


[CDC Home](#)
[CDC Search](#)
[CDC Health Topics A-Z](#)

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[NIOSH Home](#)
[NIOSH Search](#)
[Site Index](#)
[Contact Us](#)

[NIOSH Home](#) > [Safety and Health Topics](#) > [Skin Exposures and Effects](#) > [Int. Conference on Occupational & Environmental Exposures of Skin to Chemicals](#) > [Abstracts](#)



Proceedings of the International Conference on Occupational & Environmental Exposures of Skin to Chemicals: Science & Policy

Hilton Crystal City September 8-11, 2002

Improved Method to Measure Alkoxyacetic Acid in Urine: A Solid Phase Extraction -Gas Chromatography - Mass Spectrometry Method for 2- Butoxyacetic Acid (the Human Metabolite of 2-Butoxyethanol), 2-[(2H9)Butox acetic acid, and 2-(3-Methylbutoxy)acetic acid

Site Contents

- [Main Page](#)
- [General Information](#)
- [Conference Agenda](#)
- [Posters](#)
- [Attendees](#)
- [Authors](#)
- [Course Information](#)
- [Vendor Exhibits](#)
- [Products](#)
- [Workshop Discussion Paper \(Version of 20 August 2002\)](#)
- [Disclaimer](#)

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Historically, the analysis of polar alkoxyacetic acid metabolites has been problematic, involving laborious extraction and derivatization (e.g., Smallwood et al., 1988. Appl. Ind. Hyg. 3(2), 47-50). Thus, NIOSH researchers developed a method to biomonitor alkoxyacetic acids, based on a published method for butoxyacetic acid (Shih et al., 1999. J. Occup. Environ. Med. 56(7), 460-467). Shih et al. had eliminated the derivatization step and its imprecision contribution in measuring butoxyacetic acid by using a free fatty acid phase (FFAP) capillary column in the gas chromatography/mass spectrometry (GC/MS) system. In addition, the NIOSH method eliminated the need for the laborious liquid extraction by replacing it with solid phase extraction (SPE), using a pH-resilient anion exchange resin. This improved method was then optimized for the measurement of butoxyacetic acid (BAA), using novel deuterated butoxyacetic acid (dBAA) as an isotopically diluted internal standard and novel 2-methylbutoxyacetic acid (MBAA) as a recovery/instrumental performance standard.

Previous BAA methods used internal standards such as pentoxyacetic acid, 3-chloropropionic acid, or dichlorobenzene to enhance accuracy and precision. The isotope dilution internal standard, dBAA improved upon these previous candidates. The two novel compounds were prepared using a synthesis method previously applied to make other alkoxyacetic acids, and their properties were characterized. MBAA was used as an instrument performance standard and recovery standard by adding it to each sample extract injected, and then monitoring the instrument's

response to it during the study.

The GC/MS analytical instrumentation consisted of a Hewlett Packard HP-6890 gas chromatograph, an HP-5973 mass spectrometer, and an HP-7673 automatic liquid sampler. The 2- μ L samples were injected at the fastest rate in splitless mode. Injection port temperature was 250 °C. The carrier gas was ultra pure helium, used a constant flow mode of 1.0 ml/min. The injection port purge flow was set at 50 ml/min for a 2.00-minute time period. The GC oven temperature was held at 80 °C for 2 minutes and, then, ramped at 2.0 °C/min until it reached 105 °C, after which it was stepped up as rapidly as possible to 240 °C for a 15-min bake.

MBAA and dBAA were synthesized, mixed with commercially available BAA, and separated by gas chromatography on an FFAP wall coated open tubular (WCOT) capillary column that had an $l = 50$ m, $d_c = 0.200$ mm, and $d_f = 0.3$ μ m. The standards dBAA, BAA, and MBAA were baseline resolved, eluting at $t_R = 13.55$, 13.78, and 15.20 min, respectively. The chromatographic efficiency of MBAA was calculated at $N = 360,000$. The analytes were detected by using mass spectrometry (MS) in the selective ion mode (SIM) and monitored at the target and qualifying ion at m/z 66 and 96 for dBAA, 57 and 60 for BAA, and 70 and 101 for MBAA. The instrumental response for BAA amounts from 0.04 ng to at least 200 ng was linear, having a limit of detection (LOD) of 0.04 ng.

Two standard solutions of BAA in urine were prepared at 500 ppm and 0 ppm, and both made with 10-ppm dBAA. The 500-ppm and 0-ppm BAA urine solutions were mixed in varying proportions to generate 41 urine solutions of BAA, ranging in concentrations from 500 to 0.01 ppm, each with 10-ppm dBAA. In a similar manner external calibration standards were prepared from a 100-ppm and 0-ppm BAA solution in acetonitrile and 2% formic acid, both solutions with 10-ppm dBAA. The 100-ppm and 0-ppm calibration solutions were mixed in varying proportions to generate 37 calibration solutions ranging from 100 to 0.01 ppm, each with 10-ppm dBAA. The urine standard solutions were processed by SPE. Both the urine extract and calibration standards were analyzed by GC/MS in a 2- μ L injection sequence, alternating between an extract and a standard. The urine extracts and calibration solutions were randomized within the alternating sequences.

Figure 1 shows the resulting chromatograms from injecting the dBAA, BAA, and MBAA calibration solution mixture under the conditions of two different column lengths. In both systems all three components were separated. Note that BAA was resolved from dBAA. Figure 2 shows the mass spectrum of components taken at the center of their respective chromatographic peaks. The BAA spectrum showed fragment ions at m/z of 57, 56, 60, 73, and 87; the dBAA spectrum showed fragment ions at m/z of 66, 64, 62, 96, and 82; and the MBAA showed fragment ions at m/z of 71, 70, 69, 55, and 101.

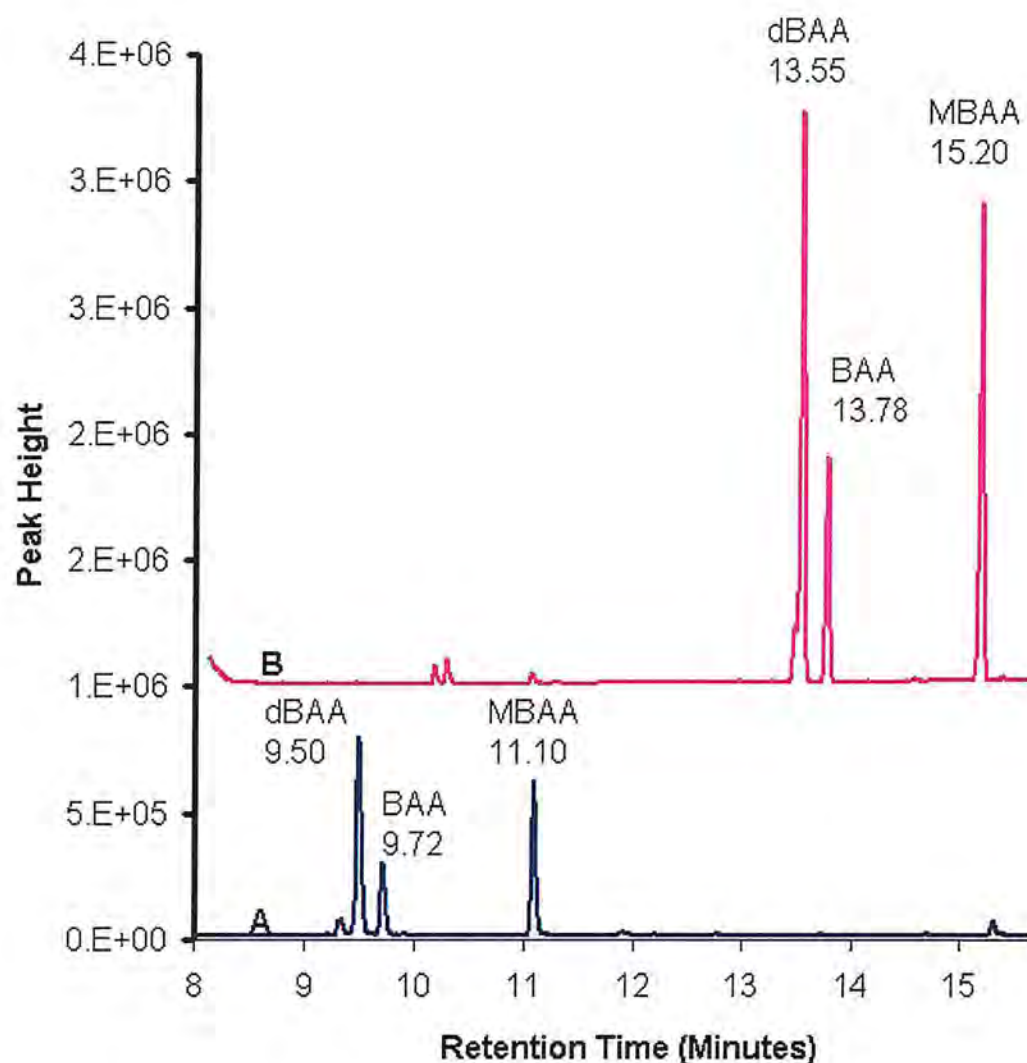


Figure 1: Chromatograms of dBAA, BAA, and MBAA in acetonitrile/2%formic ac from 2 different columns, (A) a 30 m, 0.25 mm, 0.25 μ m HP-FFAP, and (B with response offset of 1E+06 units) 50 m, 0.20 mm, 0.3 μ m HP-FFAP. Helium carrier had a constant flow of 1.0 ml/min, and the detection was done using MS-Total Ion Count (TIC).

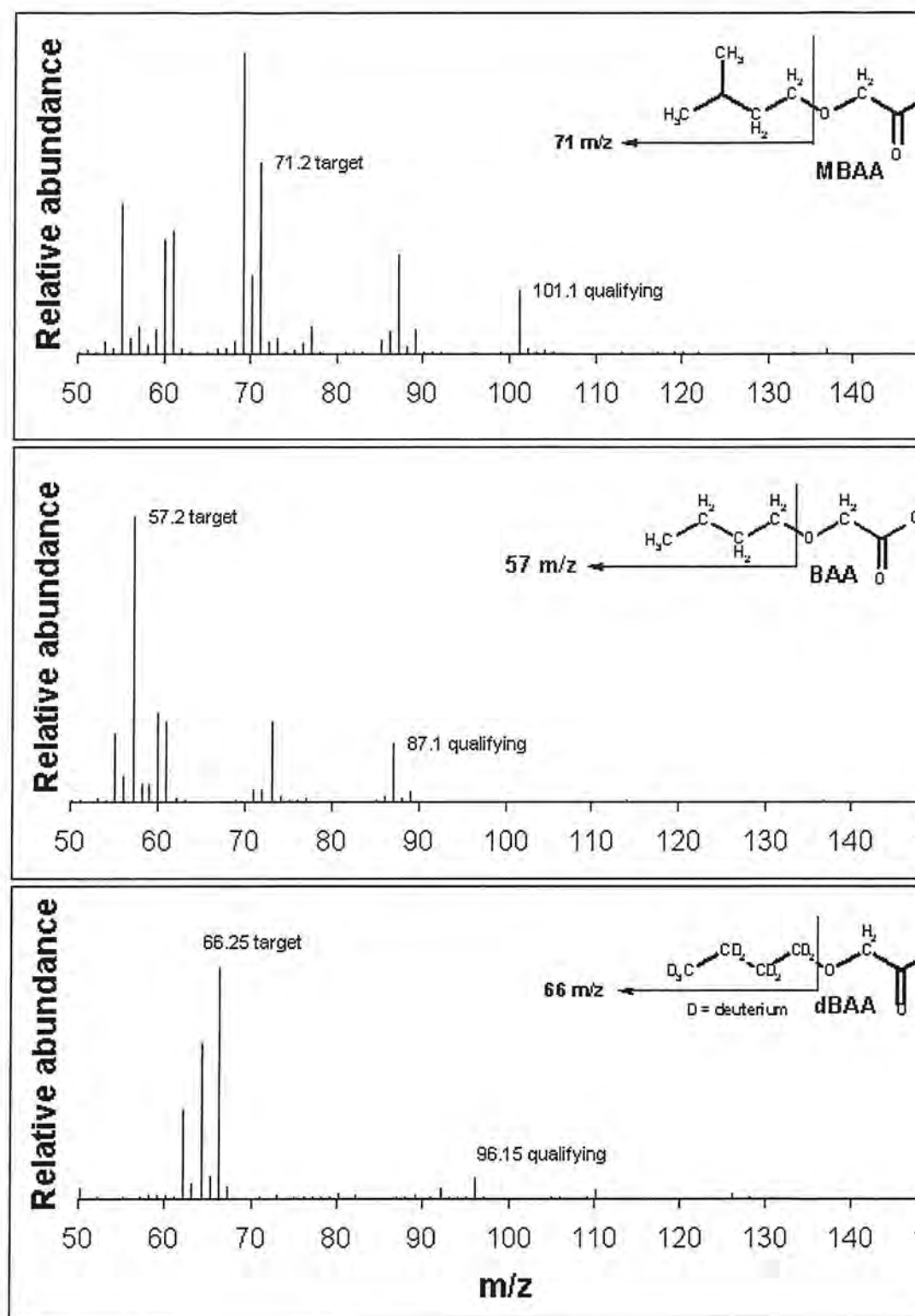


Figure 2. The mass spectra of dBAA (perdeuterobutoxyacetic acid), BAA (2-butoxyacetic acid), MBAA (2-(3-methylbutoxy)acetic acid). The most abundant fragment is the alkyl moiety of the molecule in each case of fragmentation.

An SPE-GC/MS biomonitoring method was developed to measure BAA, dBAA, or MBAA acid in urine extract. Two SPE approaches were developed; a 1-minute

extraction, using 30 mg of Strong Anion Exchange (SAX) resin and resulting in an LOD of 0.7 ppm; and a 30-minute extraction, using 500 mg of SAX resin and resulting in an LOD of 0.2 ppm. The 30-minute extraction is to be reported later. Using a FFAP GC column, extracts could be directly injected without derivatization and separated in 15 minutes. The method's linear response was verified from 0.7 to 500 ppm using spiked urine samples, mass spectrometric detection, isotopically diluted internal standard, and a recovery standard.

Sample Preparation and Solid Phase Extraction Procedure: The urine samples were acidified, spiked with internal standard, and passed through an SPE cartridge. A 1-ml urine sample was mixed with 0.275 ml of formic acid and 0.1 ml of 137.5-ppm dBAA internal standard to make a urine solution with 20% formic acid and 10 ppm dBAA. A 30-mg/1-ml SPE cartridge was placed in the vacuum manifold stepped through the following procedure. Step 1: the SPE cartridge was conditioned by elution with 1 ml of methanol. Step 2: the SPE cartridge was further conditioned by elution with 1 ml of water. Step 3: the urine mixture was loaded onto the cartridge and slowly drawn through the extraction bed, almost at a gravity-pulled rate. Step 4: the SPE cartridge was rinsed with 1 ml pH 7 buffer. Step 5: SPE cartridge was rinsed with 1 ml methanolic solution in pH 7 buffer. Step 6: the cartridge then was dried by a flow of air for 15 minutes. Step 7: the BAA and dBAA were eluted off the SPE cartridge with 2 1-ml portions of a 98 % methanol and 2% formic acid solution into 15-ml tube. Step 8: 1 ml of the extract was transferred to a GC vial. Step 9: 0.1 ml of 110-ppm MBAA was added to the extract as a recovery/instrumental performance standard. The extract was analyzed for BAA, dBAA, and MBAA by GC/MS.

These extraction parameters were chosen for optimum analyte concentration, not recovery efficiency. A recovery of 100% could be obtained using 0.1 ml urine, but would result in 1:20 dilution and a lower analyte concentration. Maximum analyte concentration was obtained using 1.0-ml urine, but it resulted in a 20% recovery. Theoretically, there should be very little difference between the recoveries of BAA and dBAA. Hence, the isotope dilution method corrects for incomplete recovery.

In Figure 3, the instrumental response to each of the urine extracts and each of the calibration standards is plotted. The calibration standards had a linear response to concentration determined by linear regression analysis, which produced a linear equation of $Y = 11219X - 2752$, where Y = peak height response, X = ng of BAA in the original solution. The correlation coefficient was $r^2 = 0.9923$, and the LOD = 0.1 ng. The LOD was determined from the linear regression data using the equation $LC = (3 * Se_y) / m$, where $Se_y = 5404$ and $m = 11219$ height units/ng. When the calibration solution response was normalized to the internal standard response, the LOD dropped to 0.1 ng.

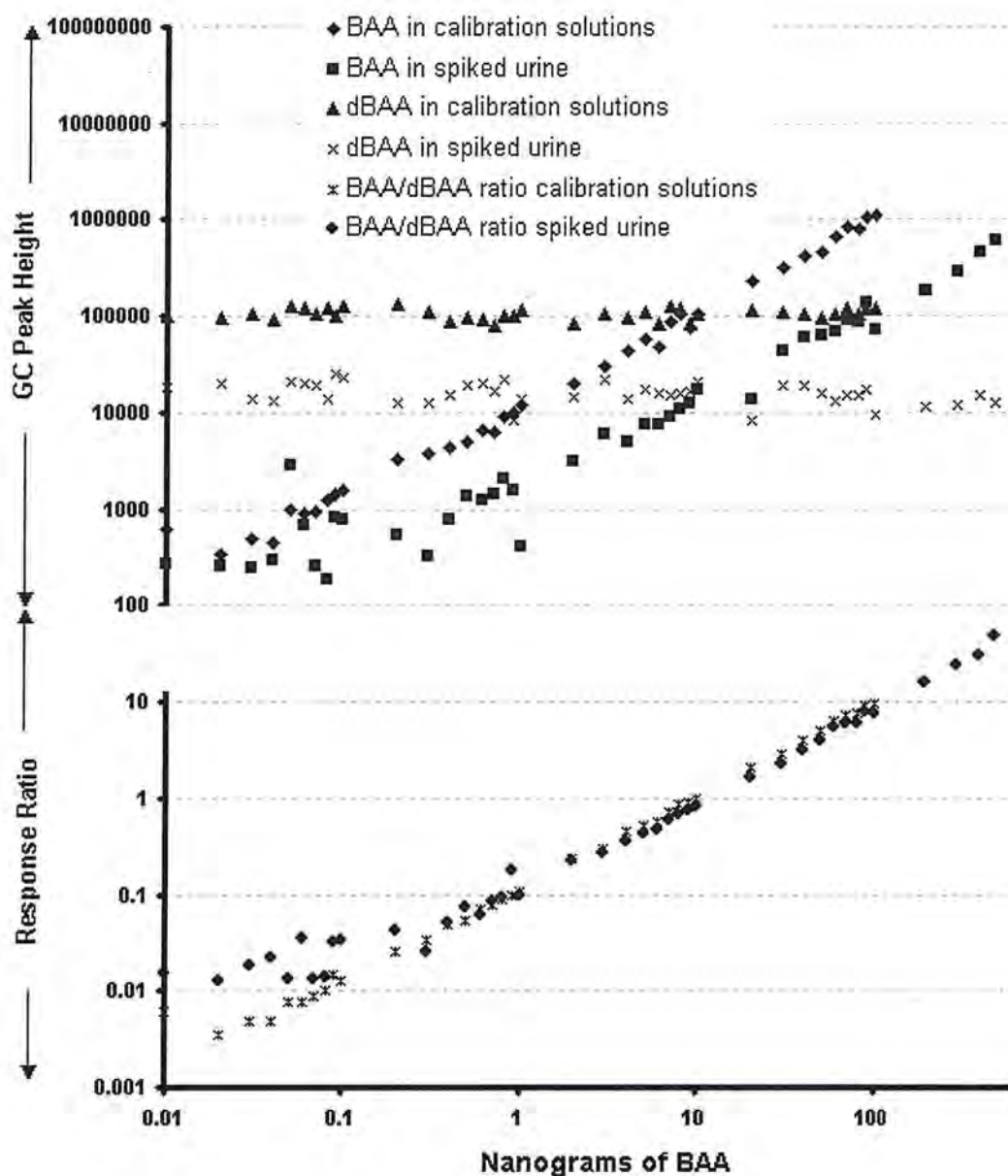


Figure 3: Analytical characterization plots for the 30-mg SPE method. The 40 calibration samples and 40 spiked urine samples ranged in BAA concentrations from 0.01 to 1000 ng per 2- μ l. Internal standard, dBAA, was present in each sample at 10 ppm. The calibration samples were injected directly into the GC-MS, and the spiked urine samples went through the SPE method first. The horizontal plots are the calibration dBAA and spiked urine dBAA responses. The upper two sloped plots are the calibration BAA and spiked urine BAA responses. The lower two sloped plots are the response ratios of calibration BAA to calibration dBAA and the response ratios of the spiked urine BAA to spiked urine dBAA.

Like the calibration standards, the spiked urine solutions produced a linear response to concentration, determined by linear regression analysis, resulting in a linear equation of $Y = 1143X + 155$ where Y = peak height response, X = ng in original

solution. The correlation coefficient was $r^2 = 0.9846$, and the LOD = 6 ng. When the spiked urine solutions response was normalized to the internal standard response, the LOD dropped to 1 ng.

In summary, an improved method to measure alkoxyacetic acids in urine was developed that uses a one-minute solid phase extraction procedure. The 30-g/1-ml SPE cartridge was selected for convenience and economy and because it is also available in 96-well format for high throughput. The gas chromatography mass spectrometry instrumentation used a FFAP capillary column that eliminated derivatization of the analyte for analysis, and the method was modeled after Shih et al. (1999, *J. Occup. Environ. Med.* 56(7), 460-467). The method was developed using the human metabolite of 2-butoxyethanol, 2-butoxyacetic acid. The novel synthesis and use of the isotopically diluted internal standard 2-[(2H9)butoxy]acetic acid and the recovery standard 2-(3-methylbutoxy)acetic acid enhanced the method's precision and accuracy. The synthesis of 2-[(2H9)butoxy]acetic acid and 2-(3-methylbutoxy)acetic acid was a one-step synthesis based on the synthetic procedure of J.R. Pribish, (*J. Labelled Compd. Rad.* 36/3 (1995) 225) using economically available reagents. The method was characterized using spiked urine samples. The primary improvements of this method are simplicity and speed.

[Return](#)



[NIOSH Home](#) > [Safety and Health Topics](#) > [Skin Exposures and Effects](#) > Int. Conference on Occupational & Environmental Exposures of Skin to Chemicals



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Present the Proceedings for

The International Conference on Occupational & Environmental Exposures of Skin to Chemicals: Science & Policy

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Site Contents

- [Main Page](#)
- [General Information](#)
- [Conference Agenda](#)
- [Posters](#)
- [Attendees](#)
- [Authors](#)
- [Course Information](#)
- [Vendor Exhibits](#)
- [Products](#)
- [Workshop Discussion Paper \(Version of 20 August 2002\)](#)
- [Disclaimer](#)

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[CDC Home](#)

[CDC Search](#)

[CDC Health Topics A-Z](#)



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[NIOSH Home](#)

[NIOSH Search](#)

[Site Index](#)

[Contact Us](#)

[NIOSH Home](#) > [Safety and Health Topics](#) > [Skin Exposures and Effects](#) > [Int. Conference on Occupational & Environmental Exposures of Skin to Chemicals](#) > [General Information](#)



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Site Contents

- [Main Page](#)
- [General Information](#)
- [Conference Agenda](#)
- [Posters](#)
- [Attendees](#)
- [Authors](#)
- [Course Information](#)
- [Vendor Exhibits](#)
- [Products](#)
- [Workshop Discussion Paper \(Version of 20 August 2002\)](#)
- [Disclaimer](#)

The National Institute for Occupational Safety and Health (NIOSH) co-sponsored this inaugural conference to bring together dermatologists, occupational hygienists, laboratory researchers, policy makers and other to focus on the science, knowledge gaps and policy opportunities related occupational and environmental exposures of the skin to chemicals.

The site was the Hilton Crystal City at Ronald Reagan National Airport hotel. The main conference was followed by a one-day workshop focusin on specific research and public health opportunities for decreasing the burden of skin exposures to chemicals in both workplaces and the gener environment.

Approximately 135 individuals attended. A second conference is expecte in 2004.

[Return](#)