrometer (200 MHz). Spectra were recorded using deuterated chloroform as the solvent and tetramethylsilane as the internal reference at 0.0 ppm on the δ scale. For nitrocellulose, deuterated acetone was used as the solvent. All gel permeation chromatographies were realized with a Waters chromatograph using a Waters pump 590, Waters automatic injector 712 WISP and a Waters differential refractometer 410 as the detector. GAP samples (200 μL) were injected and analysed with a series of Water Ultrastyragel columns (7.8 x 300mm) 10 μm, 1 μm , 0.05 μm and 0.01 μm. An universal calibration curve using different molecular weight polystyrenes was used for the molecular weight determination of the samples.

^{14}C-Hexamethylenetetramine (HMTA) (2)

^{14}C-formaldehyde (1, 1 mCi, 43.6 mCi/mmole) was quantitatively transferred in a three-neck flask (50 mL) with unlabelled aqueous formaldehyde (37%) (7.84g, 0.0966 mole), and the flask was immersed in a water bath at 18°C. Concentrated ammonium hydroxide (5mL) was added dropwise for 30 minutes in order to maintain a temperature between 28 and 35°C. This solution was heated at 33-35°C for 24 hours and then transfered to a flask (250 mL) where water was added to precipitate the product. The glassware was thoroughly washed to avoid contamination and the rinse water is added to the flask. Water was evaporated to yield ^{14}C-HMTA (2, 2.25 g, quantitative, mp: 265-272°C (decomp)). IR: υ_{max} (KBr) cm^{-1}: 2960, 2940, 2880, 1460, 1440, 1370, 1240, 1000, 810, 670. ^{1}H NMR : δ ($CDCl_3$) ppm : 4.73 (all protons,s). ^{13}C NMR : δ ($CDCl_3$) ppm : 74.83 (all carbons).

^{14}C-Hexahydro-1,3,5-trinitro-1,3,5-triazine (^{14}C-RDX) (3)

In a three-neck flask (100 mL) equipped with a reflux condenser and a magnetic stirrer, fuming nitric acid (39.4 g) was cooled at 5°C and ^{14}C-HMTA (2, 2.25 g , 0.016 mole) was added slowly over a period of 1 hour in order to keep the mixture temperature between 5-10°C. The temperature of the solution was then increased to 15-20°C and held there for 3 hours. The solution was then poured into a beaker containing water (40 mL) to precipitate the ^{14}C-hexogen. The solid was filtered, washed thoroughly with water, with a sodium carbonate solution (5%) and again with water until neutral pH was achieved. The RDX was dried at room temperature for 2 days. Dried ^{14}C-hexogen (3) was collected (2.0 g, 0.009 mole, 56% yield) (mp: 201-208°C, lit. 202°C, ref.15, lit. 204°C, ref.6) and flegmatized with ethanol (8%). IR:υ_{max}(Acetone)cm^{-1}: 3070, 1590, 1310, 1270, 1010, 930-920, 790. ^{1}H NMR: δ ($CDCl_3$) ppm: 6.01 (all protons). ^{13}C NMR: δ ($CDCl_3$) ppm: 60.31 (all carbons). The total activity for this ^{14}C-RDX was 120 μCi.

^{14}C-2,4,6-Trinitrotoluenes (^{14}C-TNTs)(5 and 7)

In a three-neck flask (25 mL) equipped with a reflux condenser and a magnetic stirrer, concentrated sulfuric acid (6 g) was cooled at 0°C. A tube of ^{14}C-toluene-ring (4, 1 mCi, 10.2 mCi/mmole) was cooled to -80°C in an acetone-dry ice bath. After pouring freshly distilled toluene (2 g) over the tube seal, it was broken so that ^{14}C-toluene-ring was diluted with unlabelled toluene; the mixture was then carefully transferred to the flask previously prepared.

When the temperature of the solution reached 0°C, nitric acid (50 %, 4 g) was carefully added dropwise. Upon completion of the addition, the mixture was heated at 35°C for 6 hours and then transferred into a separatory funnel (125 mL) to stand for 12 hours.

The aqueous phase was discarded and the organic phase was transferred in a three-neck flask (100 mL) at 0°C. The separatory funnel was successively washed with concentrated sulfuric acid (11.2 g), nitric acid (50%, 4 g) and fuming sulfuric acid (20% oleum, 19.0 g) which were carefully added dropwise to the flask in order to keep the temperature of the mixture below 10°C. When this was completed, the mixture was heated at 90°C for 8 hours. The flask was then cooled down to 0°C, and fuming sulfuric acid (20% oleum, 30 g) and fuming nitric acid (2 g) were successively and carefully added dropwise to the mixture. The solution was heated to 95°C for 6 hours.

The mixture was extracted with methylene chloride (3 x 50 mL) and the organic phases were mixed and washed successively with a saturated sodium bicarbonate (2 x 100 mL) and water (3 x 100 mL). The organic phase was separated and evaporated to yield an oil which when in contact with ethanol yielded solid crude ^{14}C-TNT-ring. This solid was recristallized in ethanol and filtered to yield pure ^{14}C-TNT-ring (5, 2.4 g, 49% yield) (mp:80.6-81.2°C, lit. 80.8°C, Ref.6). Further work on the filtrate yielded an additional quantity of ^{14}C-TNT-ring (0.57 g, mp: 80.1-80.8°C) to give a total reaction yield of 61%. IR:υ_{max}(CH_2Cl_2)cm^{-1}: 3120, 2900, 1610, 1550, 1400-1350, 1200, 1180, 1090, 940, 920, 910, 800. ^{1}H NMR: δ ($CDCl_3$) ppm: 2.72 (3H, CH_3, s), 8.85 (2H, meta aromatic H, s) ^{13}C NMR: δ ($CDCl_3$) ppm: 15.8 (CH_3), 122.3 (meta carbons), 134.1 (C-CH_3), 145.8 (para carbon), 151.7 (ortho carbons).The total activity for the ^{14}C-TNT-ring was 1.581 mCi.

The same procedure was repeated with ^{14}C-toluene-methyl (6, 1 mCi, 9.5 mCi/mmole) and after recrystallization, ^{14}C-TNT-methyl was isolated (7, 2.32 g, 47% yield, mp: 80.8-81.1°C). The spectroscopic analysis for this product was the same as for the ^{14}C-TNT-ring. The total activity for the ^{14}C-TNT-methyl was 395 μCi.

^{14}C-Cellulose (9)

^{14}C-cellulose was produced from a mixture of ^{14}C-glucose (8, 1 mCi, 4.1 mCi/mmole) and unlabelled glucose (16 g) in static batch culture reactor by *Acetobacter xylinum. A. xylinum* was cultivated on the medium of Masaoka et al. (Ref.22) and consisted of 2 % bactopeptone, 0.5 % yeast extract, 0.5 % glucose, 0.1 % $MgSO_4$-$7H_2O$, 0.2 % ethanol (95 %) and 1.5 % agar (pH 6.0). A bacterial suspension was prepared in 0.85 % NaCl from agar plates cultures of *A. xylinum*, and was used to inoculate 20 bioreactors (500 mL) each containing 100 mL of the above medium modified to contain 0.8 % glucose but no agar. Each flask received ^{14}C-glucose to yield 1 x10^8 dpm prior to sealing the flasks. The flasks were incubated under static conditions at room temperature for 20 days, and 30 cc of the atmosphere in each flask were exchanged with sterile air or pure oxygen daily to ensure aerobiosis.

^{14}C-cellulose was harvested and purified by separating the cellulose pellicles from the culture broth and cells. Washed pellicles were boiled in a 2 % NaOH solution for 30 min, and then rinsed 3 times with distilled water. The cellulose was then frozen overnight at -20°C to extract the liquid. After thawing, the cellulose was again rinsed, boiled in NaOH (2 %) for 30 minutes, and washed in changes of distilled water until it reached neutral pH. After filtration, the cellulose was frozen at -20°C for 12 hours and lyophilized. The isolated ^{14}C-cellulose (9, 1.81 g, 11 % yield) was poured into ethanol and cut into fragments before the nitration.

^{14}C-Nitrocellulose (10)

The ^{14}C-cellulose (9, 1.81 g) was transfered in a flask (500 mL) with ethanol. The solvent was removed by evaporation, and the flask was put under high vacuum to remove the last traces of ethanol. A mixture of concentrated nitric acid (88 g, 70 %) and fuming sulfuric acid (132 g, 20% oleum) was cooled at 25 °C and poured into the flask containing the ^{14}C-cellulose. The temperature of the flask was maintained at 25 °C for 18 minutes while the suspension was stirred occasionally. The mixture was then transfered to a beaker containing water (1600 mL), and the flask was thoroughly rinsed. The suspension in the beaker was stirred, filtered and rinsed many times with water. ^{14}C-nitrocellulose was transfered again into water, stirred, and filtered. The solid was washed with a sodium carbonate solution (1800 mL, 1%) followed by water until neutral pH. ^{14}C-nitrocellulose was collected, dried at 60 °C for one hour, and dried on filter paper for 12 hours. The dried ^{14}C-nitrocellulose (10, 2.9 g, 91% yield) was phlegmatized with ethanol. IR:υ_{max}(Film)cm^{-1}: 3525, 2960, 2900, 1660, 1630, 1430, 1380, 1280, 1150, 1060, 990, 830, 740, 680. ^{1}H NMR: δ (Acetone- D$_6$) ppm: 4.20 (1 H, H-5, s), 4.30 (1 H, H-4, s), 4.55 (1 H, H-6$_a$, s), 4.65 (1 H, H-6$_b$, s), 5.20 (2 H, H-2 and H-1, s) 5.75, (1 H, H-3, s). ^{13}C NMR: δ (Acetone-D$_6$) ppm: 71.1 (C-6), 72.2 (C-5), 77.8 (C-4), 78.9 (C-2), 80.2 (C-3), 100.8 (C-1). Elemental analysis of the ^{14}C-nitrocellulose revealed a nitrogen content of 12.6 %, thus, this nitrocellulose was a pyrocellulose. The total activity of this ^{14}C-nitrocellulose was 140 μCi.

^{14}C-1,3-Dichloro 2-propanol (12)

Unlabelled glycerol (4 g, 0.043 mole) was weighed into a flask (25 mL) and ^{14}C-glycerol (11, 4 x 250μCi, 9.6 mCi/mmole) was quantitatively transferred into the flask with diethyl ether. Diethyl ether and water were distilled and the product was dried under vacuum (0.1 torr) at 90°C for 16 hours. Acetic acid (0.25 g, 6%) was added to the flask and gaseous hydrochloric acid was slowly passed through at 90°C for 48 hours. The mixture was dissolved with methylene chloride and the aqueous phase was separated and extracted with methylene chloride (4 x 20 mL). The combined organic phases were evaporated to yield a product which after vacuum distillation (14 torr, 63-64°C) was isolated to give pure ^{14}C-1,3-dichloro 2-propanol (12, 4.6 g, 0.0357 mole, 83% yield). IR:υ_{max}(NaCl)cm^{-1}: 3350, 2960, 1640, 1430, 1290-1240, 1070, 1050, 960, 880, 870, 750, 700. ^{1}H NMR: δ (CDCl$_3$) ppm: 3.64 (4H, CH$_2$Cl, d; ^{3}J= 5.5 Hz), 4.02 (1H, C$\underline{H}$OH, p; ^{3}J= 5.3 Hz). ^{13}C NMR: δ (CDCl$_3$) ppm: 46.13 (CH$_2$Cl), 71.27 (CHOH).

^{14}C-Epichlorohydrin (^{14}C-ECH) (13)

In a flask (100 mL) with a magnetic stirrer, were added ^{14}C-1,3-dichloro 2-propanol (12, 4.57 g, 0.0354 mole), diethyl ether (50 mL) and finely ground sodium hydroxide (1.70 g, 0.0425 mole, 1.2 eq.). The mixture was refluxed for 20 hours, cooled, and filtered. Diethyl ether was distilled and the ^{14}C-epichlorohydrin obtained (13, 3.28 g, 0.0354 mole, quantitative) was used without further purification. IR:υ_{max}(NaCl)cm^{-1}: 3080, 3020, 3000, 2940, 2880, 1480, 1460-1440, 1400, 1280, 1140, 980, 940, 860, 770, 730. ^{1}H NMR: δ (CDCl$_3$) ppm: 2.66 (1H, 1H of CH$_2$-epoxide, ddt; ^2J=4.8 Hz, ^3J=2.4 Hz, ^4J=0.8 Hz), 2.87 (1H, the other H of CH$_2$-epoxide, tt; ^2J=4.8 Hz, ^3J=4.5 Hz, ^4J=0.5 Hz), 3.22 (1H, CH-epoxide, m), 3.55 (2H, CH$_2$Cl, dt; ^3J= 5.2 Hz, ^4J= 0.8 Hz). ^{13}C NMR: δ (CDCl$_3$) ppm: 45.3 ($\underline{C}$H$_2$CL), 47.2 ($\underline{C}$H$_2$O), 51.6 ($\underline{C}$HO).

^{14}C-Glycidyl azide polymer (^{14}C-GAP) (14)

In a three-neck flask (50 mL) ECH, DMF and sodium azide were mixed in a ratio of 1:1:1. Thus, ^{14}C-epichlorohydrin from the preceding step (13, 3.28 g, 0.0354 mole) was dissolved in DMF (3.3 g) and sodium azide (3.3 g, 1.5 eq.) was added. The mixture was heated to 85°C, and stirred at that temperature for 2 hours, it was then heated at 100°C for 40 hours. After the 24 hours of heating at 100°C, an additional portion of DMF (3.3 g) was added. When the reaction was completed, the mixture was cooled to room temperature and methylene chloride (50 mL) was added. The organic phase was washed with brine water (4 x 50 mL). The aqueous phases were combined and extracted with methylene chloride (100 mL). The organic phases were combined and evaporated to yield ^{14}C-glycidyl azide polymer (14, 2.77 g, 84%). IR:υ_{max}(NaCl)cm^{-1}: 3430, 2920, 2880, 2100, 1450, 1290, 1120, 940. ^{1}H NMR: δ (CDCl$_3$) ppm: 3.2-4.1 (all protons, m). ^{13}C NMR: δ (CDCl$_3$) ppm: 52.0 ($\underline{C}$H$_2$N$_3$), 68.0, 73.0 ($\underline{C}$H$_2$O), 78.9 ($\underline{C}$HO). The total activity for the ^{14}C-GAP was 626 μCi.

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