Monitoring Natural Endocrine Disruptors in Water
and Pesticides in Conventional Cereal Products

by

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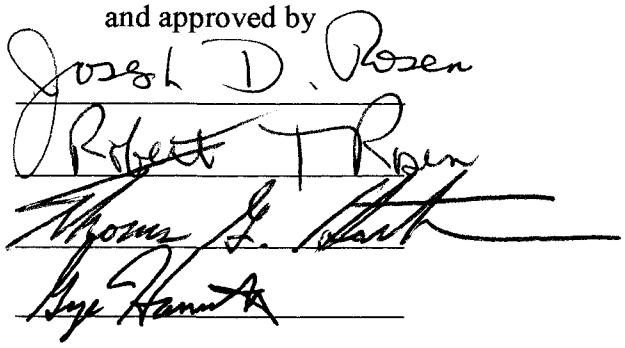

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ABSTRACT OF THE DISSERTATION

Monitoring Natural Endocrine Disruptors in Water
and Pesticides in Conventional Cereal Products

By Jae Hyock Kwak

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To determine the presence of sterols, suspected endocrine disruptors, in drinking and river water, a sensitive analytical method was developed. Sterols were extracted by solid-phase extraction (C_{18} disk) and determined by gas chromatography/ion trap mass spectrometry. Sterols were silylated to improve sensitivity and chromatography. The sensitivity was 10 parts per trillion (ng/L) and the recovery of cholesterol-d_5 from 1 L water (spiked at 0.5 ppb) was 93.1 ± 20.6%. Of 126 well water samples analyzed, 41 contained at least one of the following phytosterols: brassicasterol, 22-dehydrocholesterol, campesterol, stigmasterol, β-sitosterol and fucosterol, in concentrations ranging from 3 ng/L to 6.8 μg/L. Only 2 contained coprostanol (13 ng/L and 3.5 ng/L). No sterols were found in tap water. All river waters analyzed, contained cholesterol, coprostanol and phytosterols, in concentrations ranging from 3 ng/L to 2 μg/L.

In addition, another analytical method was developed to determine the concentrations of sterols in meat samples. Campesterol and sitosterol were detected in all samples analyzed, in the range of 65 – 448 ng/g and 17 – 2,222 ng/g meat, respectively. Except in chicken, coprostanol was found in all samples, ranging from 87 to 607 ng/g meat.

Commercially available sterols (cholesterol, campesterol, stigmasterol and sitosterol) and a river water extract were tested for endocrine disruption. They were positive for the test.

To determine the concentration of pesticides in conventional processed foods, analytical methods were developed. Pesticides were extracted with acetonitrile/water, cleaned up by liquid-liquid extractions and solid phase extractions, and analyzed by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). In GC/MS analysis, 65 percent of pesticides spiked in cereal at 100 ppb showed recoveries of 70-130%. Limits of detection in most pesticides ranged from less than 1 to 10 ppb. Some carbamate insecticides and phenylurea herbicides were monitored by LC/MS since they were thermally degraded during GC analysis. Methomyl, monuron, neburon and siduron had good recoveries, ranging from 70 to 121%. Methomyl, siduron and thiodicarb could be detected at less than 15 ppb, and others at less than 75 ppb in toasted oats. No pesticides were detected in 20 conventional cereals.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1. Presence of Sterols, Suspected Endocrine Disruptors, in Water

INTRODUCTION

The publication of the book in 1996, "Our Stolen Future", has caused a great deal of public concern that environmental chemicals, mainly PCBs, pesticides and plasticizers, raise developmental problems (decreased sperm counts in humans, changes in sex, etc) in wildlife and in humans. The authors concentrated on synthetic estrogen disruptors, but not on naturally occurring ones such as plant or animal sterols. β-Sitosterol and coprostanol are two such materials. The former is found in tree bark, while the latter is a metabolite of cholesterol found in contaminated water and sewage. In this study, the concentrations of several sterols were determined for their presence in river and drinking water.

BACKGROUND

There is growing concern that environmental chemicals act as hormones, disrupting endocrine systems in wildlife and in humans. These endocrine disruptors have been considered to mimic hormones and block hormone receptors, consequently causing hormone-activated cancers (breast and prostate cancer) and developmental problems (decreased sperm counts in humans, changes in sex) (Colborn *et al.*, 1996). PCBs, pesticides and plasticizers are well-known ones. In addition to synthetic chemicals, natural compounds, such as phytoestrogens, plant sterols and mycotoxins, have been claimed to be endocrine disruptors (National Research Council, 1999).

Wood is one of rich sources of plant sterols. Even though the concentrations of sterols in wood are less than 0.1 percent (Obst, 1998), when the sterols are released into rivers during paper manufacture, their concentrations in river are high enough to have biological effects to

aquatic animals. Paper mill effluents have been claimed to have harmful effects on fish, including reduced gonadal development, delayed sexual maturity, decreased levels of plasma sex steroids, and increased hepatic mixed-function oxygenase activity (Andersson *et al.*, 1988; Munkittrick *et al.*, 1992). In addition, the elongation of anal fin in female fish, a characteristic of masculinization, has been observed (Cody and Bortone, 1997; Bortone and Cody, 1999). Among the proposed chemicals causing this endocrine disruption to fish, sterols are the most probable with high concentration ranging from 71 to 535 μg/L in paper mill effluents (Cook *et al.*, 1997).

β-Sitosterol, a major plant sterol, has been claimed to be an endocrine disruptor. *In vitro* studies showed that β-sitosterol bound to fish estrogen receptor (Tremblay & Van Der Kraak, 1998) and to human estrogen receptor in yeast transformants (Gaido *et al.*, 1997). β-Sitosterol also induced cell proliferation in human breast cancer cells (Mellanen *et al.*, 1996; Mäkelä *et al.*, 1995). MacLatchy and Van Der Kraak (1995) reported that β-sitosterol reduced the levels of plasma sex steroids, such as testosterone and 17β-estradiol, in fish. They suggested that β-sitosterol might inhibit the enzyme that converts cholesterol to steroid hormones, or compete with cholesterol, the precursor of steroid hormones, interfering with the biosynthesis of steroids. In addition, β-sitosterol induced the production of vitellogenin (an egg yolk protein), an estrogen-dependent process, in immature and male fish (Mellanen *et al.*, 1996; Tremblay and Van Der Kraak, 1998). β-sitosterol also showed estrogenic activity in mammals. It stimulated uterine growth, an estrogen-dependent process, in mice (Elghamry and Hänsel, 1969) and in rats (Malini and Vanithakumari, 1993).

Coprostanol is a metabolite of cholesterol produced by intestinal microorganisms in mammals and it is found in contaminated water and sewage. It has been demonstrated as a possible indicator of fecal contamination in water and sediment (Walker *et al.*, 1982). Good correlations have been obtained between coprostanol levels and coliform counts from water samples near a sewage outfall in the Clyde Estuary in Scotland (r^2=0.9671; Goodfellow *et al.*,

1977), from sediment samples in Sydney, Australia (r^2=0.8831; Nichols *et al.*, 1993), and from water samples in the Ohio River (r^2=0.9743; Tabak *et al.*, 1972). Coprostanol also showed estrogenic activity in freshwater mussel, increasing the production of vitellins and inhibiting the binding of estradiol to receptor (Gagné *et al.*, 2001). The estrogenic activity of β-sitosterol and coprostanol may come from the structural similarity to estrogen (Figure 1).

Natural endocrine disruptors may be more serious than synthetic ones. Humans are exposed to high levels of natural endocrine disruptors from foods (Safe, 1995). In addition to foods, water could be a source of natural endocrine disruptors since water comes in contact with plant and animal feces. Thus, the levels of natural endocrine disruptors in water should be determined to examine the potential for human exposure from sterols in water.

OBJECTIVE

To determine the concentrations of plant and animal sterols, suspected endocrine disruptors, in river and drinking water, and additionally in foods in order to examine the potential for human exposure from water. This work will provide data for future work by epidemiologists.

HYPOTHESIS

Water could contain plant and animal sterols, suspected natural endocrine disruptors, since water comes in contact with plants and animal feces.

EXPERIMENTAL

1. Materials

1.1. Chemicals

Sterol standards, such as coprostanol, cholesterol, campesterol, stigmasterol, sitosterol, were purchased from Sigma (St. Louis, MO). Surrogates (cholesterol-2,2,4,4,6-d_5 and sitostanol-5,6,22,23-d_4) were obtained from Medical Isotopes, Inc. (Pelham, NH). The silylation agent, N,O-*bis*-(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane, was purchased from Sigma (St. Louis, MO). Chrysene-d_{12}, an internal standard, was obtained from Supelco (Bellefonte, PA). Organic solvents (HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA).

1.2. Solid Phase Extraction (SPE) Cartridges

Bakerbond Octadecyl (C-18) Speedisk (50 mm diameter) cartridges were purchased from J.T.Baker (Phillipsburg, NJ).

1.3. Samples

One hundred twenty six well water samples were collected between July 1, 1998 and May 28, 2002 and received from NJDEP. Five river water samples were collected from the Raritan River, in New Jersey between November, 1998 and January, 1999 and received from a water company. One river water sample (our own collection) was collected from the Raritan River on September 2, 2001. Food samples were obtained from dining halls in Rutgers University between April and May, 2001.

2. Instrumentation

2.1. GC/MS

Gas chromatography was carried out using a Varian (Walnut Creek, CA) 3400 gas chromatograph interfaced to a Finnigan MAT (San Jose, CA) Magnum ion trap mass spectrometer. Coprostanol and other sterols were chromatographed on DB-5 or DB-17 (Agilent Technologies, Wilmington, DE) fused silica capillary columns (30 m × 0.25 mm (i.d.), 0.25 μm film thickness). Helium was used as a carrier gas with 25 cm/s linear velocity. The injector was held at 260°C. Initially, the split valve was closed and then opened 30 seconds after the injection. After that, the valve kept open until each analysis finished. The DB-5 column was temperature-programmed from 150°C to 260°C at 15°C/min, and then to 269°C at 2.5°C/min, and then to 300°C at 3.8°C/min where it was held for 4 min. The DB-17 column was temperature-programmed from 150°C to 275°C at 15°C/min, and then to 300°C at 2.5°C/min where it was held for 4 min. The temperature of the transfer line between column and mass spectrometer was 280°C.

The mass spectrometer was operated by electron ionization (EI) mode and chemical ionization (CI) mode. The manifold temperature was 210°C. Emission current was 12 μA and multiplier voltage was 1900 V. Mass scan range was set at 213 to 487 amu at 0.5 sec/scan. The electron energy was 70eV for EI mode. In CI mode, methane was used as a reagent gas at a source pressure that gave a ratio of 1:1 for m/z 17 (CH_5^+) to m/z 29 ($C_2H_5^+$) and a ratio of 10:1 for m/z 17 to m/z 16 (CH_4^+).

3. Sample Preparation

3.1. Extraction of Sterols from Water Samples

Water sample (1L or 4L) was spiked with 20 μL of the surrogate solution (25 ng/μL cholesterol-2,2,4,4,6-d_5 in dichloromethane) and mixed thoroughly using an ultrasonic cleaner

(Fisher Scientific, Pittsburgh, PA). The sample was then poured into a Bakerbond Octadecyl (C-18) Speedisk (50 mm diameter) that was previously conditioned first with 15 mL acetonitrile and then with 15 mL distilled water. The disk was then washed with 30 mL of 20% acetonitrile/water solution and extracted first with 30 mL acetone and then with 30 mL dichloromethane. Water sample bottle was rinsed with 30 mL dichloromethane to extract remaining sterols inside the bottle. The rinsing was combined with the extracts from the disk. They were evaporated to near dryness using a rotary evaporator. The extracts were dissolved in 6 mL dichloromethane, and filtered through a nylon membrane syringe filter (0.2 μm, Pall Gelman), and evaporated under a gentle stream of nitrogen. Trimethylsilyl ether derivatives were prepared by adding 200 μL *bis*-trimethylsilyltrifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane to the extracts. After 15 minutes at 60°C, the reaction mixture was evaporated to dryness under the nitrogen stream, keeping it warm in order to minimize condensation. Fifty μL of the internal standard solution (8 ng/μL chrysene-d_{12}) and enough dichloromethane were added to bring a total volume to 250 μL. Two μL was injected into GC/MS system.

3.2. Extraction of Sterols from Food Samples

Extraction was based on the method of Bligh and Dyer (1959) and saponification procedure was based on AOCS Official Method Ca 6a-40 (1990). Five grams of meat sample were spiked with 250 μL of the surrogate solution (200 ng/μL sitostanol-5,6,22,23-d_4 in dichloromethane) and homogenized with 15 mL chloroform–methanol solution (v/v, 1:2) in a Waring blender for 2 minutes. Five mL chloroform was then added to the homogenate. After blending for 30 seconds, 5 mL of distilled water was added and blending continued for additional 30 seconds. The extract was filtered and the filtrate was transferred to a 125 mL separatory funnel. After allowing a few minutes for the phase separation, the lower phase

(chloroform fraction) was collected and evaporated using a rotary evaporator. The extract was then dissolved in 10 mL petroleum ether, and washed with distilled water to remove water-soluble materials. The petroleum ether solution was divided into 5 fractions. Four mL methanol was added to one out of five fractions and the resulting mixture was saponified with 2 mL of 50% potassium hydroxide solution in a cap tube at 70°C for 1 hour. The hydrolysate was then extracted with 2 mL petroleum ether 5 times. The petroleum ether extracts were combined, and transferred to a 60 mL separatory funnel, and washed with distilled water until emulsions were removed. After the evaporation of the extracts under a gentle stream of nitrogen, trimethylsilyl ether derivatives were prepared by adding 500 μL *bis*-trimethylsilyltrifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane to the extracts. After 30 minutes at 60°C, the reaction mixture was evaporated to dryness under the nitrogen stream, keeping it warm in order to minimize condensation. Fifty μL of the internal standard solution (10 ng/μL chrysene-d_{12}) and enough dichloromethane were added to bring a total volume to 500 μL. Two μL was injected into GC/MS system.

4. Identification and Quantitation

Sterols were identified by injecting sterol standards into GC/MS, comparing the retention times and mass spectra. Commercially unavailable sterols, such as 22-dehydrocholesterol, brassicastanol and fucosterol, were tentatively identified by comparing the relative retention times on gas chromatogram and the mass spectra of the literature from Jones and coworkers (1994).

The concentrations of sterols were calculated by the calibration curve. Coprostanol, cholesterol, campesterol, stigmasterol and sitosterol were prepared at concentration of 100 ng/μL in methylene chloride, respectively. A 2 ng/μL standard sterol solution was prepared by mixing and diluting the 100 ng/μL each sterol solution. 10, 25, 50, 100, 250, 500, 1000 and 1500 μL of

the 2 ng/μL standard sterol solution were taken into each vial. The sterol solutions then were derivatized, evaporated, and injected into GC/MS. A calibration curve was generated for each sterol compound by dividing the quantitation ion area of each sterol by the quantitaion ion area of the internal standard, chrysene-d_{12} (m/z 240), and by plotting each response factor against its corresponding amount in nanograms.

5. Recovery Studies

Recoveries were obtained from eight 1 L distilled waters spiked at 100 ng/L coprostanol and 500 ng/L cholesterol-2,2,4,4,6-d_5 levels. The spiked waters were mixed thoroughly using an ultrasonic cleaner (Fisher Scientific, Pittsburgh, PA) for 10 minutes prior to extraction. The waters were then prepared by the extraction procedure described before. Recovery was calculated by dividing the area ratio obtained from the spiked water by the ratio from the equivalent standard concentration. An additional 5 recoveries were obtained from 4 L distilled water spiked with 25 ng/L coprostanol and 125 ng/L cholesterol-d_5.

6. Sensitivity Determination

Limits of detection were defined as a peak area of 1,000 in the quantitation ion of each sterol. The area count of 1,000 is the lowest measurable limit. This value was calculated by the Finnigan MAT computer system. To increase the sensitivity, the mass scan range was set at 213 to 487 amu.

7. Estrogenic Activity of Sterols and River Water Sample

The estrogenic activity of sterols and a river water sample (our own collection) collected from the Raritan River on September 2, 2001, was evaluated by using the *Saccharomyces cerevisiae* strain PL3 (*ura*3-Δ1,*his*3-Δ200,*leu*2-Δ1,*trp1::3ERE-URA3*) (DiPaola *et al.*, 1998). Cells were seeded in 96-well plates in 190 μL of medium lacking uracil. 10 μL of serial dilutions

(200 μM, 20 μM, 2 μM and 200 nM in ethanol) of each sterol standard (cholesterol, campesterol, stigmasterol and sitosterol) were added to the well plates, and growth was examined for four days. A 10 μL out of a 1000 μL river water extract was also evaluated. The river water extract was prepared from 1 L river water collected from Raritan river, New Jersey, by using the solid phase extraction described before. The concentrations of sterols in the extract were also determined.

RESULTS

1. Derivatization

Sterols can be analyzed by gas chromatography without derivatization. In low concentration, however, they are hard to detect because of their polar hydroxyl group (Figure 2A). We supposed that sterol concentration would be very low because sterols are generally insoluble in water. Given this, a more sensitive detection method needed to be developed. When the hydroxyl group is converted into the trimethylsilyl group, sensitivity and chromatography are much improved. (Figure 2B). Such a conversion is illustrated in Figure 3: the hydroxyl group of coprostanol is reacted with a derivatizing agent such as trimethyl chlorosilane to form trimethylsilyl coprostanol (TMS-coprostanol). The major advantage of derivatization is a more symmetrical peak shape, which translates into better separation from other sterols and matrix impurities as well as improved quantification. The major disadvantage of derivatization is the introduction of an additional step that leads to a slower overall analysis time and an additional source of lower precision. In order to decide between the two possibilities, limit of detection (LOD) comparisons were made between derivatized and underivatized sterols. The LOD for underivatized coprostanol was 5 ng in the EI mode as compared to 0.2 ng for TMS-coprostanol. In the CI mode, the LOD for coprostanol was 10 ng while the LOD for the derivatized coprostanol was again 0.2 ng. Results for other sterols were similar. All samples were derivatized since a sensitive detection method was required in this study.

2. Mass Spectra of TMS-coprostanol and TMS-cholesterol

The EI mass spectrum of TMS-coprostanol is shown in Figure 4. The molecular ion of this material is 460 amu but is too unstable to be recorded. The peak at 370 m/z is due to loss of $HOSi(CH_3)_3$; the peak at 355 m/z is due to loss of an additional methyl group; the peak at 257

m/z is due to loss of the C_8H_{17} side chain; the peak at 215 m/z is due to cleavage of the five membered ring (loss of propylene). The EI mass spectrum of TMS-cholesterol is shown in Figure 5. In contrast to the coprostanol derivative, TMS-cholesterol does exhibit a molecular ion at m/z 458. The peak at 443 m/z is from loss of a methyl group; m/z 368 is due to loss of $HOSi(CH_3)_3$ from m/z 458; m/z 353 is loss of another methyl group from m/z 368. The ion at m/z 329 is presumed to arise from loss of octane side chain from the 443 ion. The peaks at m/z 255 and 213 are analogous to the TMS-coprostanol peaks at m/z 257 and 215. The retention times and mass spectral data of TMS-sterols are summarized in Table 1.

The CI mass spectrum of TMS-coprostanol is shown in Figure 6. Chemical ionization is much gentler than electron ionization, resulting in less fragmentation. In the case of TMS-coprostanol, the expected protonated molecular ion at m/z 461 is not seen; instead there is a small ion at m/z 459. We believe that this ion is formed from the protonated molecular ion by loss of hydrogen gas. In any event, the m/z 459 ion loses $HOSi(CH_3)_3$ to give the only large peak, m/z 369. TMS-cholesterol (Figure 7) has a similar mass spectrum, two mass units lower.

The advantage of EI over CI is that better analytical precision can be achieved. The advantage of CI over EI is less interference from other chemicals so that sensitivity for environmental samples may be higher if analyses are conducted in the CI mode. Limits of detection were compared for TMS-coprostanol in both modes and found to be same (0.2 ng on column). In this study, EI was mainly used for the determination of sterols.

3. Mass Chromatography

Mass chromatography is a computer technique that is used for quantification and enhancement of sensitivity. The effluents from the gas chromatograph are converted to ions in the mass spectrometer where they are scanned from 60 to 500 amu. All this data is stored in the computer and recalled. A plot of the ions formed versus retention time can be printed out as shown in the lowest chromatogram of Figure 8. This is called a total ion chromatogram that is a

typical gas chromatogram in GC/MS. Instead of detection being due to flame ionization or electron capture, it is the result of ions generated in the mass spectrometer. In addition to measuring all the ions, the computer can be instructed to print out the whereabouts of only those ions of interest (quantitation ions) to the analyst. These are called mass chromatograms. In the example shown in Figure 8, we have instructed the computer to print out (in addition to the total ion chromatogram) only three of the major EI ions of TMS-cholesterol (m/z 368, 353 and 458) and two of the major EI ions of TMS-coprostanol (m/z 370 and 257).

Mass chromatography provides two advantages in quantitation over other detectors. One is sensitivity. As shown in Figure 9, it is hard to see the peak of coprostanol in total chromatogram at 100 parts per trillion spiked level. But in mass chromatogram by plotting the base peak (quantitation ion) of coprostanol only, coprostanol is clearly detected. The other advantage is the quantitation of compounds having similar retention times on gas chromatogram. Cholesterol and cholesterol-d_5 cannot be quantified due to the similar retention times in total ion chromatogram (Figure 10). However, they can be quantified by using mass chromatogram by plotting base peaks of cholesterol and cholesterol-d_5 (Figure 10). The quantitation ion for each sterol is summarized in Table 1.

4. Surrogate and Internal Standard

A surrogate was used to correct for losses of analyte during the sample preparation. The surrogate and analyte should be very similar in chemical structure and there should be no chance that the surrogate will be found in nature. Cholesterol-2,2,4,4,6-d_5 is ideal for this purpose.

An internal standard is used to correct for the changes in run to run sensitivity of the GC/MS system. It is added to the analyte just before the GC/MS determination. Quantification of the analyte is determined from the ratio of the area counts obtained from the analyte divided by the area counts obtained from the known amount of internal standard. The internal standard must have (in addition to a low or zero chance of being found in water and food) a retention time

close to that of the analyte and must exhibit good chromatographic behavior (sharp peaks, no tailing) on the analytical column chosen for the analysis. Chrysene-d_{12} was chosen for the sterol analysis.

5. Extraction of Sterols from Water Samples

Liquid-liquid extraction has been used to extract organic compounds from water. Organic compounds, being less polar than water, would preferentially associate with the organic solvent. The solvent and the compounds would then be separated from the water. While effective, this method requires large amounts of organic solvents (that have to be disposed of) and is time-consuming.

Solid Phase Extraction (SPE) is a technique by which water is passed through adsorbent where the analytes of interest bind while allowing the water to pass through. Of the several SPE adsorbents evaluated, octadecyl (C_{18}) was best. We supposed that sterol concentration would be trace because sterols are generally insoluble in water. In order to detect at trace level, large volumes of water would have to be analyzed. Thus, instead of the usual SPE mini-column, the J.T. Baker Speedisk™ that has the capacity to absorb analytes from large quantities of water using a flow rate of 200 mL per minute at 25" Hg, was used. After passing water sample through the disk, the disk was washed with 30 mL of 20% acetonitrile/water solution to remove polar compounds. Acetone and dichloromethane were used to elute sterols from the disk since they are evaporated easily and rapidly. The method developed is simple and fast, and handles large amounts of water, and uses small amounts of organic solvents.

6. Recovery and Sensitivity

While the recovery of coprostanol in 1L water was 79.2% at 100 ppt level with 19.17% of coefficient of variation, the recovery in 4L water (at 25 ppt level) was reduced to 57.0% with 22.98% of coefficient of variation. Recoveries of cholesterol-d_5 were 93.1% in 1L water (at 100

ppt level) and 49.6% in 4L water (at 25 ppt level). The coefficients of variation were 22.15% and 20.36%, respectively.

The analyticl method of sterols is quite sensitive. Figure 11 shows the mass chromatogram at m/z 370, the base peak of coprostanol, obtained from analysis of a 0.1 ppb spike using a 1L sample. Since area counts of 1,000 are measurable, we calculate that 0.0116 ppb can be detected. When a 4 L water spiked at 25 ppt was extracted (Figure 12), an area of 13,391 counts was obtained for the mass chromatogram at m/z 370, resulting in a sensitivity of 2 parts per trillion.

7. Analysis of Sterols in Water Samples

Of the 126 well water samples analyzed, 41 contained sterols. Cholesterol was found in 40 well water samples in the range of 3.1 – 295 ng/L; β-sitosterol in 39 well water samples in the range of 3.5 ng/L – 6.8 μg/L; stigmasterol in 32 water samples in the range of 5 ng/L – 4.2 μg /L; campesterol in 31 water samples in the range of 3.0 – 112 ng/L. Other sterols, such as brassicasterol, 24-ethylcoprostanol, 22-dehydrocholesterol, fucosterol, were also detected. Coprostanol, however, was found in only two samples (Newton [collected 7/12/00] at a concentration of 13 ng/L and Good Sheppard (a church well in Fort Lee, New Jersey) [collected 2/21/01] at a concentration of 3.5 ng/L), which indicated that fecal contamination was rarely found in New Jersey' wells. Figure 13 shows sterols detected from Good Sheppard (2/21/01) sample. Results of the sample analyses are tabulated in Table 2. Table 2 also contains a compilation of recovery data based on the surrogate compound, cholesterol-d_5. The average recovery was 65.74 ± 31.4%. No sterols were found in treated well water.

Six river water samples collected from Raritan river, were analyzed for sterols. All samples contained coprostanol and a variety of phytosterols (Table 3). Coprostanol ranged from 3 to 123 ng/L. Cholesterol was detected in the range of 0.4 – 1.7 μg/L. β-sitosterol ranged from 152 to 1996 ng/L. Other sterols, such as stigmasterol, campesterol, brassicasterol, 24-

ethylcoprostanol, 22-dehydrocholesterol, fucosterol, were also detected. Figure 14 shows sterols detected from a sample collected from the Raritan river on September 2, 2001.

8. Analysis of Sterols in Food Samples

Five meat samples (ham, meat loaf, BBQ chicken, beef stew and Italian meatball) were analyzed for sterols. Sterols in food samples were extracted by the method of Bligh and Dyer (1959) that has been used as a simple and reproducible procedure for lipid extraction. Heat was not involved during the extraction, preventing deterioration of lipids. Saponification step was added to extract esterified sterols.

The concentrations of sterols in food samples are tabulated in Table 4. All food samples analyzed contained cholesterol, campesterol and sitosterol. Cholesterol was not quantified since the levels of cholesterol were out of the detection range, but it was estimated at milligram levels per gram of meat. Campesterol was detected in the range of 65 – 448 ng/g meat. Sitosterol ranged from 17 to 2,222 ng/g meat. Coprostanol was found in the range of 87 – 607 ng/g meat, except in BBQ chicken where it was not found. Lanosterol, a precursor of cholesterol, was detected, but not in ham. Figure 15 shows sterols detected from an Italian meatball collected from Rutgers university dining hall on May 10, 2001. Table 4 also contains a compilation of recovery data based on the surrogate compound, sitostanol-5,6,22,23-d_4 at the level of 10 μg/g meat. The average recovery was 38.2 ± 11.1%.

9. Estrogenic Activity of Sterols and River Water Sample

Ten μL of each 200 nM ethanol solution of four commercially available sterols (corresponded to 772 pg cholesterol, 800 pg campesterol, 824 pg stigmasterol and 828 pg sitosterol) and a 10 μL Raritan River water extract (our own collection) that contained 0.42 ng coprostanol, 13 ng cholesterol, 3 ng campesterol, 2 ng stigmasterol and 8 ng sitosterol, were positive for estrogenic activity, using *S. cerevisiae* strain PL3 in a transcriptional activation

assay [DiPaola *et al.* (1998)]. This strain carries a URA3 gene whose expression is induced by the activation of the human estrogen receptor by a ligand. The assay can also distinguish between binding to alpha and beta estrogen receptors. Binding to the β-, but not to the α-receptor was observed, and it appeared to be very weak when compared to that of 17β-estradiol (which also bound very strongly to the α-receptor). This growth-based assay is not quantitative. To evaluate relative activity between 17β-estradiol and sterols, further serial dilutions will be necessary until no growth is detected.

DISCUSSION

We are always exposed to natural endocrine disruptors from environment and food. Our results showed that more than 25% of New Jersey's well waters and all river water analyzed, were contaminated with a variety of sterols, suspected to be endocrine disruptors. The levels ranged from parts per trillion to parts per billion. On the other hand, according to our pesticide monitoring results (unpublished) in Raritan river for 6 years, synthetic endocrine disruptors, such as vinclozolin, dieldrin, DDT and methoxychlor, have not been detected. Several herbicides, such as atrazine and metolachlor, have been found in the river at parts per trillion levels during the spring and summer. The exposure of sterols from foods is much greater than from waters. Our results showed that meat samples analyzed, contained sterols at parts per billion levels.

Natural endocrine disruptors could be more powerful than synthetic ones. β-Sitosterol has 40 time higher relative binding activity to estrogen receptor than DDT, and 20 times higher than methoxychlor (Gaido *et al.*, 1997).

New Jersey has 400,000 private wells serving 1 million people (New Jersey Department of Environmental Protection). Disinfection is required in community wells, but not in private wells. People who drink well water without filtering ingest sterols, suspected endocrine disruptors.

Chapter 2. Determination of Pesticide Residues in Processed Conventional Foods

INTRODUCTION

The increased consumers' demand for organic food is partially caused by the public fear of pesticides. Many consumers are willing to pay higher price for organic food. Numerous comparative studies have been carried out in Europe, reporting that no difference has been observed in quality, and that organic foods are not completely free of pesticide residues (Woese *et al.*, 1997). In the U.S., however, only a few reports have been published. According to a study funded by Consumers Union (Baker *et al.*, 2002), 27 percent of organic products and 79 percent of conventional products had pesticides. However, the levels of pesticides detected in both types of foods were all below the federal limits. The critical problem of the study was that they intentionally chose samples (apples, peaches, green peppers and tomatoes) where pesticides have been found more often than in other foodstuffs. In this study, therefore, pesticides were monitored from other foodstuffs, such as processed foods.

BACKGROUND

The sales of organic foods have been increased from $847 million in 1991 to $7.8 billion in 2000 (Economic Research Service/USDA, 2001), growing 20 percent to 25 percent each year since 1990 (Murphy, 1997). The growth has, in part, leaned on the public fear of pesticides. Many consumers are willing to pay higher price for organic food, believing that organic products are healthier and safer than conventional ones.

'Organic' is a labeling term that indicates products produced under the **Federal Organic Foods Production Act of 1990**. The **Organic Foods Production Act** states that to be considered as "an organically produced agricultural product," the product should be raised and

handled without synthetic chemicals such as synthetic pesticides or fertilizers. The Act authorized USDA National Organic Program to set up national standards for organic agricultural products and the **final national organic standards rule** was announced on Dec. 20, 2000. The rule clarifies organic labeling criteria and forbids the use of synthetic fertilizers and pesticides, sewer-sludge fertilizers, growth hormones, antibiotics, genetically modified organisms and irradiation (Federal Register, Dec. 21, 2000).

In Europe, numerous comparative studies on organic and conventional foods have been reported concerning food quality and pesticide residues. There is an extensive review that covers 150 comparative studies on a wide variety of foodstuffs (Woese *et al.*, 1997). In the U.S., however, only few reports have been published. In nutritional and sensory quality, both European and American studies showed no consistent difference to be observed (Consumers Union, 1998 and Woese *et al.*, 1997).

In terms of pesticide residues, both studies demonstrated that organic products were not completely free of pesticide residues. Pesticides occasionally show up on organic food. However, there is a trend towards lower levels of pesticides in organic fruits and vegetables, according to the extensive European review (Woese *et al.*, 1997). The levels of pesticides detected from both organic and conventional samples were almost all below the federal limits.

The presence of pesticide residues in organic food may result from pesticide drift and environmental contamination. When pesticides are applied, only 10 to 15 percent hits on the target organisms and the rest is spread into air, soil and water (Hurst *et al.*, 1991). Organochlorine pesticides, one of persistent organic pollutants, have been a serious problem in environmental contamination. They do not easily break down in the environment and have harmful effects on nature. These compounds are long lasting and disperse through the environment. Therefore, they are detectable even now in fruit and vegetables, even though they

have not been used for many years. Unless a farm has been practicing organic methods through the 1940's and 1960's, it is unlikely that its produce will be free of organochlorine pesticides.

The comparative study regarding pesticide residues funded by Consumers Union were quite surprising, reporting that 27 percent of the organic products and 79 percent of the conventional products had traces of pesticides (Baker *et al.*, 2002). This doesn't mean that any of the produce is unsafe. Again, the levels of pesticides detected from both samples were all below the federal limits. In the levels of residues detected from both types of samples, actually, there is no big difference at all, as shown in Table 5.

The critical problem of the study was that they intentionally chose samples (apples, peaches, green peppers and tomatoes) where pesticides have been found more often than in other foodstuffs. According to the results of FDA pesticide regulatory monitoring program (1996-1999, Table 6), 67% of apples, 74% of peaches, 43% of peppers and 46% of tomatoes contained pesticide residues. On the other hand, the percentages of positive residues in sweet corn, onions, broccoli, watermelon, cabbage and peas were 1, 10, 11, 11, 12 and 15, respectively.

Table 5. Levels of pesticide residues detected from organic and conventional samples (Baker *et al.*, 2002)

Pesticides	**Crop**	**Mean of Positives (ppm)**		**EPA Tolerance Level (ppm)**
		Organic	**Conventional**	
Azinphos methyl	Apple	0.032	0.103	1.5
Benomyl	Apple	0.078	0.076	7
Benomyl	Peach	0.051	0.067	15
Benomyl	Tomato	0.064	0.078	5
Carbaryl	Apple	0.029	0.055	10
Phosmet	Peach	3.3	0.63	10
Thiabendazole	Apple	0.042	0.804	10

In this study, therefore, pesticides were monitored from other foodstuffs, such as processed foods in order to allow consumers to assess the health benefits derived from organic foods.

Table 6. Results of FDA pesticide monitoring in domestic food samples (1996-1999)

Foods	Total Samples	Samples with Residues	Percent of Positives
Sweet corn	391	3	1
Onions	135	13	10
Broccoli	87	10	11
Watermelon	192	22	11
Cabbage	248	31	12
Peas	292	45	15
Peppers, sweet	234	101	43
Tomatoes	608	277	46
Apples	813	541	67
Peaches	565	420	74

OBJECTIVE

To determine the concentration of pesticides in processed conventional foods in order to allow consumers to assess the health benefits derived from organic foods.

EXPERIMENTAL

1. Materials

1.1. Chemicals

Pesticide standards were obtained from Chem Service (West Chester, PA) and Polyscience (Niles, IL). Acenaphthene-d_{10}, naphthalene-d_8 and phenanthrene-d_{10} were purchased from Supelco (Bellefonte, PA). Atrazine-d_5 was obtained from Cambridge Isotope Laboratories (Andover, MA). HPLC grade organic solvents were purchased from Fisher Scientific (Springfield, NJ) and EM Science (Gibbstown, NJ).

1.2. Solid Phase Extraction (SPE) Cartridges

Bakerbond SPE™ Amino (NH_2) (1000 mg, 6 mL) cartridges were purchased from J.T.Baker (Phillipsburg, NJ). CarboPrep™ 90 (500 mg, 6mL) cartridges were obtained from Restek corporation (Bellefonte, PA).

1.3. Samples

Corn flakes and toasted oats were purchased from local supermarkets. The samples used for recovery studies were determined to be free of pesticides.

2. Instrumentation

2.1. GC/MS

Gas chromatography was carried out using a Varian (Walnut Creek, CA) 3400 gas chromatograph interfaced to a Finnigan MAT (San Jose, CA) Magnum ion trap mass spectrometer. Pesticides were chromatographed on DB-5 (Agilent Technologies, Wilmington, DE) fused silica capillary columns (30 m x 0.25 mm (i.d.), 0.25 μm film thickness). Helium was used as a carrier gas with 25 cm/s linear velocity. The injector was held at 260°C. Initially the

split valve was closed and then opened 30 seconds after the injection. After that, the valve kept open until each analysis finished. The column temperature was held initially 60°C for 2 minutes and increased to 260°C at 6°C/min where it was held for 12 min. The temperature of the transfer line between column and mass spectrometer was 260°C.

The mass spectrometer was operated in the chemical ionization (CI) mode. Methane was used as a CI reagent gas at a source pressure that was tuned to a ratio of 1:1 for m/z 17 (CH_5^+) to m/z 29 ($C_2H_5^+$) and a ratio of 10:1 for m/z 17 to m/z 16 (CH_4^+). The manifold temperature was 210°C. Emission voltage and current were 1900 V and 12 μA, respectively. The masses were scanned from 70 to 450 amu at 1 sec/scan.

2.2. LC/MS

A Shimadzu SCL-10A HPLC System (Shimadzu Co, Kyoto, Japan) interfaced to a Quattro LC tandem quadrupole mass spectrometer (Micromass UK Limited, Manchester, United Kingdom) was employed. The analyses and quantification were carried out by Masslynx 4.0 XP workstation. The separation was performed on a Supelco (Bellefonte, PA) LC-18 (250mm x 2.1mm, 5 μ particle size) column. The mobile phase was initially 100% water. After 5 minutes, it was programmed linearly to 30% acetonitrile (v/v, in water) at 15 minutes, 50% acetonitrile at 25 minutes and to 100% acetonitrile at 30 minutes. After that, it was isocratically held for 5 minutes. The flow rate was 0.3 mL/min.

The mass spectrometer was operated in electrospray ionization (ESI) positive ion mode. The source and the desolvation temperature were held at 140 and 300°C, respectively. Nitrogen was used as a desolvation gas and the flow was 350 L/hr. The capillary and cone voltage were set at 4 kV and 20V, respectively. MS/MS experiments were performed by multiple reaction monitoring (MRM) mode using argon as a collision gas. The collision energy was adapted for each pesticide.

3. Sample Preparation

Sample preparation was based on Jin (1999) and FDA Pesticide Analytical Manual 302 E4 (1994). Ten grams of ground samples were spiked with 50 μL of the surrogate solution (50 ng/μL atrazine-d_5 in acetone) and extracted with 200mL of 70% acetonitrile (v/v, in water) in an ultrasonic cleaner (Fisher Scientific, Pittsburgh, PA) for 15 minutes. The extract was then filtered and 4 g sodium chloride was added for the phase separation. The upper organic phase was collected and evaporated. The extract was then transferred to a 250 mL separatory funnel and 50 mL of 65% acetone (v/v, in water) and 0.5 g sodium chloride were added. After mixing them, 50 mL dichloromethane and 50 mL petroleum ether were added. They were followed by liquid-liquid extraction. The lower aqueous phase was followed by another liquid-liquid extraction with 40 mL dichloromethane. The upper organic phase from the 1st liquid-liquid extraction and the lower organic phase from the 2nd liquid-liquid extraction were combined and evaporated. The dried extract was dissolved in 25 mL acetonitrile and was passed through an amino cartridge (1g, 6mL, J.T.Baker, South Plainfield, NJ) that had previously been conditioned with 6mL of acetonitrile. The 'non-retaining' solution was collected and evaporated. The dried solution was then dissolved in 35 mL of 12% acetonitrile (v/v, in water) and was passed through a CarboPrep™ 90 cartridge (500mg, 6mL, Restek, Bellefonte, PA). The carbon cartridge had previously been conditioned first with 6mL of acetonitrile and then with 6mL of water. The carbon cartridge was then eluted with 4mL acetonitrile and then 8 mL of acetonitrile/toluene (3:1). The eluates were collected and evaporated. For the GC/MS analysis, 50 μL of the internal standard solution (40 ng/μL of acenaphthene-d_{10}, naphthalene-d_8 and phenanthrene-d_{10}) and enough acetone were added to the extract to bring a total volume to 500 μL. Two μL was injected into the GC/MS system. For the LC/MS analysis, acetonitrile was used to bring the total volume instead of acetone. Ten μL was injected into the LC/MS system.

4. Identification and Sensitivity Determination

Pesticides were identified by injecting pesticide standards into GC/MS and LC/MS, comparing the retention times and mass spectra. Limits of detection were defined as the computer-calculated signal to noise (S/N) ratio of 3 in the quantitation ions of each pesticide at 100 ppb fortification level in each food item in the GC/MS analysis. The signal to noise (S/N) ratio of 3 is the lowest measurable limit. In LC/MS analysis, limits of detections were estimated from the calculated signal to noise ratio of 5 in MRM determination of each pesticide at 50 ppb fortification level.

5. Recovery Studies

Recoveries were performed at 100 ppb (1 μg/10 g) fortification levels for both GC/MS and LC/MS analyses in three different cereal samples. The samples were prepared by the extraction procedure described before. Recovery was calculated by dividing the area ratio obtained from the spiked samples by the ratio from the equivalent standard concentration.

RESULTS AND DISCUSSION

1. Pesticide Analysis by GC/MS

The retention time and quantitation ion(s) for each pesticide and internal standards are given in Table 7. Chemical ionization (CI) was used instead of electron impact ionization (EI). Since electron impact ionization uses a higher energy (70eV) than chemical ionization does, it may lose molecular ion of each analyte and have extensive fragmentation. Chemical ionization, on the other hand, is a mild ionization technique and produces only several ions, mainly M+1 ion, for each analyte. It, therefore, has less interference from matrix and reaches the desired sensitivity (Mattern *et al.*, 1990). According to Mogadati *et al.* (1999), the combination of chemical ionization mass spectrometer and capillary column retention time of gas chromatogram, is often convincing enough in validating the presence of an analyte, even though CI mass spectra are much less definitive than EI mass spectra.

The recoveries of pesticides in cereals at 100 ppb level are presented in Table 8. Sixty-three percent of pesticides spiked in corn flakes at 100 ppb showed recoveries of 70-130% and 83% of the pesticides gave percent coefficients of variation between 2.2 and 20%. In toasted oats spiked at 100 ppb, 66% of pesticides were recovered in the 70-130% range and 68% showed percent coefficient of variation between 0 and 20%. As a group, triazine herbicides exhibited higher recoveries than other pesticides. Of the 10 triazines in this study, 9 triazines were recovered in the 70-109% range. Figure 16 shows mass chromatograms of the triazines recovered from a toasted oat spiked at 100 ppb. Mass chromatograms of organochlorine and carbamate insecticides recovered from a toasted oat spiked at 100 ppb are given in Figure 17 and 18, respectively. Figure 19 shows mass chromatograms of organophosphate insecticides recovered from a corn flake at 100 ppb spike levels.

Estimated limits of detection of the pesticides (at a signal to noise = 3) are also presented in Table 8. Of the eighty-seven pesticides, 18% could be detected at less than 1 ppb and 74% at less than 10 ppb in corn flakes. The sensitivity was even much higher in toasted oats than in corn flakes. In toasted oats, 38% could be detected at less than 1 ppb, and 92% at less than 10 ppb.

The use of GC/MS and the sample cleanup procedure resulted in obtaining the low limits of detection. By the sample preparation procedure, we were able to remove interfering materials from sample matrix. For the extraction of pesticides, 70% acetonitrile (v/v, in water) was chosen in order to avoid extracting very non-polar compounds, such as lipids. The purposes of adding sodium chloride were to separate phases into acetonitrile and water, and to help phase-separation between organic and aqueous phase easily. An amino cartridge was used as a sacrificial cartridge to remove polar interferences. We suppose that some polar pesticides may be lost in this step.

Dichlobenil and butylate had poor recoveries from cereal samples, but they were detectable at less than 6 ppb. Phenamiphos, phosdrin and tebuthiuron could not be quantified due to tailing peak or overlapping with the interferences from sample matrix. Endosulfan sulfate and bromacil in toasted oats were not quantified for the same reason. Most of them, however, were detectable at less than 10 ppb. Trichlorfon was not recovered, but dichlorovos, a breakdown product, was detected.

2. Pesticide Analysis by LC/MS

Some carbamate pesticides (aldicarb sulfone, aldicarb sulfoxide, methomyl, oxamyl and thiodicarb) and some phenylurea herbicides (monuron, neburon and siduron), are thermally degraded during GC analysis and monitored by LC/MS. Table 9 summarizes molecular weights, base peaks, collision energies and MRM transitions of the 9 pesticides and atrazine-d_5, an internal standard. Base peaks were all protonated molecular ions except oxamyl and aldicarb sulfone that had each ammonium adduct $[M+NH_4]^+$. Aldicarb sulfone and oxamyl were not well

separated under our HPLC conditions. They, however, can be quantified by using mass chromatography.

The SIM and MRM mass chromatograms for a toasted oat sample spiked with pesticides at 100 ppb level, are shown in Figure 20 and Figure 21, respectively. Multiple reaction monitoring (MRM) was used to increase sensitivity and to remove interfering peaks from sample matrix. The signal to noise ratio advantage of MRM over SIM is quite clear.

The recoveries of pesticides in toasted oats at 100 ppb level are presented in Table 10. Methomyl, monuron, neburon and siduron had good recoveries, ranging from 70 to 121%. Coefficients of variation (CV) were ranged from 8 to 24% except aldicarb sulfone and oxamyl that had very low recoveries. Estimated limits of detection of the pesticides (at a signal to noise = 5) are also presented in Table 10. Methomyl, siduron and thiodicarb could be detected at less than 15 ppb, and others at less than 75 ppb in toasted oats.

3. Sample Analysis

No pesticides were detected in 20 conventional cereals analyzed in this study. Our results indicate, at least in this limited study, that conventional cereals have no pesticide residues, or otherwise the residue levels are far below the EPA tolerance levels. Similar results have been observed by FDA pesticide regulatory monitoring program that is far more extensive survey. Actually in most conventional foods, the residue levels are far below the EPA tolerance levels.

CONCLUSION

To determine the presence of sterols, 126 well water samples, 6 river water samples, and 5 food samples were analyzed. Coprostanol was found in only 2 wells, which indicated that fecal contamination was rarely found in well water samples in New Jersey. However, more than 25% of the wells contained phytosterols, in concentrations ranging from 3 ng/L to 6.8 μg/L. Sterols were found in the river water samples at parts per trillion levels. The concentrations of sterols in foods are much higher with parts per billion levels. Our results have demonstrated that people are always exposed to sterols, suspected endocrine disruptors from water and food. Including ours, many *In Vitro* studies have demonstrated the endocrine disruption activity of sterols. Therefore, more researches will be needed to determine the *in vivo* effects of sterols in animals.

In order to assess the health benefits derived from organic foods, the concentrations of pesticides in processed conventional foods were determined. No pesticides were detected in 20 conventional cereals. Our results indicate, at least in this limited study, that conventional cereals have no pesticide residues, or otherwise the residue levels are far below the EPA tolerance levels. Similar results have been observed by FDA pesticide regulatory monitoring program that is far more extensive survey. Actually in most conventional foods, the residue levels are far below the EPA tolerance levels.

Table 1. Retention time and mass spectral data of TMS-sterols

Sterols	Retention Time (min)		Mass spectral data	
	DB-5	DB-17	Quantitation Ions (m/z)	Other major ion fragments (m/z)
Coprostanol	18:08	13:35	370	355, 257, 215
Epicoprostanol	18:40	13:51	370	355, 257, 215
cis-22-dehydrocholesterol*	19:02		366	456, 441, 351, 327, 255
trans-22-dehydrocholesterol*	19:24	14:48	366	456, 441, 351, 327, 255
Cholesterol	20:14	15:03	368	458
Cholesterol-d_5	20:22	15:08	373	463
Brassicastanol*	21:07	15:47	380	470, 455, 365, 255
Campesterol	22:35	16:33	382	472, 367, 343, 255
Stigmasterol	23:15	16:57	394	484, 379, 355, 255
Sitosterol	24:40	17:44	396	381, 357, 255
Fucosterol*	25:10	18:07	386	484, 371, 355, 296, 281, 257

* : tentatively identified

Table 2. Analytical results for well water samples

Sample (Date Received)	Sample Volume (L)	Sample Preparation Date	GC/MS Analysis Date	Recovery (%)	Sterols detected (ng/L)
Beverly #15 (6/22/99)	1	7/2/99	8/24/99	90	
Beverly #32 (6/22/99)	1	7/2/99	8/24/99	115	
South River Infiltration Gallary (6/23/99)	1	7/13/99	8/24/99	134	Cholesterol : 171 Stigmasterol : 155 Campesterol : 45 Sitosterol : 924 Fucosterol : ca 36
South River #2 (6/23/99)	1	7/13/99	8/24/99	37	Cholesterol : 163 Stigmasterol : 201 Campesterol : 47 Sitosterol : 1897 Fucosterol : ca 136
South River #5 (6/23/99)	1	7/13/99	8/25/99	157	Cholesterol : 173 Stigmasterol : 85 Campesterol : 17 Sitosterol : 370 Fucosterol : ca 150
Sayreville A (6/23/99)	1	7/20/99	8/21/99	69	
Sayreville K (6/23/99)	1	7/20/99	8/21/99	56	
Sayreville S (6/23/99)	1	7/20/99	8/24/99	105	
Sayreville T (6/23/99)	1	7/20/99	8/24/99	105	
Perth Amboy #5 (6/23/99)	1	7/13/99	9/10/99	30	
Perth Amboy #6 (6/23/99)	1	7/13/99	9/10/99	38	
Perth Amboy #7 (6/23/99)	1	7/13/99	9/10/99	44	
Perth Amboy (6/23/99) #2(#9R)	1	7/13/99	8/20/99	102	Cholesterol : 295 Stigmasterol : 692 Campesterol : 112 Sitosterol : 6757 Fucosterol : ca 391
E.Town (6/28/99) Hummocks 4A	1	7/22/99	8/27/99	131	
E.Town (6/28/99) Maple Glen	1	7/22/99	8/27/99	128	
E.Town (6/28/99) Quinton	1	7/22/99	9/ 7/99	100	

Table 2. Analytical results for well water samples (continued)

Sample (Date Received)	Sample Volume (L)	Sample Preparation Date	GC/MS Analysis Date	Recovery (%)	Sterols detected (ng/L)
Mt. Olive Juckett (7/13/99)	1	7/22/99	9/ 7/99	117	
Newton #1 (7/13/99)	1	7/26/99	9/ 8/99	89	
Newton #1 (7/20/99)	1	7/26/99	9/ 8/99	126	
Beverly #15 (7/22/99)	1	7/26/99	9/ 9/99	77	
Beverly #32 (7/22/99)	1	7/26/99	9/ 9/99	66	
E.Town (7/26/99) Jefferson Park	1	7/28/99	10/ 3/99	45	
South River #2 (7/22/99)	1	7/28/99	10/ 4/99	12	
South River #5 (7/22/99)	1	7/28/99	10/ 4/99	39	
South River Infiltration Gallary (7/22/99)	1	7/28/99	10/ 5/99	7	
Newton #1 (7/26/99)	1	8/6/99	10/ 5/99	45	Cholesterol 30 Campesterol 21 Stigmasterol 30 Sitosterol 17
Newton #1 (8/04/99)	1	8/6/99	10/ 6/99	55	
E.Town (8/04/99) Maple Glen	1	8/6/99	10/ 6/99	76	Cholesterol 65 Stigmasterol 65 Sitosterol 251 Fucosterol ca 76
Mt. Olive (8/04/99)	1	8/6/99	10/ 6/99	54	Cholesterol 43 Stigmasterol 31 Sitosterol 76 Fucosterol ca 33
Perth Amboy #5 (8/18/99)	1	9/30/99	10/ 7/99	33	
Perth Amboy #2(9R) (8/18/99)	1	9/30/99	10/ 7/99	53	
Perth Amboy #6 (8/18/99)	1	9/30/99	10/ 8/99	34	
Perth Amboy #7 (8/18/99)	1	9/30/99	10/ 8/99	34	
Sayreville A (8/19/99)	1	10/11/99	10/25/99	46	

Table 2. Analytical results for well water samples (continued)

Sample (Date Received)	Sample Volume (L)	Sample Preparation Date	GC/MS Analysis Date	Recovery (%)	Sterols detected (ng/L)
Sayreville K (8/19/99)	1	10/11/99	10/26/99	95	Cholesterol 218 Campesterol 104 Sitosterol 4
Sayreville S (8/19/99)	1	10/11/99	10/26/99	46	
Sayreville T (8/19/99)	1	10/11/99	10/26/99	62	
South River #5 (9/09/99)	1	10/13/99	10/28/99	48	
South River Infiltration Gallary (9/09/99)	1	10/13/99	10/28/99	126	
E.Town (9/09/99) Jefferson Park	1	10/13/99	10/28/99	90	
E.Town (10/6/99) Maple Glen	1	10/14/99	10/30/99	67	
E.Town (9/01/99) Hummocks 4A	1	10/14/99	11/01/99	60	Cholesterol : 90 Stigmasterol : 138 Campesterol : 26 Sitosterol : 239 Fucosterol : ca 42
E.Town Quinton (9/01/99)	1	10/14/99	11/01/99	66	
Mt. Olive (10/6/99)	1	10/14/99	11/01/99	47	
E. Town Quinton Ave. (11/17/99)	1	3/28/00	3/29/00	64	Cholesterol : 9.7 Stigmasterol : 23 Sitosterol : 25
E. Town Hummocks (11/17/99)	1	3/28/00	3/29/00	78	Cholesterol : 25 Stigmasterol : 64 Sitosterol : 8
E. Town Jefferson Park (1/5/00)	1	3/30/00	4/ 4/00	39	
Mt. Olive (1/5/00)	1	3/30/00	4/ 4/00	85	Cholesterol : 43 Stigmasterol : 325 Campesterol : 17 Sitosterol : 274 Fucosterol : ca 39
Perth Amboy #7 (1/27/00)	1	4/24/00	4/26/00	37	
Perth Amboy (1/27/00) #9(2R)	1	4/24/00	4/26/00	27	
South River #2 (1/27/00)	1	4/24/00	4/27/00	26	

Table 2. Analytical results for well water samples (continued)

Sample (Date Received)	Sample Volume (L)	Sample Preparation Date	GC/MS Analysis Date	Recovery (%)	Sterols detected (ng/L)
South River #5 (1/27/00)	1	4/24/00	4/27/00	37	
NJ-American (2/8/00) #13	1	5/1/00	5/16/00	54	
NJ-American (2/8/00) #27	1	5/1/00	5/17/00	61	
Sayreville Q (2/8/00)	1	5/1/00	5/17/00	77	
Sayreville T (2/8/00)	1	5/1/00	5/17/00	70	
Sayreville K (2/16/00)	1	5/8/00	5/19/00	66	
E. Town Quinton Ave. (2/16/00)	1	5/8/00	5/18/00	84	
E. Town Hummocks 4A (2/16/00)	1	5/8/00	5/18/00	39	Cholesterol : 3.1 Stigmasterol : 18 Sitosterol : 12 Campesterol : trace
E. Town Hummocks 9 (2/16/00)	1	5/8/00	5/18/00	53	Cholesterol : 3.1 Stigmasterol : 15 Sitosterol : 11
South River Infiltration Gallery (2/16/00)	1	5/11/00	5/23/00	83	
E. Town Maple Glen (3/15/00)	1	5/11/00	5/23/00	96	Cholesterol: 20.7 Stigmasterol: 33 Sitosterol : 32 Fucosterol : ca 22
NJ-American #15 (3/15/00)	1	5/11/00	5/22/00	80	Stigmasterol : 57 Campesterol : 3 Sitosterol : 113
NJ-American #32 (3/15/00)	1	5/11/00	5/22/00	90	
South River #5 (3/22/00)	1	10/14/00	10/17/00	45	
South River Infiltration Gallary (3/22/00)	1	10/14/00	10/20/00	50	Cholesterol 104 Campesterol 59 Sitosterol 73
Perth Amboy #7 (3/22/00)	1	10/14/00	10/18/00	54	

Table 2. Analytical results for well water samples (continued)

Sample (Date Received)	Sample Volume (L)	Sample Preparation Date	GC/MS Analysis Date	Recovery (%)	Sterols detected (ng/L)
Perth Amboy #9 (3/22/00)	1	10/14/00	10/20/00	38	Cholesterol 45 Stigmasterol 27 Sitosterol 4
Mount Olive (4/12/00)	4	10/18/00	10/21/00	108	Cholesterol 83 Campesterol 23 Stigmasterol 1184 Sitosterol 783 Fucosterol ca 580
Sayreville A (5/3/00)	4	10/18/00	10/22/00	78	
Sayreville S (5/3/00)	4	10/18/00	10/23/00	95	
Sayreville T (5/3/00)	4	10/18/00	10/23/00	74	
Elizabeth Town Hummocks 4A (4/12/00)	4	10/24/00	10/27/00	135	
Elizabeth Town Quinton Ave. (4/12/00)	4	10/24/00	10/27/00	63	Cholesterol 80 Campesterol 5 Sitosterol 8 Fucosterol trace 2 unknown sterols
Perth Amboy #7 (5/11/00)	4	10/24/00	10/27/00	56	
Perth Amboy #9 (5/11/00)	4	10/24/00	10/27/00	87	Cholesterol 15 Campesterol 8 Stigmasterol 25 Sitosterol 42
South River Infiltration Gallary (5/11/00)	4	10/30/00	11/ 3/00	58	
South River #5 (5/11/00)	4	10/30/00	11/ 3/00	44	
NJ-American Beverly #15 (5/16/00)	4	10/30/00	11/ 2/00	52	Cholesterol 16 Campesterol 14 Stigmasterol 94 Sitosterol 183 Fucosterol ca 24
NJ-American Beverly #32 (5/16/00)	4	10/30/00	11/ 2/00	45	Cholesterol 14 Campesterol 5
Elizabeth Town Maple Glen (5/16/00)	4	11/ 2/00	11/10/00	56	Cholesterol 19 Campesterol 7 Stigmasterol 146 Sitosterol 134 Fucosterol ca 42

Table 2. Analytical results for well water samples (continued)

Sample (Date Received)	Sample Volume (L)	Sample Preparation Date	GC/MS Analysis Date	Recovery (%)	Serols detected (ng/L)
Elizabeth Town Hummocks 4A (6/14/00)	4	11/ 2/00	11/10/00	58	
Elizabeth Town Quinton Ave. (6/14/00)	4	11/ 2/00	11/10/00	44	
Sayreville A (6/14/00)	4	11/ 9/00	11/15/00	27	
Sayreville S (6/14/00)	4	11/ 9/00	11/15/00	69	
Sayreville T (6/14/00)	4	11/ 9/00	11/15/00	62	
NJ-American Beverly #15 (6/27/00)	4	11/15/00	11/20/00	30	Cholesterol 76 Stigmasterol 269 Sitosterol 424 Fucosterol 33 Brassicasterol 12
NJ-American Beverly #32 (6/27/00)	4	11/15/00	11/21/00	36	Cholesterol 29 Campesterol 4 Sitosterol 131
Newton (7/12/00)	4	11/15/00	11/21/00	36	Coprostanol 13 Cholesterol 55 Campesterol 15 Stigmasterol 124 Sitosterol 104 Fucosterol ca 61 Isofucosterol trace Brassicasterol ca 3 22-dehydrocholesterol ca 7 24-ethylcoprostanol ca 10
Mount Olive Juckett (6/27/00)	4	11/21/00	11/25/00	67	Cholesterol 76 Campesterol 28 Stigmasterol 1069 Sitosterol 864 Fucosterol ca 505 Isofucosterol ca 97 Brassicasterol ca 50 22-dehydrocholesterol ca 60 2 Unknown sterols

Table 2. Analytical results for well water samples (continued)

Sample (Date Received)	Sample Volume (L)	Sample Preparation Date	GC/MS Analysis Date	Recovery (%)	Sterols detected (ng/L)
Mount Olive Tulip (6/27/00)	4	11/21/00	11/27/00	63	Cholesterol 196 Campesterol 26 Stigmasterol 2086 Sitosterol 1298 Fucosterol ca 940 Isofucosterol ca 85 Brassicasterol ca 20 22-dehydrocholesterol ca 25 Unknown sterol
Newton (8/23/00)	4	11/21/00	11/27/00	68	Cholesterol 20 Stigmasterol 114 Sitosterol 260
Blairs Town 10 Elm (8/23/00)	4	11/29/00	12/19/00	96	Cholesterol 81 Campesterol 10 Stigmasterol 42 Sitosterol 50 Fucosterol ca 37 Isofucosterol ca 11 Brassicasterol ca 7 2 Unknown sterols
Newton #1 (9/13/00)	4	11/29/00	12/20/00	89	Cholesterol 49 Campesterol 18 Stigmasterol 50 Sitosterol 155 Fucosterol ca 46 Isofucosterol trace Brassicasterol ca 11 22-dehydrocholesterol ca 5 Unknown sterol
Elizabeth Town Maple Glen (10/12/00)	4	12/ 5/00	12/20/00	43	Cholesterol 109 Campesterol 26 Stigmasterol 335 Sitosterol 1015 Fucosterol ca 380 Brassicasterol ca 13
Sayreville A (10/12/00)	4	12/ 5/00	12/21/00	43	Cholesterol 75 Campesterol 67 Stigmasterol 5 Sitosterol 5 Brassicasterol ca 33 2 Unknown sterols
Sayreville S (10/12/00)	4	12/ 5/00	12/21/00	48	

Table 2. Analytical results for well water samples (continued)

Sample (Date Received)	Sample Volume (L)	Sample Preparation Date	GC/MS Analysis Date	Recovery (%)	Sterols detected (ng/L)
Perth Amboy #7 (10/17/00)	4	12/ 8/00	1/19/01	70	Cholesterol 11 Campesterol 5 Stigmasterol 18 Sitosterol 87 Isofucosterol ca 33 Unknown sterol
Perth Amboy #9 (10/17/00)	4	12/ 8/00	1/19/01	70	Cholesterol 15 Campesterol 8 Sitosterol 0.8
South River #5 (10/17/00)	4	12/12/00	1/22/01	66	Cholesterol 72 Campesterol 23 Sitosterol 3.5 Brassicasterol ca 12
South River Infiltration Gallary (10/17/00)	4	12/12/00	1/22/01	66	Cholesterol 38 Campesterol 12 Sitosterol 5 Brassicasterol ca 6
Mount Olive (10/25/00)	4	12/12/00	1/22/01	48	Cholesterol 159 Campesterol 17 Stigmasterol 4228 Sitosterol 3427 Fucosterol ca 2373 Isofucosterol ca 222
Elizabeth Town Quinton Ave. (10/25/00)	4	12/14/00	1/23/01	96	
Elizabeth Town Hummocks 4A (10/25/00)	4	12/14/00	1/23/01	89	
A & P (2/21/01)	4	2/28/01	3/03/01	42	
Good Sheppard (2/21/01)	4	2/28/01	3/03/01	78	Coprostanol 3.5 Cholesterol 49 Campesterol 7 Stigmasterol 10 Sitosterol 12
Swartswood (2/21/01)	4	2/28/01	3/03/01	61	
Elizabeth Town Hummocks 4A (5/16/01)	4	5/17/01	5/25/01	54	
Elizabeth Town Hummocks 9 (5/16/01)	4	5/17/01	5/25/01	12	
Elizabeth Town Quinton Ave. (5/16/01)	4	5/17/01	5/25/01	22	

Table 2. Analytical results for well water samples (continued)

Sample (Date Received)	Sample Volume (L)	Sample Preparation Date	GC/MS Analysis Date	Recovery (%)	Sterols detected (ng/L)
South River #5 (5/16/01)	4	5/17/01	5/25/01	24	
Elizabeth Town Hummocks 4A (10/24/01)	4	10/28/01	10/31/01	38	
Elizabeth Town Hummocks 9 (10/24/01)	4	10/28/01	11/1/01	36	
South River #5 (10/24/01)	4	10/28/01	11/1/01	47	
South River Infiltration Gallary (10/24/01)	4	10/28/01	11/1/01	48	
Elizabeth Town Hummocks 4A (12/17/01)	4	12/19/01	12/24/01	109	
Elizabeth Town Quinton Ave (12/17/01)	4	12/19/01	1/2/02	102	
South River #5 (12/17/01)	4	12/19/01	1/2/02	126	Cholesterol 22 Sitosterol 154
South River #7 (12/17/01)	4	12/19/01	1/2/02	95	
South River #9 (12/17/01)	4	12/19/01	1/2/02	95	
NJ-American Five Road Well (1/15/02)	4	1/18/02	1/21/02	41	

Table 3. Sterol concentrations (ng/L) in river water samples

	Raritan A (Nov/1998)	Raritan B (Nov/1998)	Raritan A (Dec/1998)	Raritan A (Jan/1999)	Raritan B (Jan/1999)	Raritan C (Sep/2001)
Coprostanol	5	18	3	40	123	42
Cholesterol	408	371	405	1012	1666	1300
Campesterol	102	39	50	176	189	310
Stigmasterol	98	68	75	254	488	188
Sitosterol	171	152	205	1450	1996	792
22-Dehydro-cholesterol	ca 80	ca 200	ca 110	ca 150	ca 200	ca 280
Brassicasterol	ca 60	ca 35	ca 130	ca 70	ca 110	ca 200
Fucosterol	ca 100	ca 60	ca 70	ca 60	ca 450	ca 50
Isofucosterol	ca 60	ca 50	ca 35	ca 30	ca 100	ca 25

Table 4. Level of sterols in meat samples

Sample received	Recovery (%)	Coprostanol (ng/g)	Campesterol (ng/g)	Stigmasterol (ng/g)	Sitosterol (ng/g)
Ham (4/22/01)	49 ± 8.8	134 ± 8	90 ± 13	None	17 ± 6
Meat loaf (4/24/01)	32 ± 12.4	93 ± 7	131 ± 34	47 ± 13	309 ± 119
BBQ chicken (5/14/01)	27 ± 5.8	None	448 ± 85	176 ± 30	2054 ± 390
Beef stew (5/17/01)	54 ± 4.9	87 ± 7	64.5*	None	32.5*
Italian meatball (5/10/01)	29 ± 4.9	607 ± 73	194 ± 30	58 ± 13	2222 ± 508

* Average of two replicates

Table 7. Retention time and quantitation ion(s) of pesticides and internal standards

Analyte	Retention Time (min)	Quantitation Ion(s)
Acenaphthene-d_{10}	16:40	165
Acephate	15:35	143
Alachlor	24:36	238-240
Aldrin	26:04	255-259
Ametryn	24:52	228
Aminocarb	22:18	152
Atrazine	21:58	216
Atrazine desetyl	20:11	188
Azinphos methyl	34:54	132+160
Bendiocarb	20:31	167
Benfluralin	20:30	336
Bifenthrin	33:34	181
Bromacil	25:39	205-207
Butachlor	28:28	238-240
Butylate	15:29	218
α-BHC	21:01	217-221
Captafol	32:37	312-316
Captan	27:38	264-266
Carbaryl	24:48	145
Carbofuran	21:48	165

Table 7. Retention time and quantitation ion(s) of pesticides and internal standards (Continued)

Analyte	Retention Time (min)	Quantitation Ion(s)
Carboxin	29:41	143
Chlorbromuron	27:25	293-295
Chlordimeform	20:12	196-198
Chlorothalonil	22:49	265-269
Chlorpropham (CIPC)	20:15	172-174
Chlorpyriphos	25:55	350-354
Chlorpyriphos methyl	24:17	322-326
Clomazone	22:05	240-242
Cyanazine	26:07	214
λ-Cyhalothrin	35:32	225-227
Dacthal (Chlorthal)	26:09	331-335
DBCP	7:43	157
DDD	30:45	207-211
DDE	29:22	281-285
DDT	31:58	241-245
DEF	29:31	315
Dichlobenil	13:25	172-174
Dichlorvos	11:13	221-223
Diclofop methyl	32:28	281-283

Table 7. Retention time and quantitation ion(s) of pesticides and internal standards (Continued)

Analyte	Retention Time (min)	Quantitation Ion(s)
Dicloran	21:30	207-211
Dicofol (Kelthane)	26:31	251-253
Dieldrin	29:32	243-247
Dimethoate	21:34	199
Disulfoton (Di-syston)	23:07	89-90
α-Endosulfan	28:38	275-279
β-Endosulfan	30:35	275-279
Endosulfan sulfate	31:50	323-329
Endrin	30:13	243-247
Ethalfluralin	20:04	232
Ethion	30:47	199
Fenvalerate	44:06	167-169
Folpet	27:50	260-264
Fonofos	22:36	137
Heptachlor	24:45	335-341
Heptachlor epoxide	27:24	251-255
Hexachlorobenzene	21:06	283-289
3-Hydroxy carbofuran	24:39	163
Iprodione	33:10	273+275+330

Table 7. Retention time and quantitation ion(s) of pesticides and internal standards (Continued)

Analyte	Retention Time (min)	Quantitation Ion(s)
Isofenphos	27:27	245
3-Keto carbofuran	23:29	179
Linuron	25:38	249-251
Malathion	25:49	127
Metalaxyl	24:54	220
Methamidophos	11:35	94+142
Methiocarb	25:31	169
Methoxychlor	33:47	237-239
Metobromuron	23:52	259-261
Metolachlor	25:55	252-254
Metribuzin	24:18	215
Naphthalene-d_8	9:55	137
cis-Nonachlor	30:44	135-137
Oxadiazon	29:28	303-305
Parathion	26:17	292
Pendimethalin	27:06	212
Pentachloronitrobenzene	22:02	294-298
Permethrin	37:31	183
Phenamiphos	28:54	304

Table 7. Retention time and quantitation ion(s) of pesticides and internal standards (Continued)

Analyte	Retention Time (min)	Quantitation Ion(s)
Phenanthrene-d_{10}	22:42	189
o-Phenylphenol	17:24	171
Phosalone (zolone)	34:45	182-184
Phosdrin (mevinphos)	15:27	193
Phosmet	33:28	160
Pirimiphos methyl	25:24	306
Procymidone	27:46	284-286
Prometon	21:50	226
Prometryn	24:58	242
Propachlor	19:11	212-214
Propanil	24:14	218-220
Propazine	22:07	230
Propetamphos	22:31	222
Propoxur	19:15	111
Propyzamide (Pronamide)	22:37	256-258
Siduron	29:00	233
Simazine	21:48	202
Simetryn	24:43	214
Sulprofos	31:18	323

Table 7. Retention time and quantitation ion(s) of pesticides and internal standards (Continued)

Analyte	Retention Time (min)	Quantitation Ion(s)
Tebuthiuron	17:40	172
Terbufos	22:30	103
Terbutryn	25:24	242
Triadimefon	26:24	294
Trichlorfon	16:09	221-223
Trifluralin	20:24	290
Vinclozolin	24:30	242-244

Table 8. Pesticide recoveries at 100 ppb and limits of detection in corn flakes and toasted oats

Pesticide	% Recovery (% CV)		Limit of Detection (ppb)	
	Corn Flakes	Toasted Oats	Corn Flakes	Toasted Oats
Alachlor	92 (6.6)	107 (13.9)	3	2
Aldrin	97 (13.9)	74 (18.4)	7	10
Ametryn	89 (6.6)	94 (8.4)	8	3
Aminocarb	72 (14.1)	76 (19.7)	5	2
Atrazine	80 (10.4)	92 (3.5)	16	3
Azinphos methyl	98 (16.4)	49 (32.6)	21	12
Bendiocarb	47 (10.8)	59 (36.6)	3	2
Benfluralin	81 (3.8)	59 (37.1)	3	1
α-BHC	Not Detected	83 (15.8)	> 100	3
Bifenthrin	116 (16.8)	79 (1.3)	< 1	1
Bromacil	57 (15.8)	N A Q	32	16
Butachlor	11 (48.6)	92 (21.8)	5	< 1
Butylate	6 (10.2)	0 (173.2)	6	5
Carbaryl	33 (18.5)	43 (11.6)	27	4
Carbofuran	69 (7.2)	65 (21.5)	5	16
Carboxin	77 (20.8)	57 (44.2)	2	4
Chlorbromuron	134 (11.3)	180 (7.8)	70	37
Chlordimeform	53 (9.4)	53 (17.8)	5	4
Chlorpropham (CIPC)	106 (11.8)	80 (6.1)	14	2

Table 8. Pesticide recoveries at 100 ppb and limits of detection in corn flakes and toasted oats (continued)

Pesticide	% Recovery (% CV)		Limit of Detection (ppb)	
	Corn Flakes	Toasted Oats	Corn Flakes	Toasted Oats
Chlorpyriphos	115 (11.8)	98 (8.2)	< 1	< 1
Chlorpyriphos methyl	112 (8.9)	108 (7.5)	< 1	< 1
Clomazone	104 (7.2)	95 (9.0)	< 1	1
Cyanazine	80 (18.0)	58 (16.9)	9	4
λ-Cyhalothrin	49 (15.4)	63 (20.2)	16	4
Dacthal (Chlorthal)	122 (18.5)	127 (15.5)	< 1	< 1
DDD	89 (9.5)	49 (37.1)	3	1
DDE	91 (11.2)	38 (32.0)	3	2
DDT	34 (5.9)	38 (28.4)	91	4
DEF	106 (18.2)	85 (2.7)	< 1	< 1
Dichlobenil	4 (50.0)	2 (124.9)	< 1	3
Diclofop methyl	96 (14.1)	105 (17.0)	16	5
Dicloran	76 (13.4)	89 (5.6)	8	4
Dicofol (Kelthane)	86 (4.5)	85 (17.4)	2	< 1
Dieldrin	127 (14.7)	99 (6.7)	12	6
Dimethoate	52 (30.0)	35 (79.7)	21	9
Disulfoton (Di-syston)	78 (14.2)	75 (5.8)	2	< 1
α-Endosulfan	149 (15.9)	193 (8.7)	4	14
β-Endosulfan	127 (18.6)	105 (2.5)	5	7

Table 8. Pesticide recoveries at 100 ppb and limits of detection in corn flakes and toasted oats (continued)

Pesticide	% Recovery (% CV)		Limit of Detection (ppb)	
	Corn Flakes	Toasted Oats	Corn Flakes	Toasted Oats
Endosulfan sulfate	Not Detected	N A Q	> 100	1
Endrin	119 (16.7)	115 (6.5)	6	6
Ethalfluralin	14 (26.4)	54 (28.0)	42	< 1
Ethion	105 (14.0)	90 (4.5)	< 1	< 1
Fenvalerate	79 (5.1)	107 (15.1)	11	4
Fonofos	72 (13.4)	80 (14.3)	2	< 1
Heptachlor	118 (25.3)	108 (30.5)	< 1	< 1
Heptachlor epoxide	99 (10.8)	96 (5.5)	11	10
Hexachlorobenzene	Not Detected	29 (69.8)	> 100	1
Isofenphos	101 (13.8)	106 (7.1)	1	< 1
Linuron	40 (6.3)	89 (10.1)	15	3
Malathion	94 (9.6)	88 (2.6)	10	1
Metalaxyl	66 (16.0)	85 (22.0)	5	4
Methiocarb	48 (4.8)	64 (13.6)	10	3
Methoxychlor	131 (13.7)	65 (31.6)	12	17
Metobromuron	95 (11.1)	105 (2.4)	6	4
Metolachlor	92 (9.7)	100 (19.7)	5	1
Metribuzin	62 (32.2)	71 (33.5)	4	1
cis-Nonachlor	87 (15.7)	65 (3.9)	3	4

Table 8. Pesticide recoveries at 100 ppb and limits of detection in corn flakes and toasted oats (continued)

Pesticide	% Recovery (% CV)		Limit of Detection (ppb)	
	Corn Flakes	Toasted Oats	Corn Flakes	Toasted Oats
Oxadiazon	95 (7.4)	101 (3.0)	2	< 1
Parathion	99 (14.7)	104 (4.8)	< 1	< 1
Pendimethalin	86 (8.4)	78 (16.6)	1	1
Pentachloronitrobenzene	48 (19.9)	78 (22.5)	< 1	< 1
Permethrin	141 (7.5)	88 (5.6)	2	7
Phenamiphos	N A Q	N A Q	17	10
o-Phenylphenol	46 (15.2)	78 (10.7)	6	4
Phosalone (zolone)	98 (13.9)	98 (7.8)	5	6
Phosdrin (mevinphos)	N A Q	N A Q	4	4
Phosmet	66 (22.3)	41 (19.5)	7	6
Pirimiphos methyl	132 (16.3)	126 (9.2)	< 1	< 1
Procymidone	96 (8.3)	113 (13.4)	3	1
Prometon	84 (16.1)	109 (11.9)	5	4
Prometryn	103 (19.8)	100 (20.5)	6	2
Propachlor	64 (6.3)	75 (17.7)	1	< 1
Propanil	84 (19.8)	98 (19.4)	5	2
Propazine	85 (4.7)	97 (6.8)	6	2
Propetamphos	85 (8.5)	84 (6.1)	3	1
Propoxur	76 (6.8)	72 (14.9)	11	3

Table 8. Pesticide recoveries at 100 ppb and limits of detection in corn flakes and toasted oats (continued)

Pesticide	% Recovery (% CV)		Limit of Detection (ppb)	
	Corn Flakes	Toasted Oats	Corn Flakes	Toasted Oats
Propyzamide (Pronamide)	89 (4.9)	94 (13.1)	8	2
Siduron	96 (8.1)	92 (9.9)	54	46
Simazine	71 (9.4)	86 (0.0)	12	3
Simetryn	88 (18.5)	81 (23.2)	6	3
Sulprofos	120 (22.1)	120 (3.1)	4	< 1
Tebuthiuron	N A Q	N A Q	5	3
Terbufos	72 (14.3)	76 (17.3)	4	1
Terbutryn	95 (2.2)	100 (13.1)	4	1
Triadimefon	138 (13.7)	158 (9.2)	6	4
Trifluralin	79 (9.3)	55 (30.7)	< 1	< 1
Vinclozolin	73 (4.9)	89 (7.3)	5	2

* N A Q: Not able to quantify

Table 9. Molecular weights, base peaks, collision energies and MRM transitions of pesticides and atrazine-d_5, an internal standard, in LC/MS analysis

Analyte	MW	Base peak	Collision energy (V)	MRM transition
Aldicarb sulfone	222	240	10	240 > 223.0
Aldicarb sulfoxide	206	207	10	207 > 131.7
Methomyl	162	163	10	163 > 105.7
Monuron	198	199	20	199 > 125.6
Neburon	274	275	15	275 > 113.7
Oxamyl	219	237	10	237 > 90.0
Siduron	232	233	20	233 > 136.8
Thiodicarb	354	355	10	355 > 162.8
Atrazine-d_5	220	221	20	221 > 178.8

Table 10. Recoveries at 100 ppb and limits of detection of pesticides analyzed by HPLC/MS

Analyte	% Recovery (% CV)	Limit of Detection (ppb)
Aldicarb sulfone	19 (49.5)	75
Aldicarb sulfoxide	27 (24.4)	75
Methomyl	121 (16.7)	3
Monuron	92 (8.2)	27
Neburon	70 (16.8)	50
Oxamyl	16 (70.7)	75
Siduron	108 (5.6)	11
Thiodicarb	36 (14.2)	6

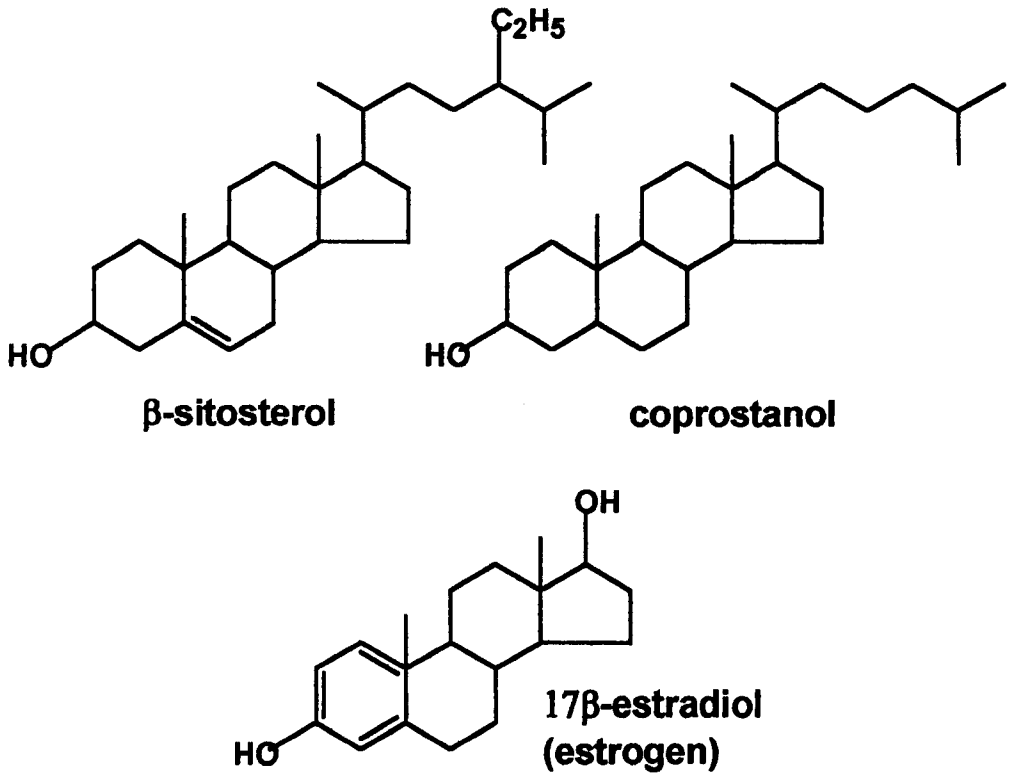

Figure 1. Structures of β-sitosterol, coprostanol and estrogen

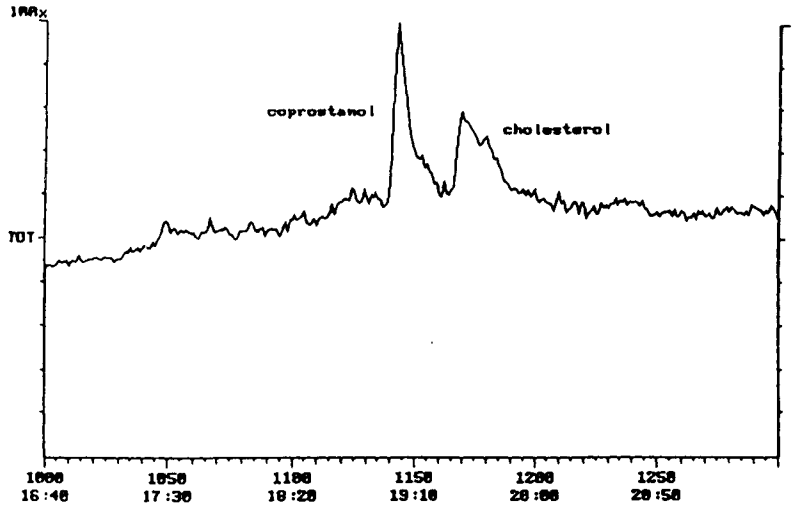

Figure 2A. Total ion chromatogram of underivatized sterols
(X axis = retention time; Y axis = ion intensity)

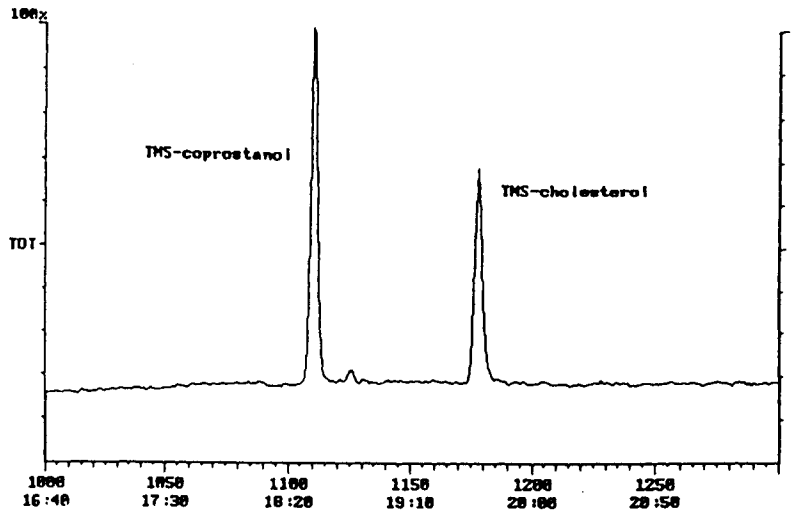

Figure 2B. Total ion chromatogram of TMS-sterols
(X axis = retention time; Y axis = ion intensity)

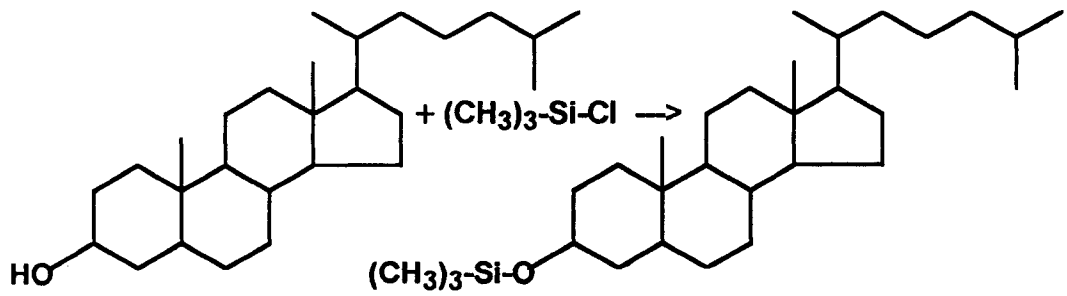

Figure 3. Structures of coprostanol and its trimethylsilyl derivative

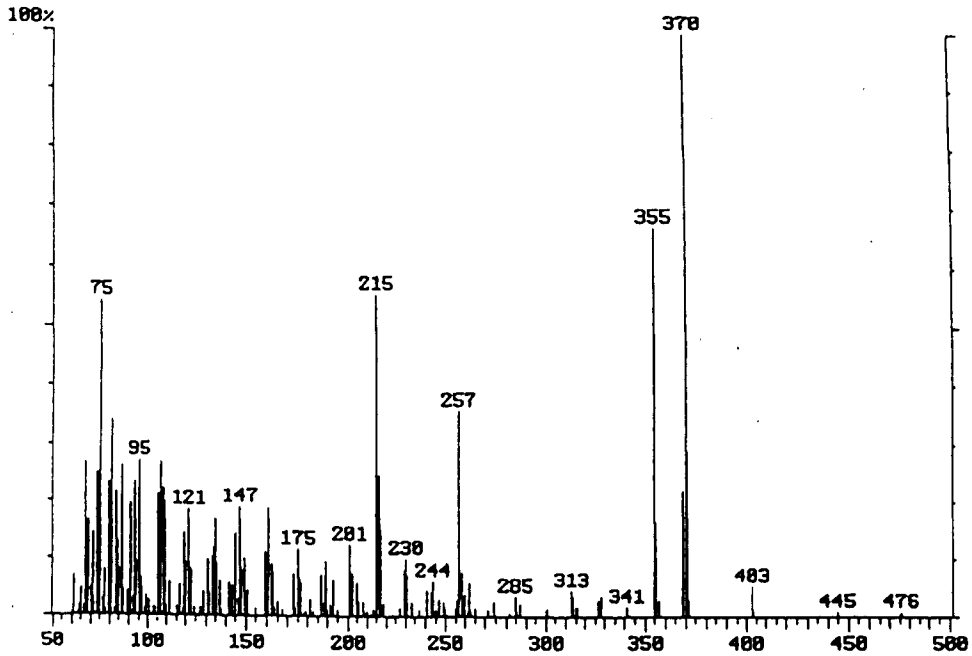

Figure 4. Mass spectrum of TMS-coprostanol in EI mode
(X axis = mass; Y axis = ion intensity)

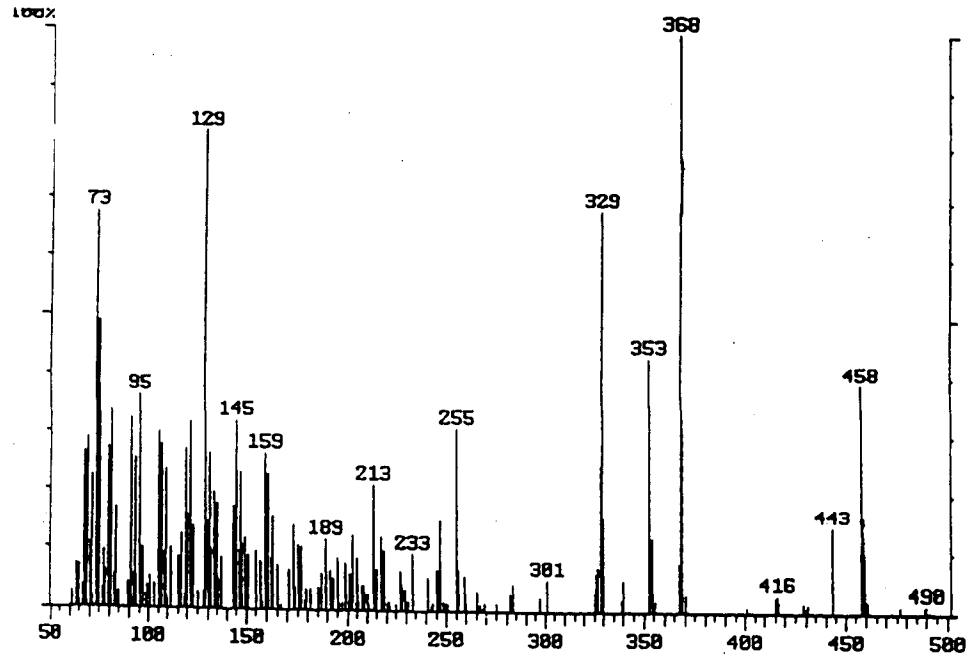

Figure 5. Mass spectrum of TMS-cholesterol in EI mode
(X axis = mass; Y axis = ion intensity)

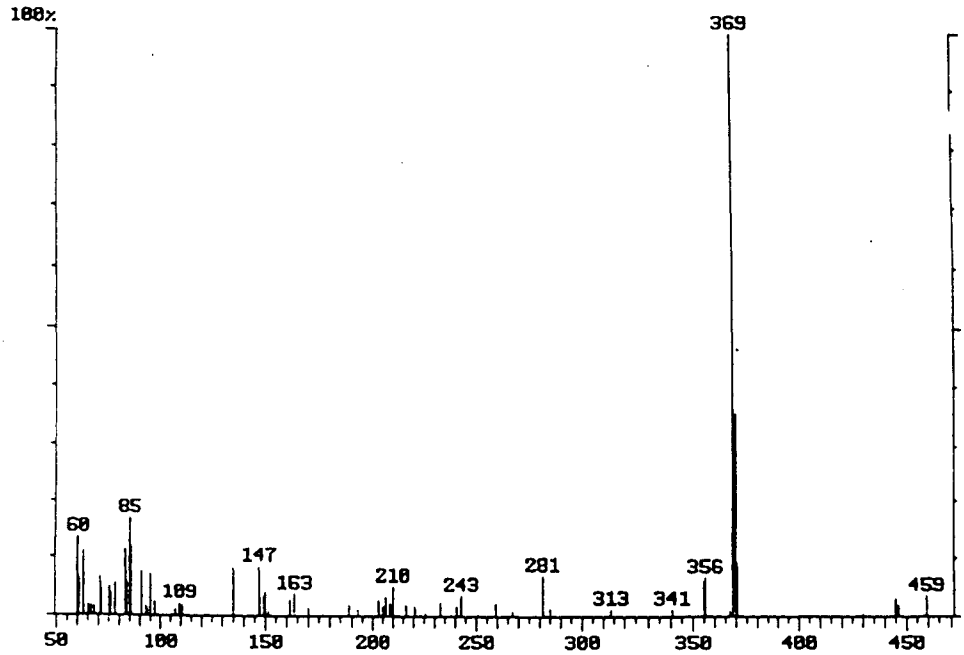

Figure 6. Mass spectrum of TMS-coprostanol in CI mode
(X axis = mass; Y axis = ion intensity)

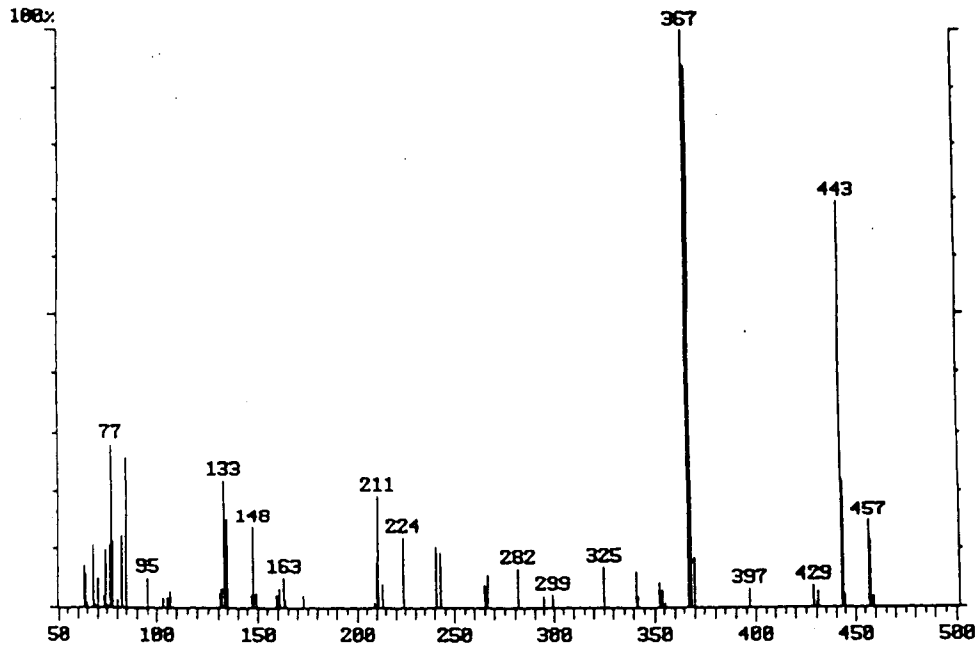

Figure 7. Mass spectrum of TMS-cholesterol in CI mode
(X axis = mass; Y axis = ion intensity)

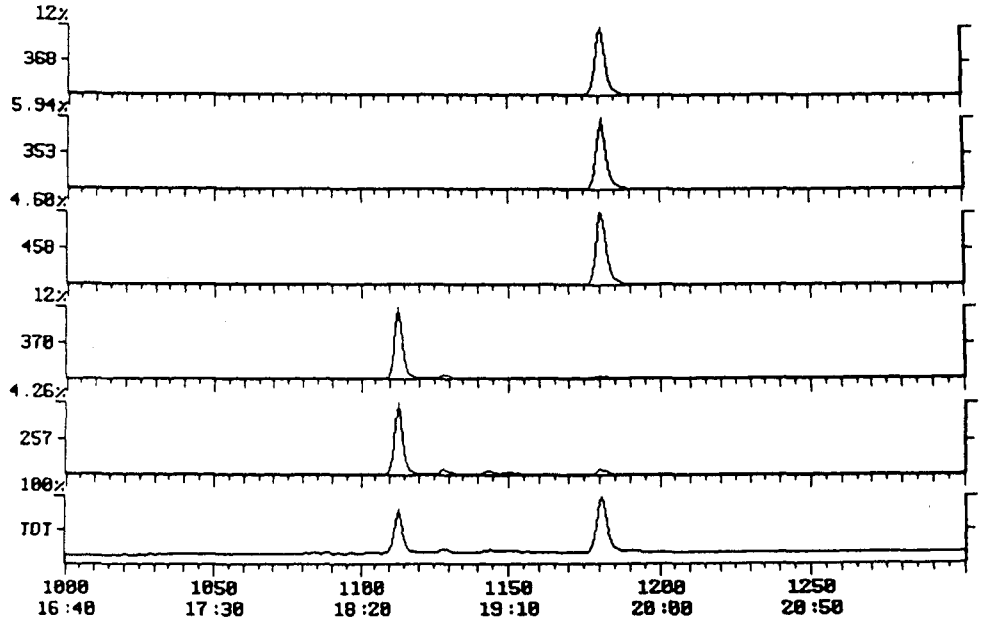

Figure 8. Mass chromatograms of TMS-cholesterol at m/z 368, 353, 458;
Mass chromatograms of TMS-coprostanol at m/z 370 and 257
Total ion current of both materials is shown in bottom trace
(X axis = retention time; Y axis = ion intensity)

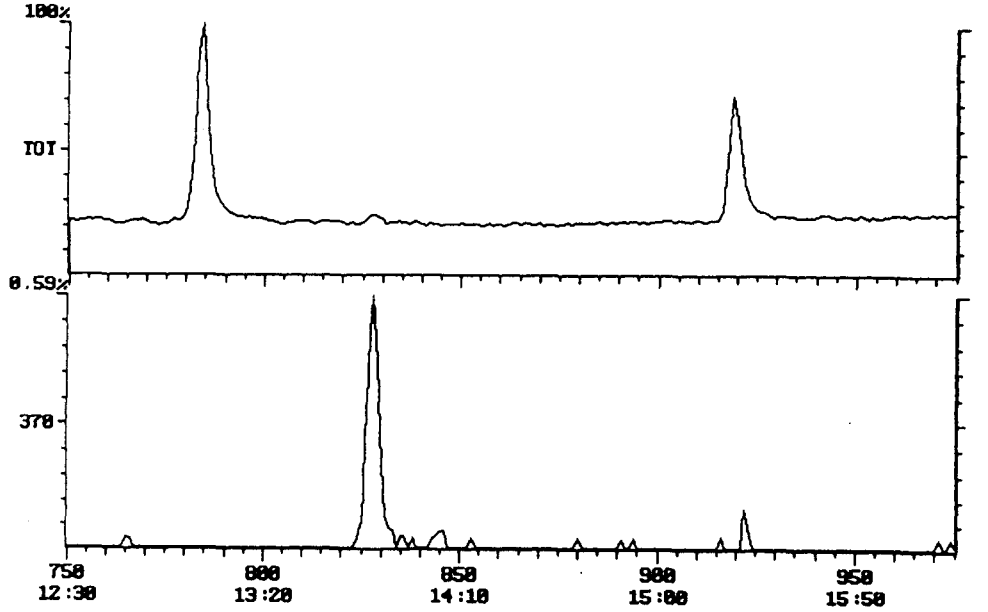

Figure 9. Mass chromatograms of TMS-coprostanol in total ion current
and at m/z 370 at 100 parts per trillion spiked level
(X axis = retention time; Y axis = ion intensity)

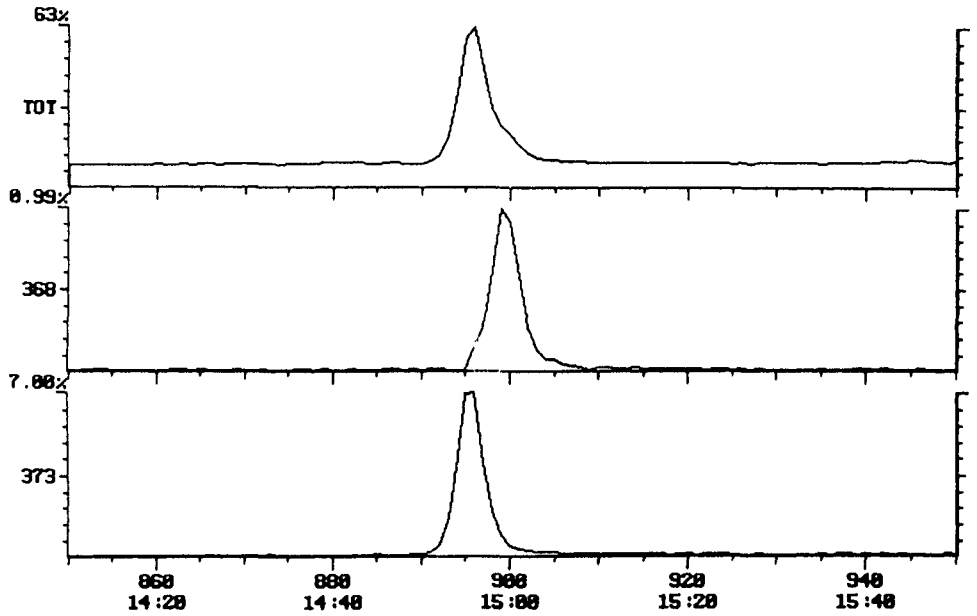

Figure 10. Mass chromatograms of TMS-cholesterol at m/z 368
and TMS-cholesterol-d_5 at m/z 373
Total ion current of both materials is shown in upper trace
(X axis = retention time; Y axis = ion intensity)

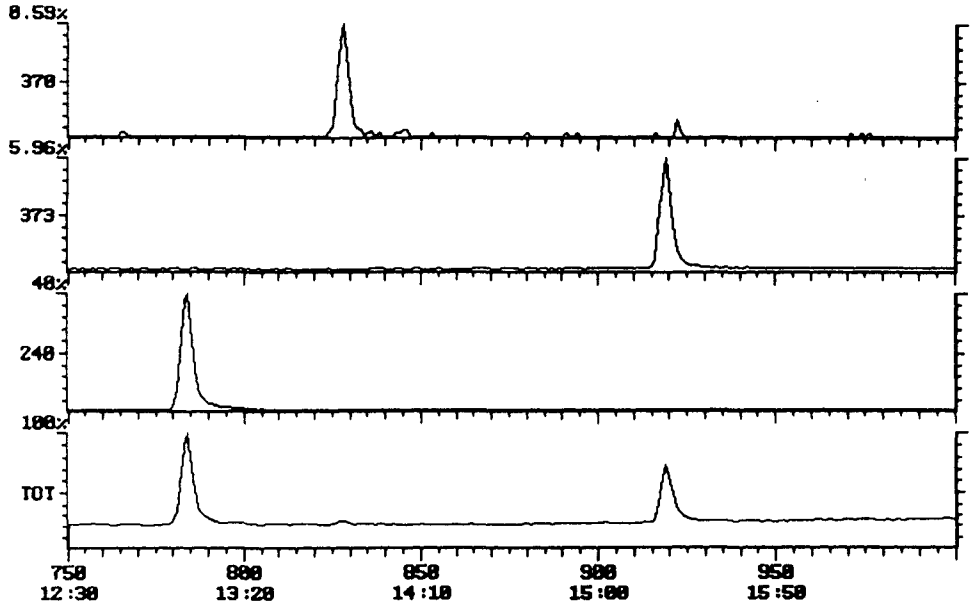

Figure 11. Mass chromatograms of coprostanol spiked into 1L water at 0.1 ppb,
cholesterol-d_5 spiked at 0.5 ppb, chrysene-d_{12}, and
total ion chromatogram (X axis = ion intensities; Y axis = retention time)

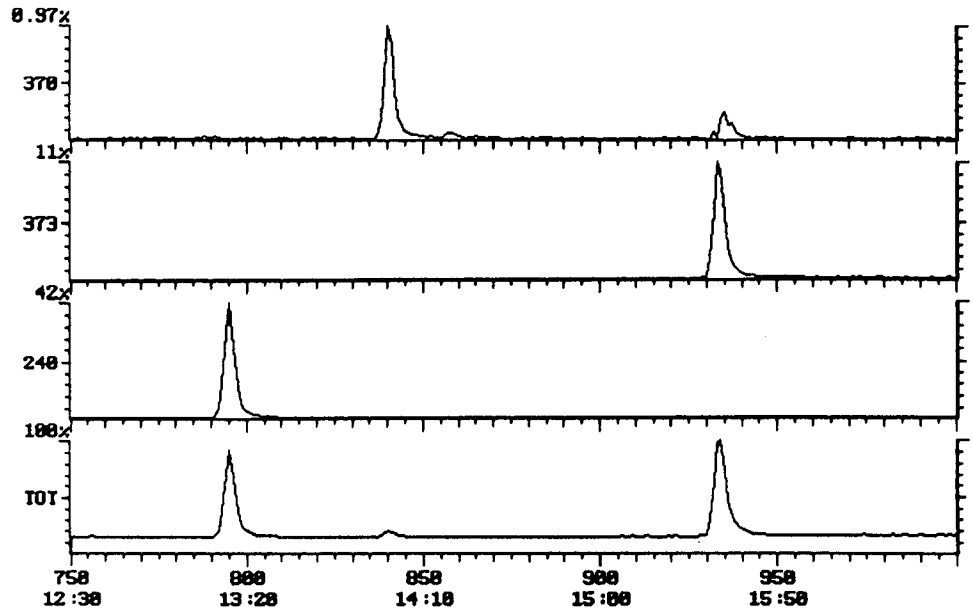

Figure 12. Mass chromatograms of coprostanol spiked into 4L water at 0.025 ppb, cholesterol-d_5 spiked at 0.125 ppb, chrysene-d_{12}, and total ion chromatogram (X axis = ion intensities; Y axis = retention time)

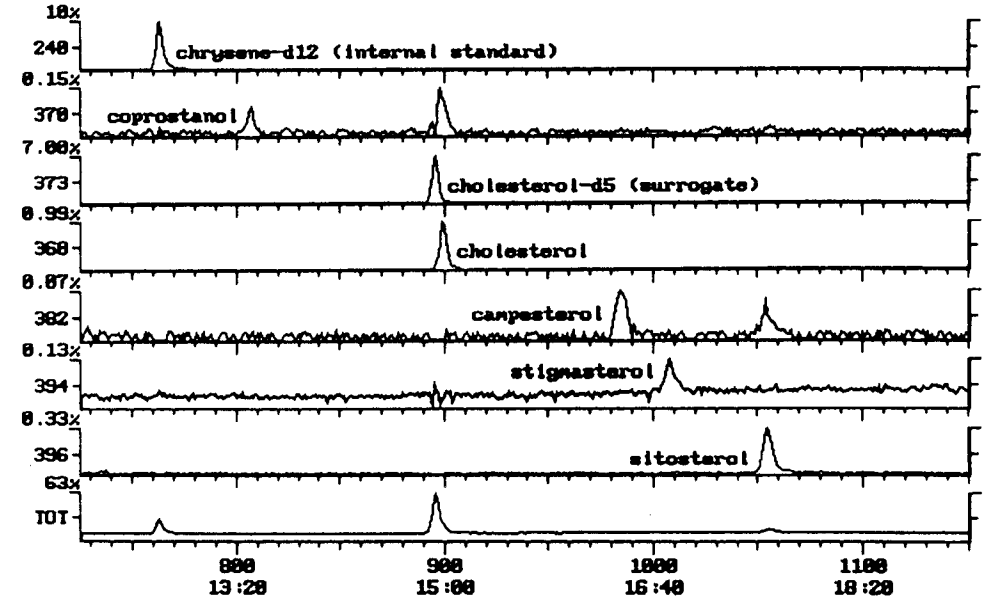

Figure 13. Mass chromatograms of sterols found in Good Sheppard (2/21/01) sample (X axis = ion intensities; Y axis = retention time)

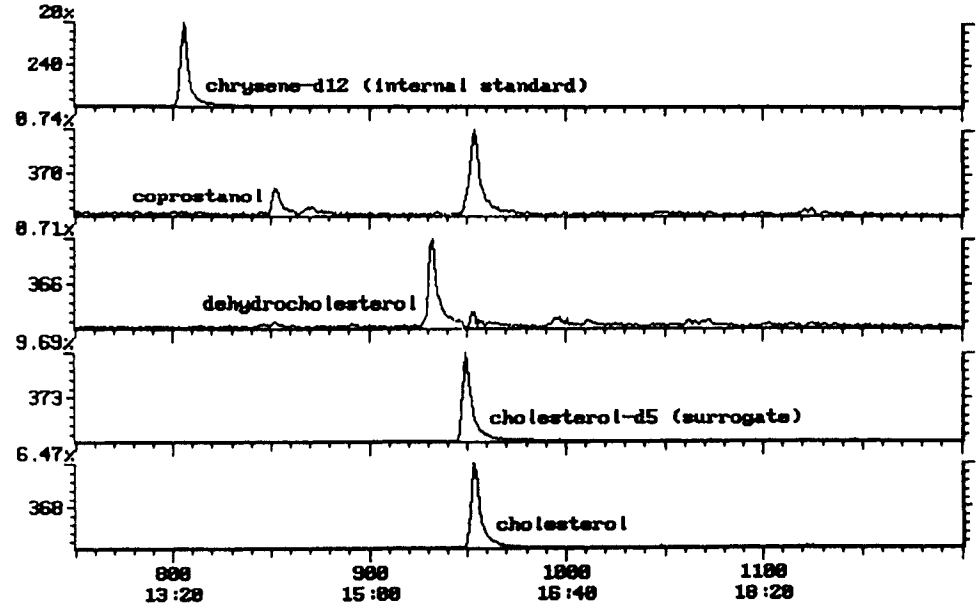

Figure 14. Mass chromatograms of sterols found in Raritan river (9/02/01) sample (X axis = ion intensities; Y axis = retention time)

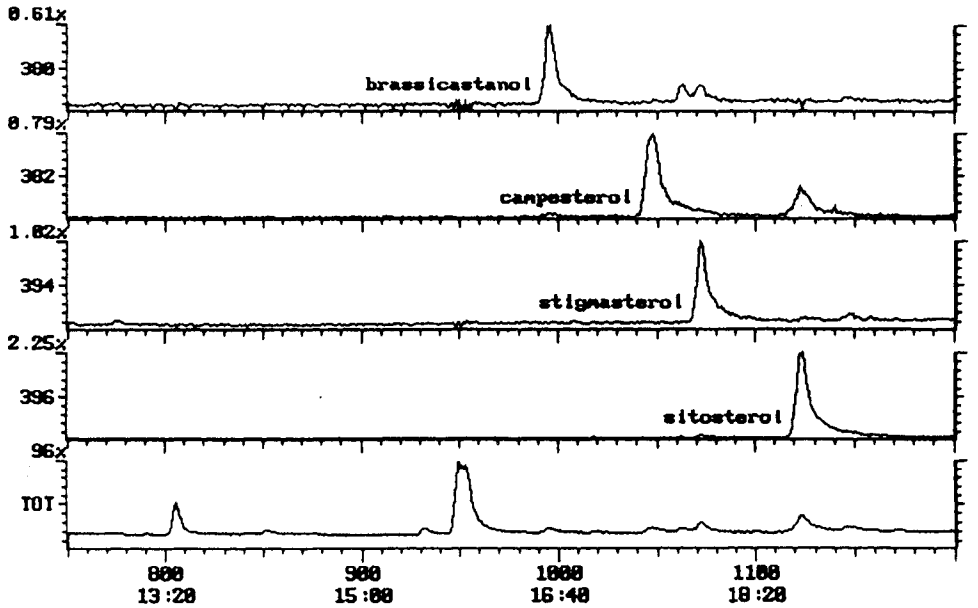

Figure 14. Mass chromatograms of sterols found in Raritan river (9/02/01) sample (continued) (X axis = ion intensities; Y axis = retention time)

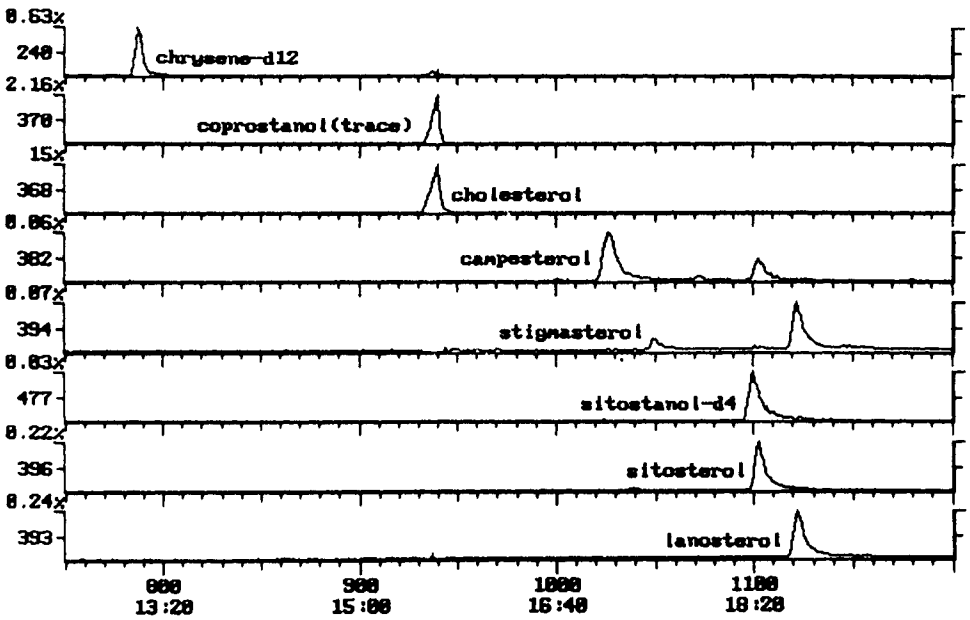

Figure 15. Mass chromatograms of sterols found in Italian meatball (5/10/01) sample (X axis = ion intensities; Y axis = retention time)

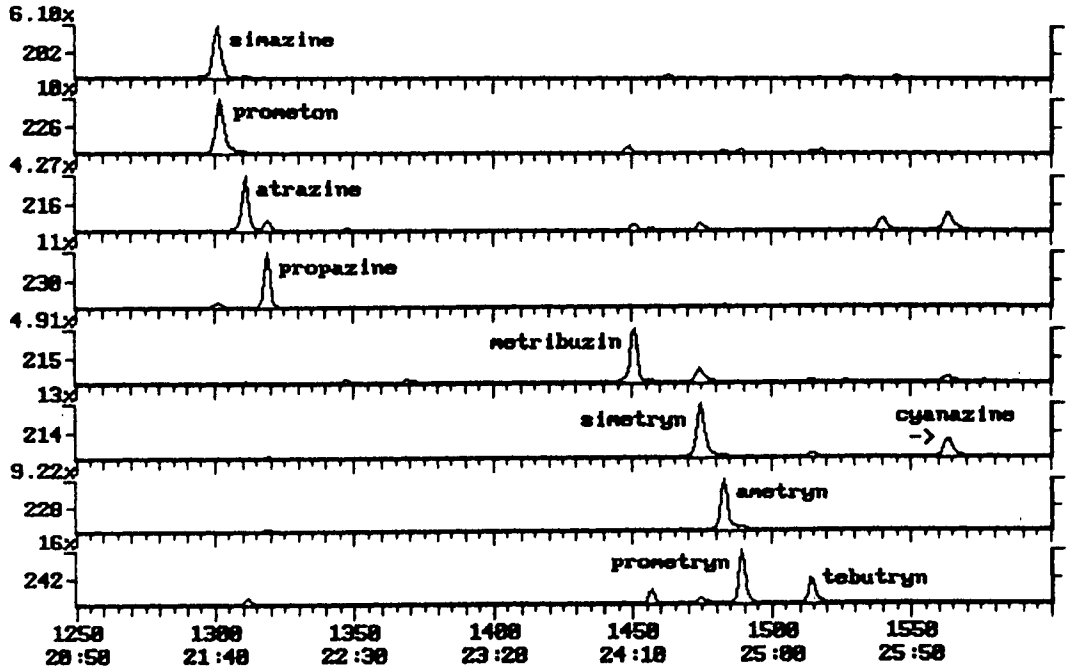

Figure 16. Mass chromatograms of triazines herbicides recovered from a toasted oat spiked at 100 ppb

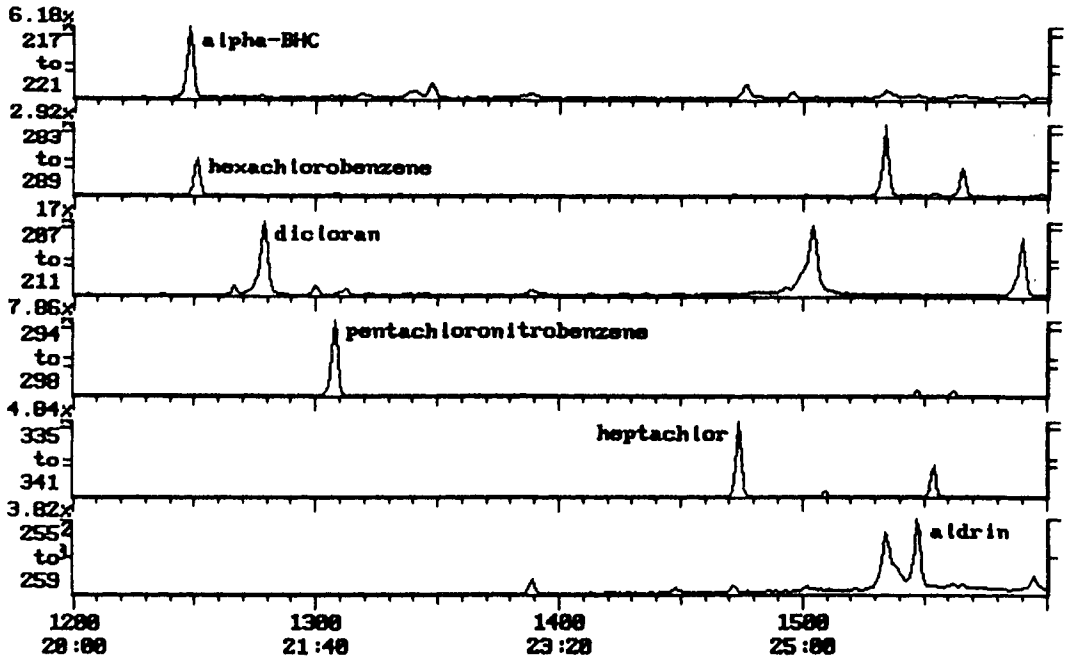

Figure 17. Mass chromatograms of organochlorine insecticides recovered from a toasted oat spiked at 100 ppb

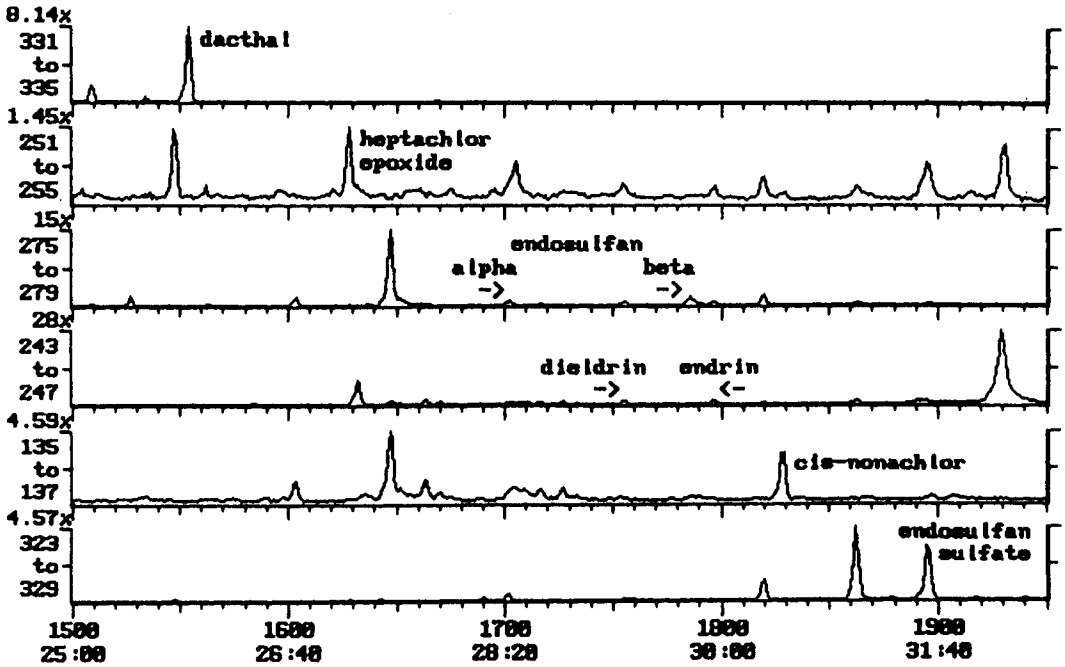

Figure 17. Mass chromatograms of organochlorine insecticides recovered from a toasted oat spiked at 100 ppb (continued)

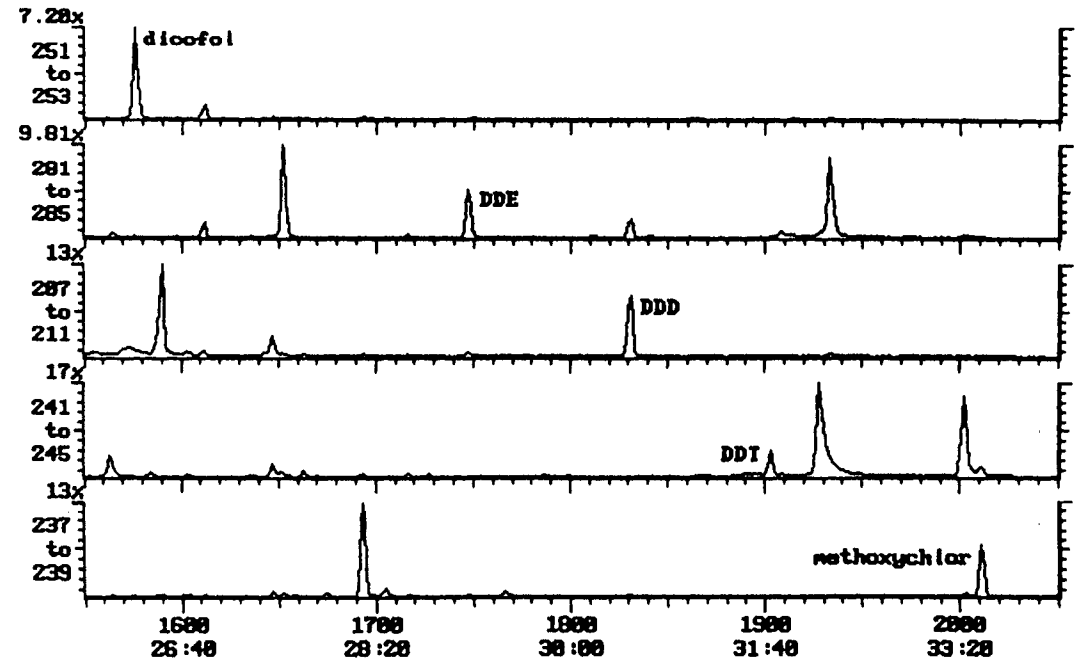

Figure 17. Mass chromatograms of organochlorine insecticides recovered from a toasted oat spiked at 100 ppb (continued)

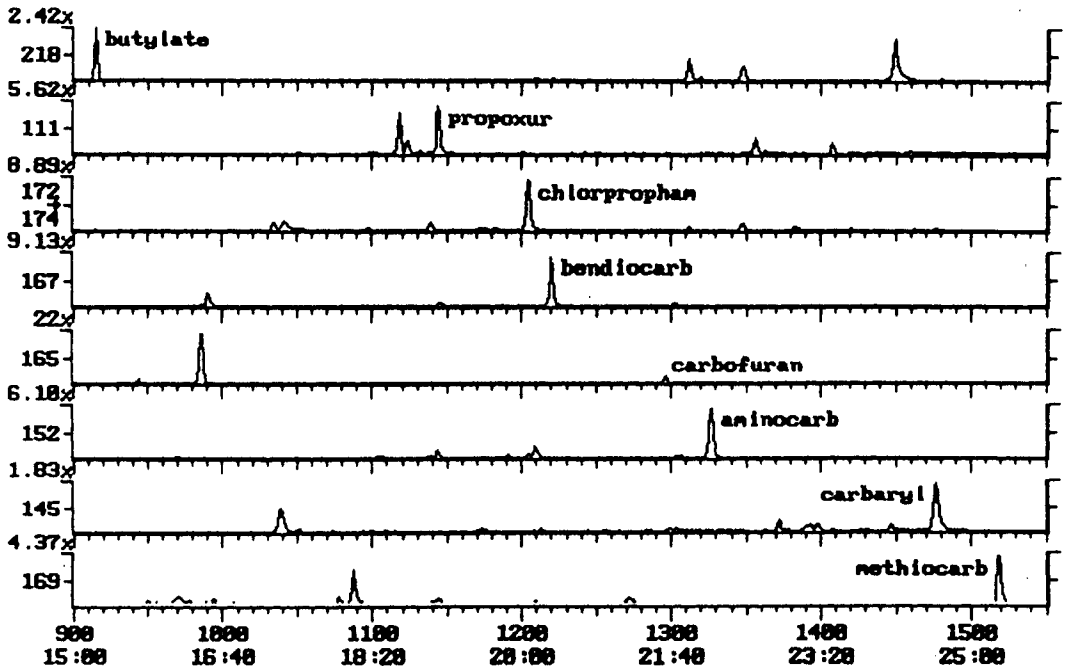

Figure 18. Mass chromatograms of carbamate insecticides recovered from a toasted oat spiked at 100 ppb

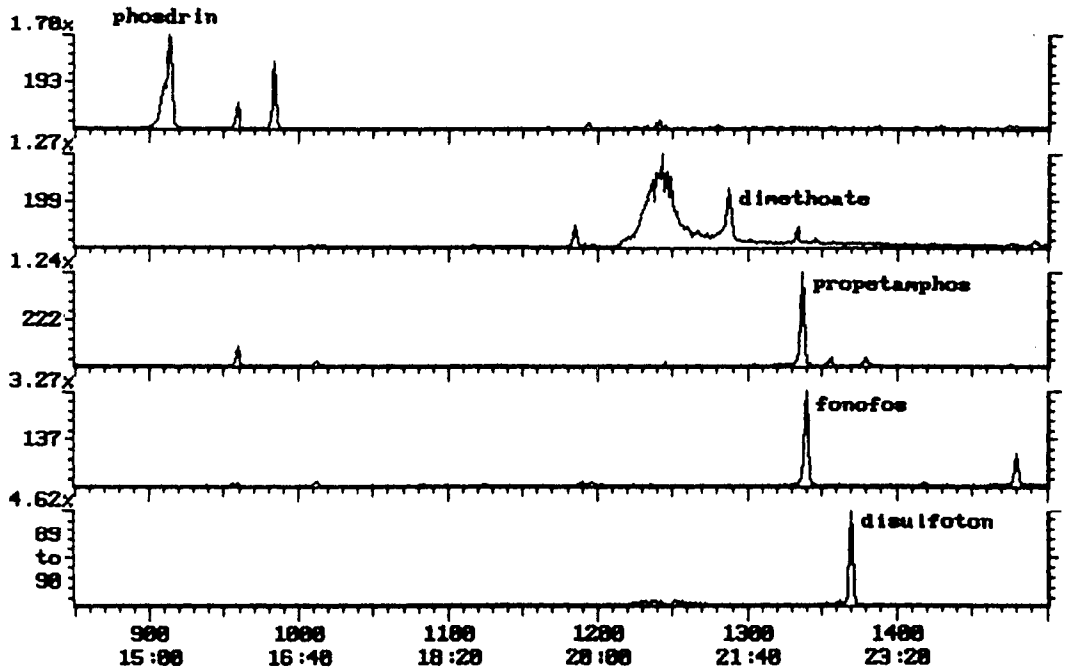

Figure 19. Mass chromatograms of organophosphate insecticides recovered from a corn flake spiked at 100 ppb

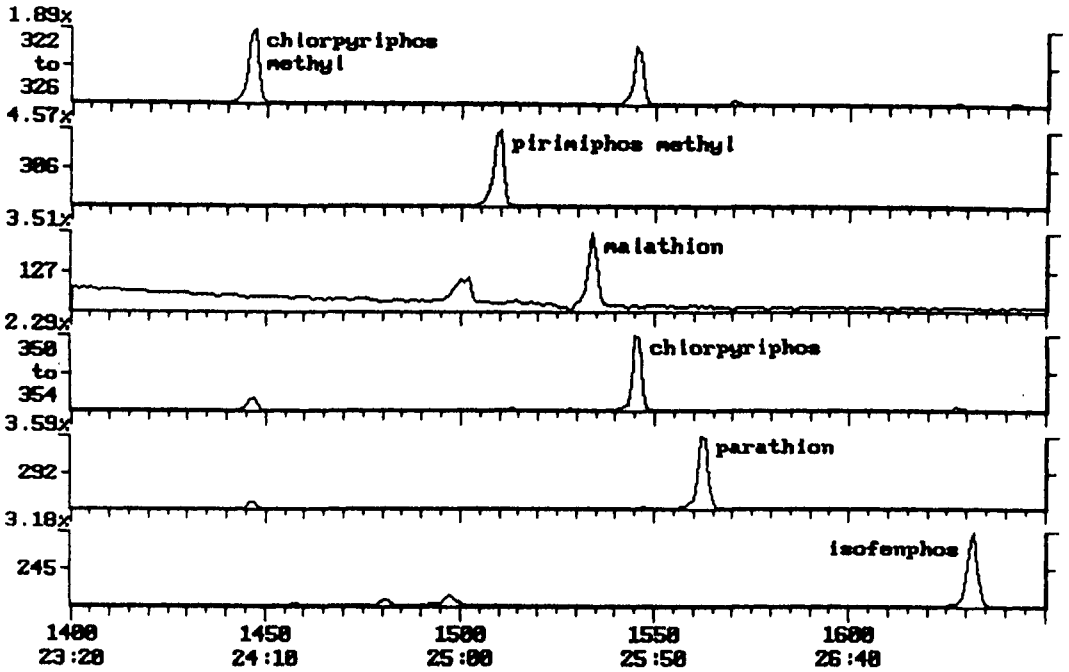

Figure 19. Mass chromatograms of organophosphate insecticides recovered from a corn flake spiked at 100 ppb (continued)

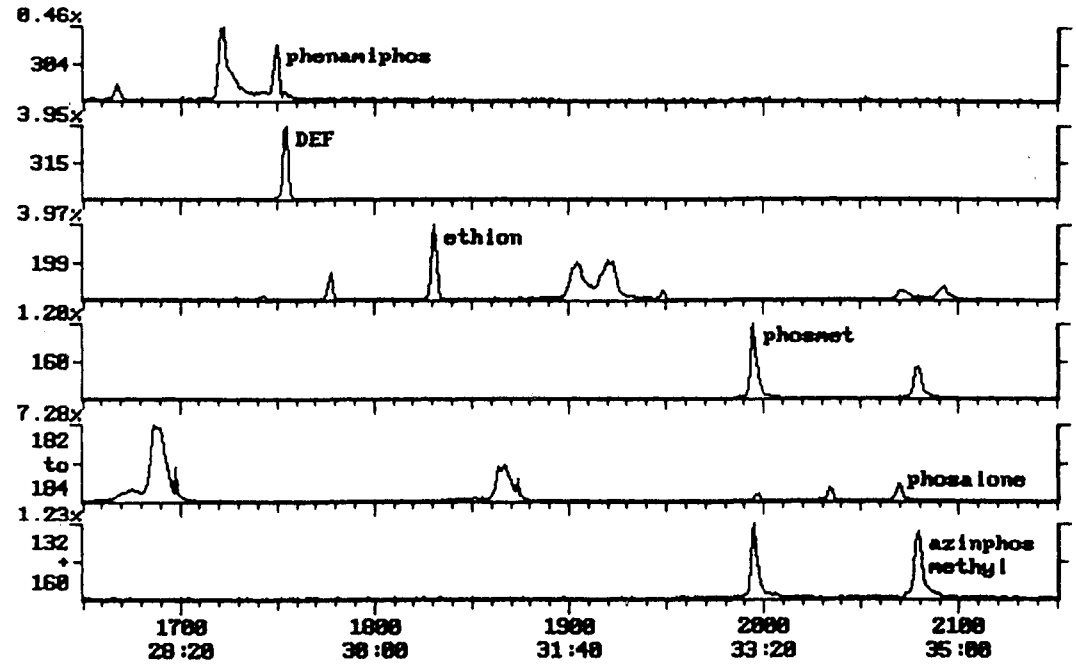

Figure 19. Mass chromatograms of organophosphate insecticides recovered from a corn flake spiked at 100 ppb (continued)

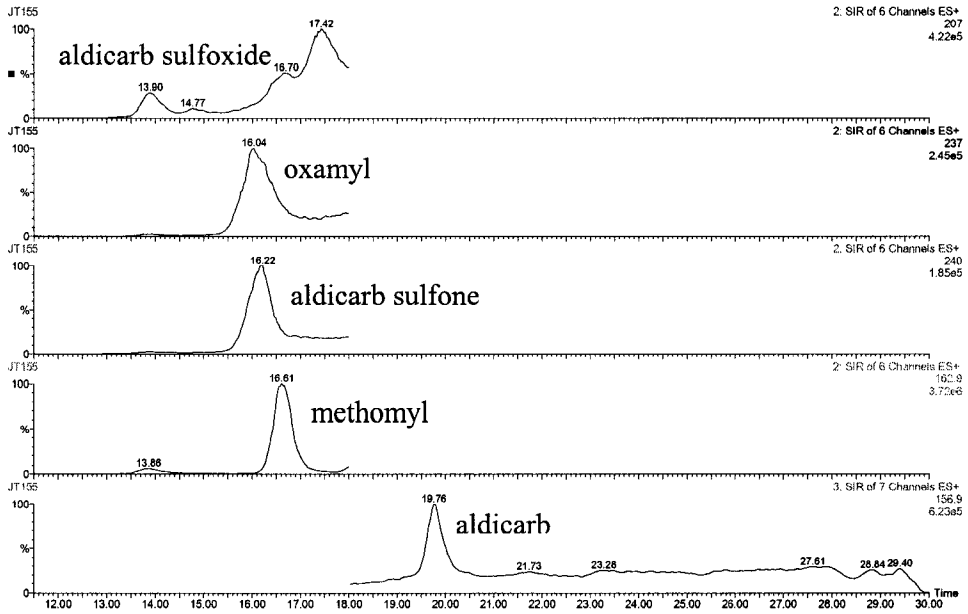

Figure 20. SIM mass chromatograms of pesticides recovered from a toasted oat spiked at 100 ppb

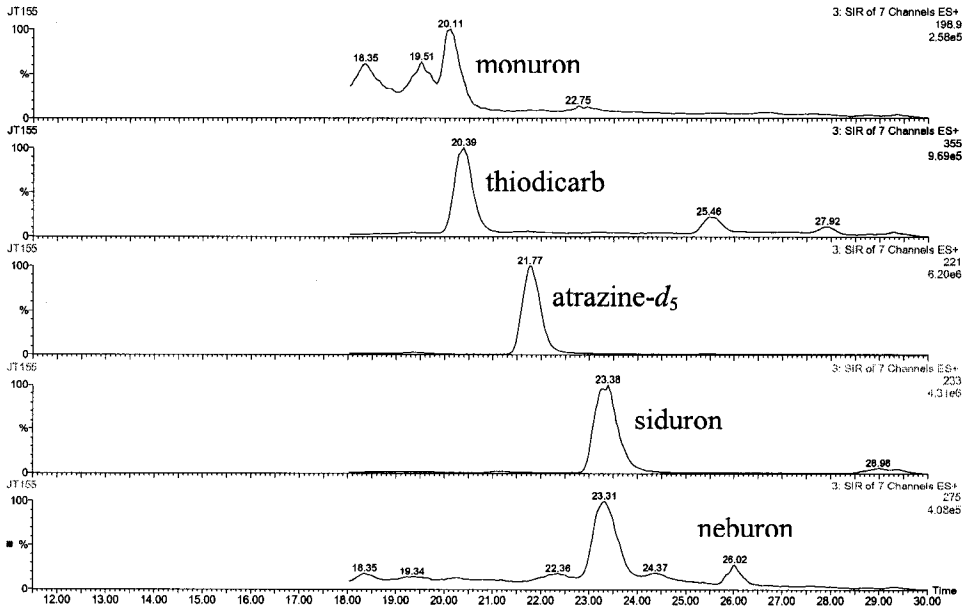

Figure 20. SIM mass chromatograms of pesticides recovered from a toasted oat spiked at 100 ppb (continued)

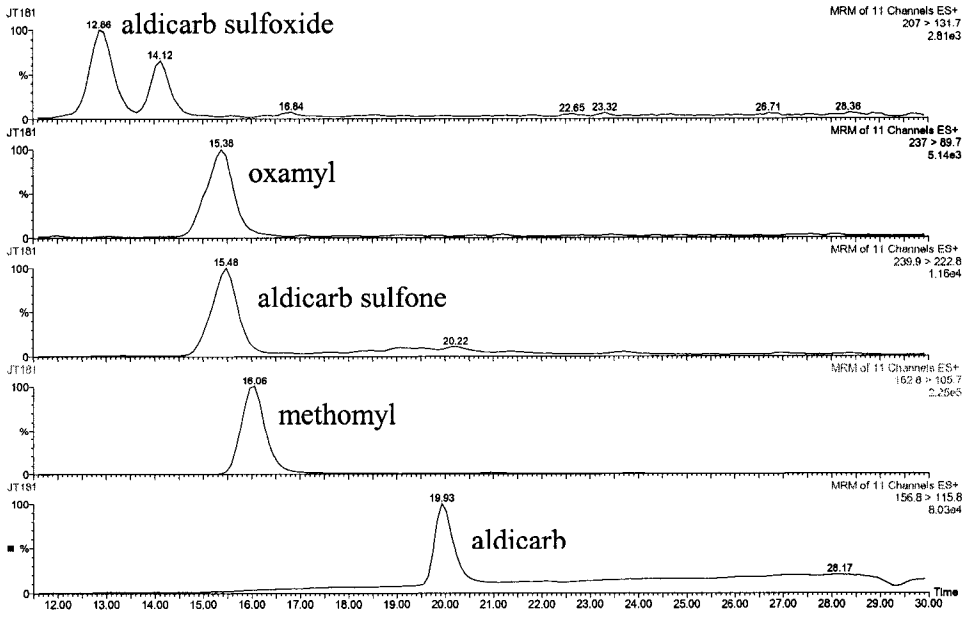

Figure 21. MRM mass chromatograms of pesticides recovered from a toasted oat spiked at 100 ppb

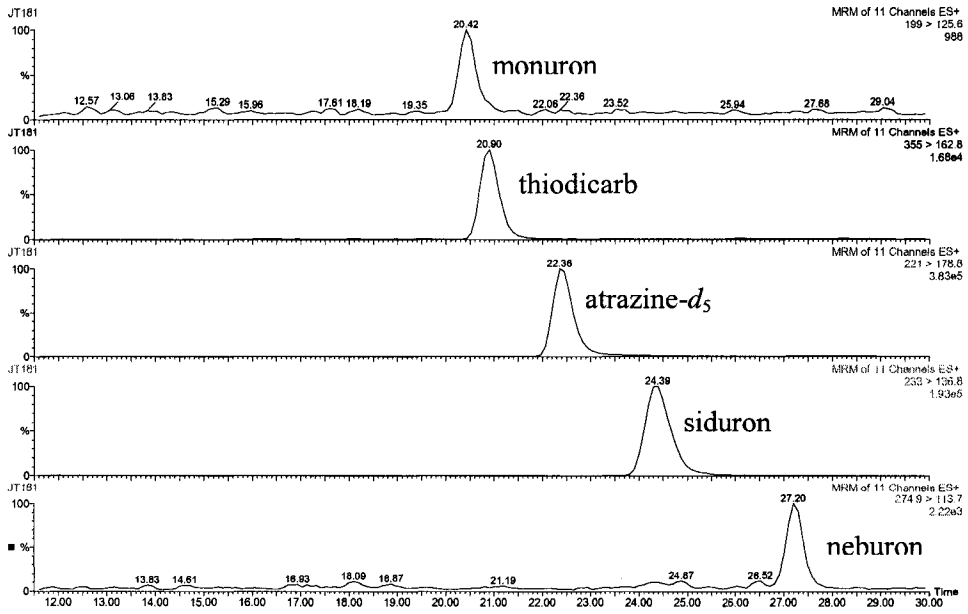

Figure 21. MRM mass chromatograms of pesticides recovered from a toasted oat spiked at 100 ppb (continued)

REFERENCES

American Oil Chemists' Society, 1990, Official Methods and Recommended Practices, 4th ed. Ca 6a-40

Andersson, T., Förlin, L., Härdig, J. and Larsson, Å., 1988, Physiological Disturbances in Fish Living in Coastal Water Polluted with Bleached Kraft Pulp Mill Effluents, Canadian Journal of Fishery and Aquatic Science, 45, 1525-1536

Baker, B.P., Benbrook, C.M., Groth III, E. and Benbrook, K.L., 2002, Pesticide Residues in Conventional, Integrated Pest Management (IMP)-Grown and Organic Foods: Insights from Three US Data Sets, Food Additives and Contaminants, 19, 427-446

Bligh, E.G. and Dyer, W.J. 1959, A Rapid Method of Total Lipid Extraction and Purification, Canadian Journal of Biochemistry and Physiology, 37(8), 911-917

Bortone, S.A. and Cody, R.P., 1999, Morphological Masculinization in Poeciliid Females from a Paper Mill Effluent Receiving Tributary of the St. Johns River, Florida, USA, Bulletin of Environmental Contamination and Toxicology, 63, 150-156

Cody R.P. and Bortone, S.A., 1997, Masculinization of Mosquitofish as an Indicator of Exposure to Kraft Mill Effluent, Bulletin of Environmental Contamination and Toxicology, 58, 429-436

Colborn, T., Dumanoski, D. and Myers, J.P., 1996, Our Stolen Future: Are We Threatening Our Fertility, Intelligence, and Survival?: a Scientific Detective Story, (New York : Dutton)

Consumers Union, 1998, Greener Greens? Consumer Reports, 63(1), 12-18

Cook, D.L., LaFleur, L., Parrish, A., Jones, J. and Hoy, D., 1997, Characterization of Plant Sterols from 22 US Pulp and Paper Mills, Water Science and Technology, 35, 297-303

DiPaola, R.S., Zhang, H., Lambert, G.H., Meeker, R., Licitra, E., Rafi, M.M., Zhu, B., Spaulding, H., Goodin, S., Toledano, M.B., Hait, W.N., Gallo, M.A., 1998, Clinical and Biologic Activity of an Estrogenic Herbal Combination (PC-SPES) in Prostate Cancer, The New England Journal of Medicine, 339, 785-791

Economic Research Service/USDA, 2001, Tracking Wholesale Prices for Organic Produce, Agricultural Outlook, October

Elghamry, M.I. and Hänsel, R., 1969, Activity and Isolated Phytoestrogen of Shrub Palmetto Fruits (*Serenoa repens* Small), a New Estrogenic Plant, Experientia, 25, 828-829

FDA, 1994, Pesticide Analytical Manual, 3rd ed.

FDA, Pesticide Program: Residue Monitoring 1996-1999, http://vm.cfsan.fda.gov/~dms/pesrpts.html

Federal Register, 2000, Final national organic standards rule, December 21

Gagné, F., Blaise, C., Lachance, B., Sunahara, G.I. and Sabik, H., 2001, Evidence of Coprostanol Estrogenicity to the Freshwater Mussel *Elliptio complanata*, Environmental Pollution, 115, 97-106

Gaido, K.W., Leonard, L.S., Lovell, S., Gould, J.C., Babaï, D., Portier, C.J. and McDonnell, D.P., 1997, Evaluation of Chemicals with Endocrine Modulating Activity in a Yeast-Based Steroid Hormone Receptor Gene Transcription Assay, Toxicology and Applied Pharmacology, 143, 205-212

Goodfellow, R.M., Cardoso, J., Eglinton, G., Dawson, J.P. and Best, G.A. 1977, A Fecal Sterol Survey in the Clyde Estuary, Marine Pollution Bulletin, 8, 272-276

Hurst, P., Hay, A. and Dudley, N., 1991, The Pesticide Handbook, Journeyman Press, London

Jin, B., 1999, Multiresidue Determination of Pesticides in Lettuce, Tomatoes and Celery by Gas Chromatography/Chemical Ionization/Ion Trap/Mass Spectrometry and Liquid Chromatography/Atmospheric Pressure Chemical Ionization/Mass Spectrometry, Ph.D. Thesis, Rutgers University, New Brunswick, NJ

Jones, G.J., Nichols, P.D. and Shaw, P.M., 1994, "Analysis of Microbial Sterols and Hopanoids", in *Chemical Methods in Prokaryotic Systematics*, M. Goodfellow and A. G. O'Donnel, eds., John Wiley, Chichester, United Kingdom

MacLatchy, D.L. and Van Der Kraak, G.J., 1995, The Phytoestrogen β-Sitosterol Alters the Reproductive Endocrine Status of Goldfish, Toxicology and Applied Pharmacology, 134, 305-312

Mäkelä, S., Poutanen, M., Lehtimäki, J., Kostian, M.-L., Santti, R. and Vihko, R., 1995, Estrogen-Specific 17β-Hydoxysteroid Oxidoreductase Type 1 (E.C. 1.1.1.62) as a Possible Target for the Action, Proceedings in Society for Experimental Biology and Medicine, 208, 51-59

Malini, T. and Vanithakumari, G., 1993, Effect of β-Sitosterol on Uterine Biochemistry: A Comparative Study with Estradiol and Progesterone, Biochemistry and Molecular Biology International, 31, 659-668

Mattern, G., Singer, G., Louis, J., Robson, M. and Rosen, J. 1990, Determination of Several Pesticides with a Chemical Ionization Ion Trap Detector, Journal of Agricultural and Food Chemistry, 38, 402-407

Mellanen, P., Petänen, T., Lehtimäki, J., Mäkela, S., Bylund, G., Holmbom, B., Mannila, E., Oikari, A. and Santti, R., 1996, Wood-Derived Estrogens: Studies *in Vitro* with Breast Cancer Cell Lines and *in Vivo* in Trout, Toxicology and Applied Pharmacology, 136, 381-388

Mogadati, P., Louis, J. and Rosen, J. 1999, Multiresidue Determination of Pesticides in High-Organic-Content Soils by Solid-Phase Extraction and Gas Chromatography/Mass Spectrometry, Journal of AOAC International, 82, 705-715

Munkittrick, K.R., McMaster, M.E., Portt, C.B., Van Der Kraak, G.J., Smith, I.R. and Dixon, D.G., 1992, Changes in Maturity, Plasma Sex Steroid Levels, Hepatic Mixed-Function Oxygenase Activity, and the Presence of External Lesions in Lake Whitefish (*Coregonus clupeaformis*) Exposed to Bleached Kraft Mill Effluent, Canadian Journal of Fishery and Aquatic Science, 49, 1560-1569

Murphy, K. 1997, There's Big Green in Organic Food, Business Week, October 6, 170

National Research Council, 1999, Hormonally Active Agents in the Environment, (Washington, D.C. : National Academy Press)

New Jersey Department of Environmental Protection, Private Well Testing Act, www.state.nj.us/dep/pwta

Nichols, P.D., Leeming, R., Rayner, M.S., Latham, V., Ashbolt, N.J. and Turner, C. 1993, Comparison of the Abundance of the Fecal Sterol Coprostanol and Fecal Bacterial Groups in Inner-Shelf Water and Sediments near Sydney, Australia, Journal of Chromatography, 643, 189-195

Obst, J.R., 1998, Special (Secondary) Metabolites from Wood, In Forest Products Biotechnology, edited by Bruce, A.M. and Palfreyman, J.W., London: Taylor & Francis, p.156

Safe, S., 1995, Environmental and Dietary Estrogens and Human Health – Is There a Problem? Environmental Health Perspectives, 103, 346-351

Tabak, H.H., Bloomhuff, R.N. and Bunch, R.L. 1972, Coprostanol: A Positive Tracer of Fecal Pollution, *In* Developments in Industrial Microbiology, American Institute of Biological Sciences: Washington, D.C., pp. 296-307

Tremblay, L. and Van Der Kraak, G., 1998, Use of a Series of Homologous *in vitro* and *in vivo* assays to Evaluate the Endocrine Modulating Actions of β-Sitosterol in Rainbow Trout, Aquatic Toxicology, 43, 149-162

Walker, R.W., Wun, C. and Litsky, W. 1982, Coprostanol as an Indicator of Fecal Pollution, CRC Critical Reviews in Environmental Control, 10, 91-112

Woese, K., Lange, D., Boess, C. and Bögl, K.W., 1997, A Comparison of Organically and Conventionally Grown Foods-Results of a Review of the Relevant Literature, Journal of the Science of Food and Agriculture, 74, 281-293

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