Fate of ^{14}C-Labeled Carbonyl Sulfide on Grains and Grain Fractions

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^{14}C-Labeled carbonyl sulfide (COS) was used to measure the amount of sorbed fumigant and alteration products on grains. Wheat, paddy rice, polished rice, mungbean, and safflower were exposed to a 60 mg L^{-1} of ^{14}COS for 7 days and then aired for 5 days. Carbonyl sulfide and/or alteration products in sugars, protein, starch, amino acids, protopectines, and hemicelluloses were undetectable. The total uptakes of radioactivity determined after fractionation and also by extraction were in the range of 36−53 ng g^{-1} COS equiv. The total radioactivity determined by autoradiography was below the detection limit of 70 ng g^{-1} COS equiv. Radioactivity in the commodities was less than 0.003% of all the radioactivity applied. The amount of retained radiolabel was measured in three ways. First, biochemical fractions such as lipids and amino acids were separated by chromatography, and the activity was determined in each component. Second, commodities were crushed and extracted in aqueous acetone until the maximum amount of radiolabel was extracted. Third, autoradiography was carried out on commodity kernels.

Keywords: *^{14}C-Labeled carbonyl sulfide; carbonyl sulfide; fumigants; residues; alteration products*

INTRODUCTION

Fumigation of stored products with chemicals such as phosphine (PH_3) or methyl bromide (MeBr) is used to prevent insect damage. However, MeBr is being withdrawn as an ozone-depleting substance, and insect resistance to PH_3 is increasing (Chaudry, 1997). Carbonyl sulfide (COS) is a potential fumigant to replace MeBr and PH_3 for stored products (Desmarchelier, 1994; Zettler et al., 1997; Plarre and Reichmuth, 1997; Weller, 1998) and has been patented by Banks et al. (1992). Studies on the fate of this chemical in grain are important for the evaluation of its safety to consumers. This paper measures the uptake of ^{14}COS on representatives of three major grain groups: cereals, legumes, and oilseeds. It describes the determination of the total radioactivity extracted by aqueous acetone, the total radioactivity measured by autoradiography, and the distribution of the label after fractionation of commodities into biochemical fractions.

EXPERIMENTAL PROCEDURES

Reagents and Apparatus. α-Amylase type X-A fungal crude from *Aspergillus oryzae* (Aqis high), protease type XIV bacterial from *Streptomyces griseus* (Aqis low), Trizma buffer solution, formic acid, and starch soluble (ACS) reagent were supplied by Sigma Aldrich, Sydney, Australia. The other chemicals were obtained from Tech Ajax, Sydney, Australia. Scintillation vials (20 mL) and scintillation fluid was obtained from Edwards Instruments Company. ^{14}C-Labeled sodium thiocyanate (NaS^{14}CN) was supplied by ICN Pharmaceuticals, Inc., Irvine, CA. The purity was >98%, specific activity was 12.1 mCi $mmol^{-1}$ (448 GBq $mmol^{-1}$), and concentration was 149 mCi mg^{-1} (5.5 MBq mg^{-1}). ^{14}C-Labeled sodium carbonate ($Na_2{}^{14}CO_3$) was supplied by DuPont NEN, Billerica, MA. The purity was >97% and specific activity was 1.00 mCi $mmol^{-1}$ (37.00 MBq $mmol^{-1}$). All reagents were analytical grade unless otherwise specified.

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Table 1. Moisture Content and Relative Humidity of Commodities at 25 °C

commodity	moisture content (%, w/w, wet basis)	RH (%)
mungbean	11.6	49.0
paddy rice	12.1	50.3
polished rice	12.1	50.6
safflower	6.1	50.3
wheat	12.3	53.7

The purity of synthetic COS was determined on a GOW-MAC (model 40-001) gas density detector (GOW-MAC Instrument Co., Madison, NJ) after separation on a 1 m × 5 mm (i.d.) Porapak Q 100/120 mesh (Alltech Associates, Sydney, Australia, Catalog No. 2702) at 105 °C and carrier (N_2) flow of 150 mL min^{-1}. The reference gas was tetrafluoroethane (>99.9% pure).

Carbonyl sulfide was determined on a Shimadzu GC6AM GC (Shimadzu Seisakusho, Kyoto, Japan) equipped with a flame photometric detector (FPD). Separation was achieved on a 1 m × 3 mm (i.d.) glass column packed with HayeSep Q (Alltech Associates, Catalog No. 2801) at 140 °C and carrier (N_2) flow of 40 mL min^{-1} at 0.8 psi.

A model LS 200 Beckman (Beckman Instrument Co.) liquid scintillation analyzer was used for scintillation counting, operating at the appropriate wavelength for the radioisotope.

Commodities Conditioning and Fumigant Dosing. Five representative grains (wheat, paddy rice, polished rice, mungbean, and safflower) were used. All commodities (1 kg) were placed into a sealed chamber (2 L) and allowed to equilibrate at 25 °C. After a period of 2 months, the commodities were removed, and moisture content and relative humidity (Table 1) were checked. Moisture content (wet basis) was measured from loss of mass in ground samples after oven-drying at 130 °C for 2 h. Relative humidity was calculated from measured equilibrium dew point observed on a cooled mirror dew point meter (MBW Elektronic AG, model DP3-D) placed in a closed loop with a 1-kg grain sample.

All conditioned grains (10 g for each variety) were separately placed in a single sealed desiccator (1.5 L) equipped with a septum. ^{14}COS (35 mL) was injected into the desiccator by gas-tight syringe to give an initial concentration of 60 mg L^{-1}, which is the maximum recommended dose of the fumigant

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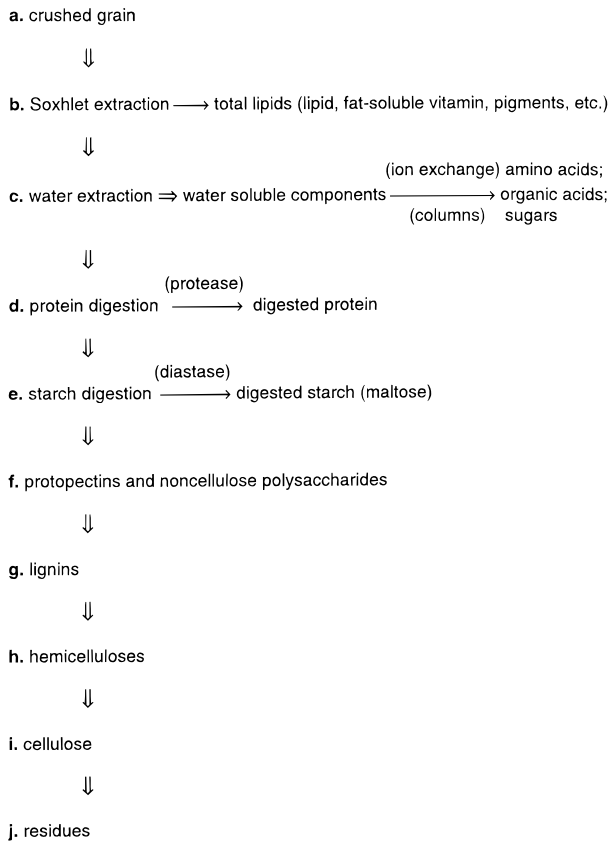

Figure 1. Scheme for the fractionation of commodities into nutrient classes.

(Desmarchelier, 1994). The grain samples were fumigated for a typical exposure period (7 days) at 25 ± 2 °C. Following the initial dose, the fumigant was circulated in the desiccator by a magnetic stir bar. After 7 days exposure, the desiccator was opened, and the samples were transferred to a fume hood and aired for 5 days at 25 ± 2 °C.

Synthesis of ^{14}C-Labeled COS. ^{14}C-Labeled COS was produced in the same way as nonlabeled COS by the method of Ferm (1957), namely, reaction of ^{14}C-labeled sodium thiocyanate with 9 M sulfuric acid. After generation, the gas was washed with a solution of lead acetate to remove hydrogen sulfide, and the gas was then passed through a barium hydroxide solution to remove carbon dioxide. Gas purity was >95% v/v, with the impurity being air.

Determination of Radioactivity in Biochemical Fractions. The method of fractionating nutrients relies on the sequential solubilizing and/or digestion of one fraction while leaving the residue as the substrate for the next extraction. The procedures listed in Figure 1 were carried out to determine the fate of applied ^{14}COS. Untreated grains were used as controls. All samples were prepared for duplicate testing.

(a) A weighed proportion of the commodity (5 g) was removed and placed in a stainless steel mortar. The sample was crushed (not finely ground) and transferred to a Soxhlet extraction thimble.

(b) The extraction thimble (containing the 5 g of sample) was placed into the Soxhlet apparatus, and the sample was extracted with 30 mL of chloroform overnight. After extraction, the solvent (containing lipid, fat-soluble vitamin, pigments, etc.) was transferred into a 50-mL volumetric flask for total lipid analysis (Canvin and Beebers, 1961). The thimble was then placed in a fume hood to allow complete evaporation of residual chloroform. The sample was then transferred to a 25-mL centrifuge tube.

(c) The sample (remaining from step b) was resuspended in 10 mL of distilled water. The sample was vortexed and allowed to soak for 1 h. The sample was centrifuged for 10 min at 3500 rpm, and the supernatant was transferred to a collection vessel. The pellet was resuspended twice in 10 mL of distilled water for a 15-min soak between each washing. The centrifuged washings were collected in the vessel (Davis and Daish, 1960; Oechel et al., 1972). This solution contained sugars, amino acids, and organic acids. The pellet was retained (for step d).

The aqueous solution (collected from above step) was passed through a series of ion-exchange columns (10 mm in diameter and 150 mm in length). The first column was Dowex-50W activated with 45 mL of 2 M HCl. The amino acids were trapped on the column while the mixture of sugars and organic acids passed through. This solution was then passed through a second column of Dowex-1 activated with 45 mL of 8 M formic acid. This column trapped the organic acids, and the sugars passed through (Davis and Daish, 1960). The final solution contains mainly sugars and was placed in a 50-mL volumetric flask.

The first column (Dowex-50W) was then washed with 45 mL of 2 M NH_4OH to elute the amino acids. The eluate, containing amino acids, was collected in a 50-mL volumetric flask for counting. The Dowex-1 column, containing the remaining organic acids, was washed with 45 mL of 8 N formic acid (Ting and Dugger, 1965). The eluate containing organic acids was collected in a 50-mL volumetric flask for counting.

(d) 24 PUK units of protease in 0.1 M Tris, pH 7.4, was added to the non-water-soluble pellet. The pellet (from step c) was resuspended by vortexing and was then digested overnight at 25 ± 2 °C. The sample was centrifuged, and the supernatant was collected in a 50-mL volumetric flask. The pellet was washed twice with two lots of 10 mL of distilled water, and the centrifuged washings were collected in the same flask as above for subsequent analysis (Nomoto et al., 1960). The solution containing digested protein was collected for subsequent analysis (counting).

(e) The pellet (from step d) was resuspended in 5 mL of distilled water and placed in a boiling water bath for 5 min. The sample was cooled, and 2 mL of 20% diastase (made in distilled water) was added and digested overnight at 25 ± 2 °C. The sample was centrifuged, and the supernatant was collected in a 50-mL volumetric flask. The pellet was washed twice with two lots of 10 mL of distilled water (Canvin and Beebers, 1961). The solution containing digestied starch was collected for subsequent analysis (counting).

(f) The pellet (from step e) was placed in a test tube, and 5 mL of 0.05 N HCl was added. The sample was heated to 100 °C for 1 h. The sample was centrifuged, and the supernatant was collected in a 50-mL volumetric flask. The pellet was washed twice with two lots of 10 mL of distilled water (Bean and Ordin, 1961). The solution containing broken down protoectins and noncellulose polysaccharides was collected for subsequent analysis (counting).

(g) The pellet (from step f) was extracted with 15 mL of sodium hypochlorite in 5 mL of water plus 0.2 mL of 12% acetic acid at 100 °C for 1 h. The sodium hypochlorite was prepared fresh. The sample was centrifuged, and the supernatant was collected in a 50-mL volumetric flask. The pellet was washed twice with 10 mL of distilled water (Bean and Ordin, 1961). The solution containing broken down lignins was collected for subsequent analysis (counting).

(h) The pellet (from step g) was extracted with 5 mL of 18% NaOH overnight at 25 ± 2 °C. The sample was centrifuged, and the supernatant was collected in a 50-mL volumetric flask. The pellet was washed with two lots of 10 mL of distilled water (Bean and Ordin, 1961). The solution containing broken down hemicelluloses was collected for subsequent analysis (counting).

(i) To obtain the remaining cellulose, the pellet (from step h) was extracted by acetylating with 5 mL of fresh acetylating reagent (glacial acetic acid, acetic anhydride, and concentrated sulfuric acid 1:1:0.05) at 100 °C for 30 min. The acetylating agent was freshly prepared. The sample was centrifuged, and the supernatant was collected in a 50-mL volumetric flask. The pellet was washed with two lots of 10 mL of distilled water (Bean and Ordin, 1961). The solution containing broken down cellulose was collected for subsequent analysis (counting).

(j) The remaining pellet was washed and resuspended with 45 mL of distilled water in a 50-mL volumetric flask for subsequent analysis.

Scintillation Counting. Liquid samples (2 mL each, collected from steps b−k) were mixed with the scintillation fluid (5 mL) in a scintillation vial and then placed in the dark. Precautions were taken to avoid complications due to photoluminescence. Four replicate samples were prepared, and each was counted 4 times and averaged. Quenching was determined on each fraction to allow correction for counting efficiency.

Analysis of Total Radioactive Residues. A scintillation counting method was used in order to get comparable results for total residues and residues contained in fractions. Samples were crushed, and 0.1 g was transferred to a scintillation vial containing 5 mL of scintillation fluid plus 0.5 mL of distilled water and 2 mL of acetone. They were then placed in the dark. The samples were counted at timed intervals. Samples were counted over a period of 5 days. Unfumigated samples of each commodity were used as controls. The levels of residue were calculated as COS equivalents. All samples were replicated 4 times.

Autoradiography. Commodity kernels treated with ^{14}COS were cut in half sections (cross and longitudinal). The sections were placed in contact with film (Fuji Medical X-ray Film) for 30 days at −75 °C. Control autoradiographs were carried out with each batch of a nonlabeled section on a film. After 30 days exposure, the sections were removed, and the film was developed.

Semiquantitative analysis of autoradiographs was carried out by serial dilutions, using ^{14}C-labeled sodium carbonate as a reference. Serial dilution was performed by adding 0.5 mL of labeled sodium carbonate (containing 0.0044 MBq/mL) in water to 0.5 mL of water. Each dilution (2 μL) was spotted on a TLC plate (Art. 5554, DC-Alufolien, Kieselgel 60 F_{254}) (3 replicates at each dilution) for taking autoradiographs under the conditions described above. Radioactivity was calculated as COS equivalents.

Preparation of Quenched Standards. In scintillation counting, quench correction was carried out by calibrating a series of progressively quenched standards with reference to an external standard of ^{14}COS in aqueous acetone. Samples were replicated 3 times, and each was counted 4 times and averaged. All radioactive residue data were converted/calculated from scintillation counting data by calibrating with the quenching standard curve.

RESULTS AND DISCUSSION

Method Evaluation. The quenching standard curve (Figure 2) was linear over the tested region. Counts per unit time were summed for each of the biochemical fractionts; these increased with the period of mixing with scintillation fluid, and the standard error between replicates decreased (Figure 3). Thus, the extraction of label from the biochemical fractions and glassware into the scintillation fluid was not instantaneous. The amount of extracted radiolabel from crushed grain also increased with extraction period, and the standard error decreased (Figure 3). In each case, results recorded were those taken after 5 days, as this was a time when the counts were at a maximum and the standard error was at a minimum.

Distribution and Uptake of ^{14}C-Containing Substances. The distribution of ^{14}C in the biochemical fractions of commodities after treatment with ^{14}COS is shown in Table 2. Amounts were calculated as nanomoles of COS equivalents per gram of commodity (g^{-1}). Residues of COS or alteration products (calculated as COS equivalents) were detected at low levels in (i) total lipids, 14.5−22.1 ng g^{-1} COS equiv; (ii) organic acids, 9.2−24.2 ng g^{-1} COS equiv; (iii) celluloses 2.4−4.8 ng g^{-1} COS equiv; and (iv) lignins (<1.0−2.1 ng g^{-1} COS equiv). The largest readings were in lipids and in organic acid fractions. They were undetectable in all commodities in the fractions of sugars, protein, starch, amino acids, protopectines, and hemicelluloses. Organic acids containing the ^{14}C label may be alteration products of COS, and formic acid is the most obvious candidate.

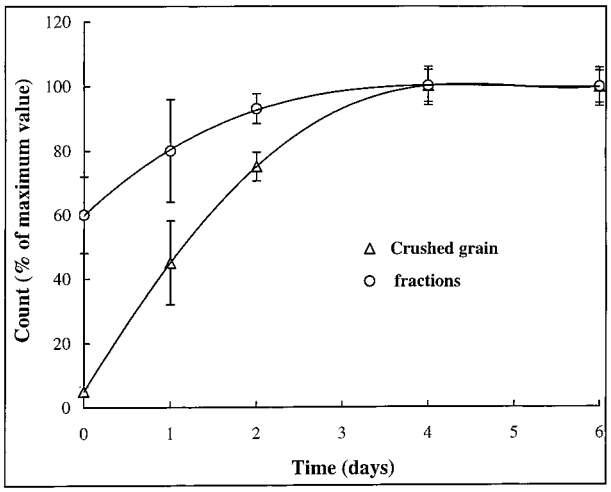

Figure 2. Counting data (% of maximum value) plotted against time (days) of sample standing in dark. After 4 days standing, the counting data from both fractions and crushed grains were stable and at maximum value.

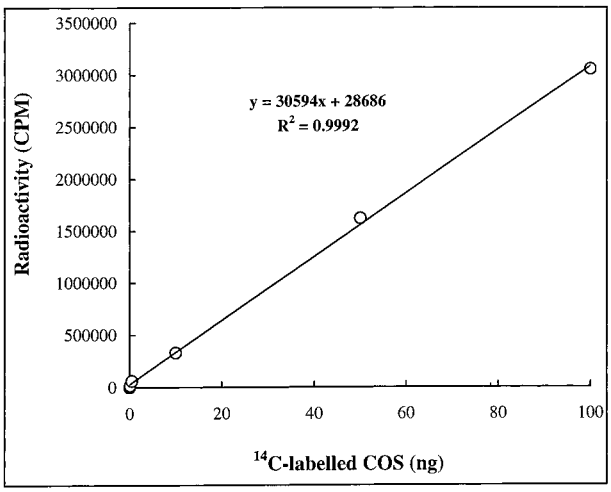

Figure 3. Quenching standard curve. Plots from scintillation counting data (CPM) against series of progressively added ^{14}COS (ng). Error bars indicate SD, $n = 3$.

The total uptakes of radioactive residues (calculated as COS equivalents) were determined in two ways. First, the COS equivalents in each fraction were summed. Second, the residue was calculated from the amount of radioactivity extracted from crushed grain. The second method involved determination of only one value, that of total radioactivity, whereas the former reading involved determination of radioactivity in each of 11 components. The cumulative error in the readings from the 11 fractions is greater than the error in determination of a single reading, although the mean values from each method of determination are similar (Figure 4). The method of evaluating residues in fractions gives information on the distribution of residues that is not obtained from an extraction/digestion in a single solvent. The total recovery from the fractions was similar to that from a single digestion.

Table 2. Radioactive Residues, Calculated as COS Equivalent, on Commodities after Exposure to ^{14}COS at 25 °C, 60 mg L^{-1} for 7 Days and 5 Days Aeration

biochemical fractions	radioactive residues, ng g^{-1} OS equiv, in				
	mungbean	wheat	polished rice	paddy rice	safflower
total lipids	19.7 ± 2.8	21.1 ± 3.1	14.5 ± 3.2	17.6 ± 2.3	22.1 ± 2.2
organic acids	9.2 ± 1.7	16.3 ± 2.3	24.2 ± 3.5	16.4 ± 2.8	17.3 ± 2.5
celluloses	2.4 ± 1.2	4.8 ± 1.8	3.2 ± 1.5	3.6 ± 1.3	4.2 ± 2.0
lignins	*a*	1.8 ± 0.8	*a*	2.0 ± 1.1	2.1 ± 1.0
protopectines	*a*	*a*	*a*	*a*	*a*
amino acids	*a*	*a*	*a*	*a*	*a*
sugars	*a*	*a*	*a*	*a*	*a*
protein	*a*	*a*	*a*	*a*	*a*
starch	*a*	*a*	*a*	*a*	*a*
hemicelluloses	*a*	*a*	*a*	*a*	*a*
residual	*a*	*a*	*a*	*a*	*a*
total amount[b]	30.6−38.7	43.8−51.0	42.0−49.8	39.6−53.4	45.6−52.8

[a] Below the limit of detection, which is 1.0 ng g^{-1} COS equiv. [b] The total amount is the sum of equivalents in each fraction. This sum includes 7−8 readings at below the detection limit.

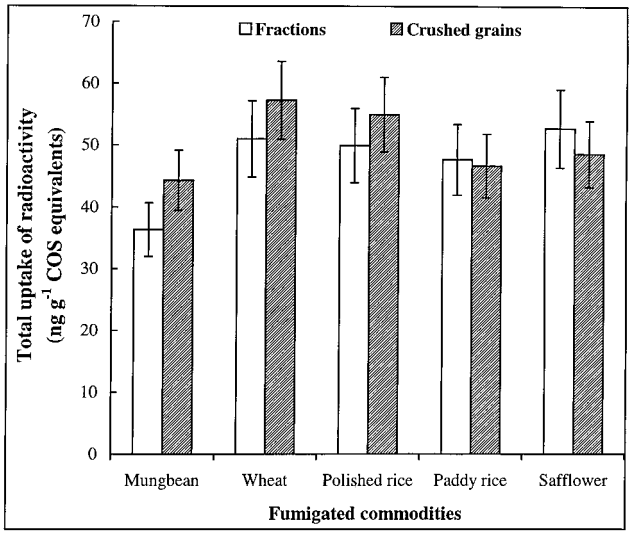

Figure 4. Total ^{14}C radioactive residues in fumigated commodities (crushed) and their fractions at 25 °C, 60 mg L^{-1} for 7 days exposure and 5 days aeration. Error bars indicate SD, $n = 4$.

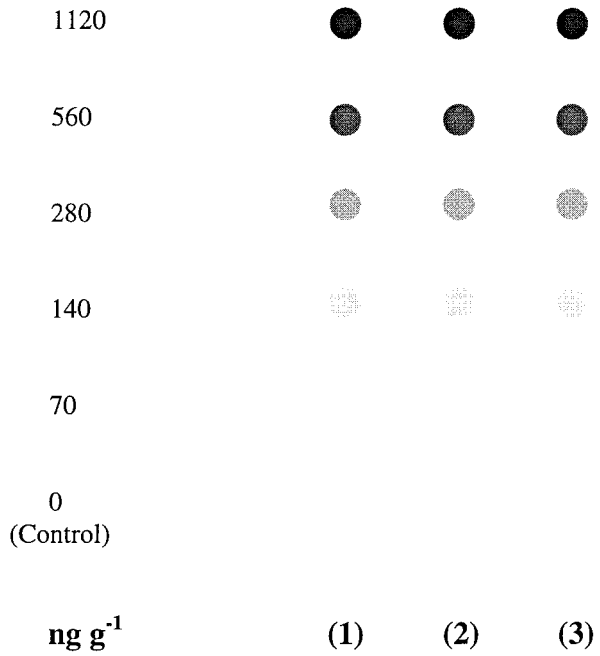

Figure 5. Schematic representation of a serial dilution autoradiograph to determine limits of detection under 30 days exposure at −75 °C.

Additional evidence of the uptake of ^{14}C by commodities was obtained from autoradiography. The method detected radioactivity down to a level equivalent to 70 ng g^{-1} COS equiv (Figure 5), but no radioactivity was detected in any autoradiograph. That is, the total uptake of radiolabel at the cut face was ≤70 ng g^{-1} COS equiv. Thus results from three methods for uptake of ^{14}C were similar.

The total amount of ^{14}COS used in fumigation was 90 mg (1.5 L × 60 mg L^{-1}), which was applied to 50 g of commodity (5 × 10 g). Thus, the maximum possible uptake of ^{14}C was 1.8 mg g^{-1} COS equiv. The total uptake of radioactivity was <70 ng g^{-1} COS equiv, that is, less than 1 part/30,000 of the total possible uptake. If all label were present as COS, this would represent a residue of approximately 70 ng g^{-1}. However, as the label is distributed among several biochemical fractions, the total amount of intact COS is much lower than this figure.

These results on low residues and alteration products are consistent with other evidence. For example, COS is less sorbed on grain than is MeBr and can be blown more easily through grain (Desmarchelier, 1994; Ren, 1997). Exposure to COS also resulted in no loss of germination (Ren et al., 1996) and no observed loss of thiamine, niacin, lysine, maltose, riboflavin, pyridoxine, or α-tocopherol (Ren, 1997). There were no irreversible reactions between COS and lipids in wheat germ oil and canola oil and no effect on total lipids (Ren et al., 1997). The reversible partitioning of COS into lipid (Ren et al., 1997) provides a useful insight into the behavior of residual COS on grain.

All these results provide an upper limit of COS residues. Because the experiments here were conducted with five different grains, if can be argued that this can be generally expected. In view of high natural COS levels, the radiolabeling demonstrates that only a very low fraction of additional residues can be expected from COS treatment.

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