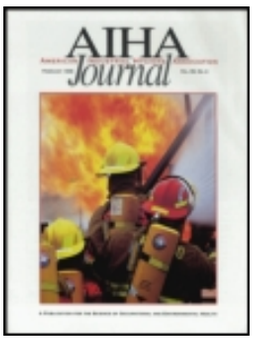

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Richard A. Fenske & Shari G. Birnbaum

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Second Generation Video Imaging Technique for Assessing Dermal Exposure (VITAE System)

AUTHORS
Richard A. Fenske[a]
Shari G. Birnbaum[b]*

[a]Department of Environmental Health, Box 357234, School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195; [b]Department of Environmental Sciences, Rutgers University, New Brunswick, NJ 09803; *Currently at the Interdepartment Neuroscience Program, Yale University, New Haven, CT 06510

Development of a second-generation video imaging technique for assessing occupational skin exposure (VITAE) is described, its performance evaluated, and new procedures for exposure quantification are presented. The current VITAE system has higher resolution in regard to both its picture element array and gray scale when compared with the prototype system. System performance was evaluated during extended field deployment: variability was 3–4% during data acquisition for individual worker evaluation sessions, and 10% over a 22-day study period. Variabilities attributable to subject positioning and image outlining procedures were 2.7 and 1.2%, respectively. Visual observations of fluorescent tracer deposition on skin were used to classify specific body regions as either exposed or unexposed, and two computer-based classification criteria were tested against the visual classifications. These criteria were generally better at minimizing false negative than false positive classification; sensitivity and predictive value reached 95 and 99%, respectively, when analysis was preceded by presampling of a subset of images. Variability in skin pigmentation was found to have a substantial effect on fluorescent tracer quantification, leading to development of new calibration procedures. Standard curves were generated by spotting a range of tracer concentrations on volunteer subjects and quantifying fluorescence with the VITAE system. These data were then grouped either by subject or by the magnitude of the background signal of the unexposed skin. The ability to control for the effects of skin pigmentation was found to be comparable for these two grouping methods, indicating that calibration curves can be developed without the creation of a unique curve for each subject.

Keywords: skin exposure, video imaging

The dermal route of exposure can contribute substantially to total dose for many chemical substances in occupational and residential environments. Recent studies have documented the significance of skin exposure across a wide variety of workplace settings.[1] Skin contamination may also be an important component of chemical exposures in residential environments, particularly for children.[2–4] Biological monitoring can assist in estimating dose for exposures that include a substantial contribution from the dermal route, but there is a clear value in assessment of dermal exposure patterns. Knowledge of the extent and distribution of skin contamination allows source characterization and development of effective exposure reduction strategies.

Progress in dermal exposure assessment has been hampered in part by a lack of accurate sampling methods.[5] Traditional methods include surrogate skin sampling through use of patches or whole body garments, and chemical removal techniques such as skin washing or wiping. However, these methods have limited accuracy and do not provide real-time information during field exposures.

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Visualization of skin exposure patterns with fluorescent tracers is a relatively new assessment method. Compounds known as fluorescent whitening agents (FWAs) were first reported to be useful tools for characterizing skin deposition of pesticide sprays in 1981.[6] Quantification of these compounds on human skin was achieved with a prototype video imaging technique for assessing exposure, or VITAE system.[7–9] Field studies with this prototype system demonstrated correlations of fluorescent tracer skin deposition with work time among timber mill workers exposed to chlorophenols,[10] and with urinary metabolite excretion following pesticide mixing and application.[11]

Despite these successes it became evident that this prototype system was extremely limited in its ability to quantify exposure. First, the computer employed had no hard drive, relying solely on two 8-inch floppy disk drives, so data collection and analysis were slow and tedious. Second, the prototype system employed the first analog-to-digital board available commercially for microcomputers; its digital resolution was limited to a matrix of 128 × 128 picture elements (pixels). This low spatial resolution produced block-like television images rather than the finer images we are accustomed to in commercial broadcasting. Third, the quantitative scale for light intensity, the gray level scale, was limited to 16 units (zero representing no light signal and 15 representing a maximum signal, with values between representing shades of gray). Thus, only large differences in fluorescent patterns on skin could be recorded.

VITAE System Chronology

A second generation VITAE system was assembled in the late 1980s to take advantage of advances in computer-based imaging analysis technology.[12] The new system greatly increased speed of operation, resolution, and quantitative range. The original VITAE software was revised substantially, and detailed laboratory studies led to new insights and calibration procedures regarding the influence of skin characteristics on fluorescence emission. These advances are described later in this article.

Beginning in 1988 the new VITAE system was deployed in the field to evaluate occupational exposures to pesticides. Studies in central Florida in the summers of 1988 and 1989 focused on the efficacy of chemical protective clothing during air-blast applications of ethion in citrus orchards[13] and demonstrated that limitations in garment design actually made so-called chemical protective clothing less effective than ordinary cotton coveralls or even cotton work shirts. A second series of studies in this same period focused on factors affecting pesticide exposure during greenhouse applications.[14,15] These studies demonstrated that proper use of unidirectional airflow within the greenhouse could reduce dermal exposure substantially, and that such use was dependent on applicator training. Also documented was the failure of several types of chemical protective clothing due to direct contact with treated foliage during applications.

The second generation system was then employed in a series of controlled field trials to estimate skin exposure to children contacting chemically treated lawns.[16] In these studies children played on turf that was treated with fluorescent tracer in place of pesticides, and adults conducted high contact activities to provide upper bound estimates of skin exposure. These exposure measurements with the VITAE system were coupled with analysis of activity patterns to help understand exposure mechanisms better. Parallel studies involving measurement of pesticide and tracer residues on turf (but no human contact) produced estimates of potential pesticide exposure for these populations.[17] A subsequent study compared whole body garment sampling and video imaging analysis of adults conducting a defined exercise program on tracer-treated turf.[18]

In the course of these field studies, the VITAE system methodology was refined and evaluated. The data presented in this article come in part from these field studies, as well as from concurrent laboratory studies, but have not been reported previously.

In the early 1990s laboratories at three other institutions adopted the new VITAE system methodology. In each case the authors' laboratory provided hardware specifications, software programs for the collection and analysis of data, and standard operating procedures for system calibration. Also, one of the authors provided on-site consultation in system theory, operation, and quantitation procedures to each laboratory. Researchers at the University of Iowa have used the system to estimate dermal exposures among golf course workers.[19] Researchers at the University of Guelph developed refinements to the calibration procedures[20] and employed the VITAE system in a field study of greenhouse applicators.[21] Researchers at TNO Laboratories in The Netherlands adopted the VITAE system in principle, but chose to use different hardware components, develop new software, and employ a different fluorescent tracer.[22] A fourth laboratory, in the United Kingdom, has also explored the use of fluorescent tracers and imaging technology, producing a novel lighting system to improve quantitative accuracy.[23] An international workshop was convened at the University of Iowa in June 1996 to discuss applications of the VITAE methodology.

The purpose of this article is to provide a review of advances in this technique and an analysis of several important methodological issues associated with quantitative use of the VITAE system. Its focus is four fold: (1) description of current operational procedures; (2) evaluation of system variability, particularly during deployment in extended field studies; (3) analysis of methods for classifying video images as either exposed or unexposed; (4) evaluation of calibration procedures with a focus on the effects of skin pigmentation.

Operational Procedures

Field Deployment

Typical use of this technique in the assessment of pesticide applicator exposure consists of the following steps.

(1) Fluorescent tracer application. A fluorescent tracer compound is introduced into the application process, either by premixing with the pesticide formulation or by direct addition into the spray tank. Handling of the tracer compound may be done by the workers themselves or by technical staff, depending on the purposes of the study.

(2) Pre-exposure worker evaluation. Prior to any contact with the fluorescent tracer, each worker in the study is evaluated with the imaging system. A layout of VITAE system components is presented in Figure 1, including both a plan and elevation view. Workers enter a darkened examination area and change into clothing provided by the investigator (black athletic shorts for men, shorts and black athletic bra for women). They stand behind a subject frame illuminated by the UV-A lamps, and follow instructions regarding positioning so that a series of digitized images can be recorded of all relevant skin surfaces. In the absence of the fluorescent tracer the skin is still visible, producing a variable pre-exposure signal, or background brightness.

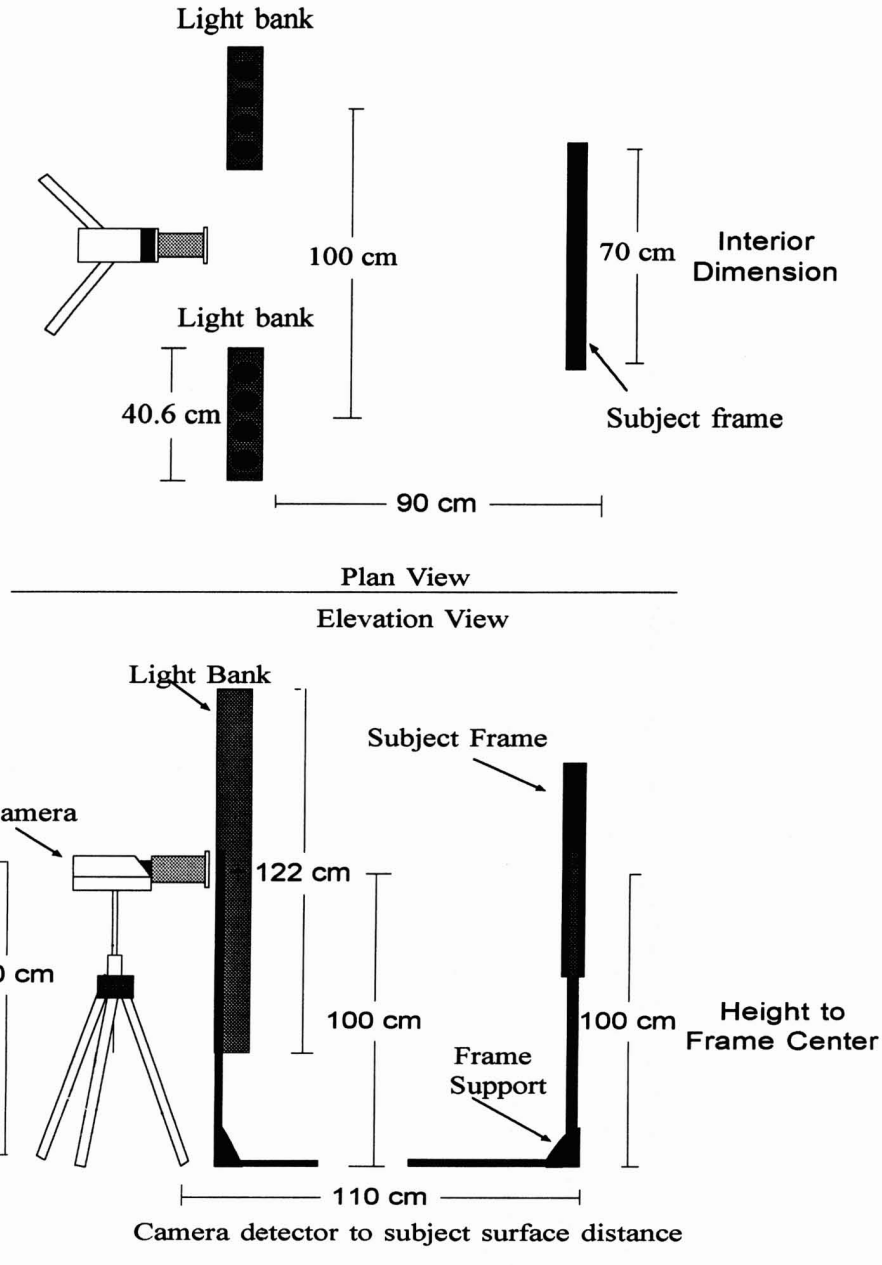

Figure 1. VITAE component layout. Subject stands or sits behind frame on right; camera is linked to computer-based analog-to-digital imaging board. Lamps each house four black light bulbs with additional UV-passing glass filters (source: reference 8).

(3) Worker exposure. When this initial examination session has ended, the worker changes back to work clothing and proceeds with pesticide application activities. Under normal interior lighting or sunlight the tracer compound is not visible, so there is no indication of exposure out of the ordinary during work.

(4) Postexposure worker evaluation. At the end of the application activities, the worker returns to the examination area and repeats the procedures described in Step 2. In this postexposure session, however, skin deposition patterns of the fluorescent tracer are likely to be evident. In addition to the collection of a second set of digital images of the skin surface, the pattern of tracer deposition is observed visually and preserved either by videotape or through a visual scoring system; that is, the amount and extent of tracer deposition is given a numerical score on a simple scale.[23] The use of a TV camera allows live viewing of the deposition patterns by the worker, so these sessions also serve as an important opportunity for workers to understand how they have been exposed and might prevent future exposures. Figure 2 illustrates the fluorescent tracer pattern detected on the hand and arm of a pesticide applicator who had been wearing protective gloves and a long-sleeved coverall during spraying.

The recording of digital images can be likened to a series of still photographs of various body parts and views; for example, the palms of the hands are captured in one image and the backs of the hands recorded in a second image. However, instead of light from the skin surface being imprinted on a photographic emulsion to produce a negative, as in still photography, the light signal is collected by a detector array within a television camera and stored as voltage. Periodically (30 times a second in this system) the voltage is converted to current and sent to a "frame grabber" housed in a microcomputer. The frame grabber converts the current from each point in the array to a digital value on a gray scale. This array of pixels with varying gray level values is the digital image. Location information for each pixel is retained so that the image can be reconstructed from its data file. The gray scale for the current system is zero to 255, with zero representing no current or light signal, and 255 representing a maximum light signal. The digital images are stored on the computer's hard drive as unique data files, each file consisting of an array of numbers that represent the original voltage array. The file can later be used to reconstruct the image on a television screen for viewing and analysis.

Quantitative Procedures

A detailed sequence of events and procedures during data acquisition and analysis is presented in Table I.

Data acquisition follows the steps outlined in the previous section. Most of the procedures conducted during data acquisition are related to quality assurance/control; that is, system warm-up, readings of ultraviolet light intensity and standard targets, and system noise adjustment. Variability during data acquisition is the first issue addressed in the experimental section of this paper.

Image preparation involves demarcating an area of interest within each image (i.e., specific anatomical region), and matching pre- and postexposure images. Polygonal boundaries are drawn

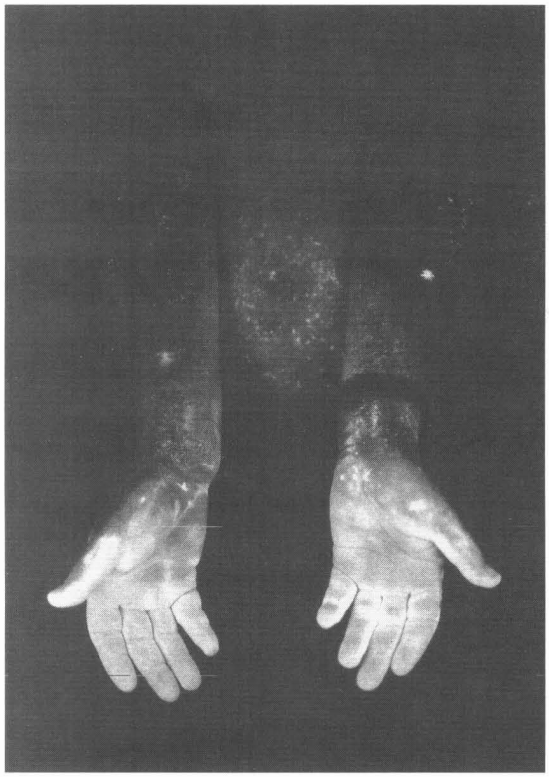

Figure 2. Fluorescent tracer exposure to the hands, forearms, and belly of a greenhouse pesticide applicator. Applicator had worn chemical protective gloves and a long-sleeved shirt while working. Protruding belly contacted foliage and equipment sufficient to cause tracer to break through shirt material.

around the body region on the postexposure image, and two anatomical reference points are selected. These reference points are then identified on the pre-exposure image, and the two images are overlaid automatically. The boundaries and reference points are saved as unique files for use in subsequent image analysis.

Image classification is aimed at separating those parts of the body that have received exposure from those that have not. The presence of tracer on the skin can be easily distinguished by the eye from background fluorescence or confounding materials such as lint; however, the VITAE system can detect only the strength and distribution of light emissions, and must distinguish these emissions from the background signal present in pre-exposure images. These characteristics have led to a relatively simple classification scheme of four categories: no exposure, high exposure, intense exposure (high intensity; spatially limited), and diffuse exposure (spatially extensive; low intensity). Intense exposure patterns may result from spray droplet deposition, splashing, or holes in protective clothing, and appear as bright spots occupying a very small fraction of the total skin surface. Diffuse exposure patterns may result from contact with fine sprays or contaminated surfaces, and appear as a widely distributed fluorescence slightly greater than background brightness.

Each histogram in Figure 3 represents the distribution of pixels across the gray level scale for a single pair of pre- and postexposure images. The pre-exposure distribution of pixels is constant across the four figures, with the median background gray level occurring between 10–20, and the range extending from 0–40. Figure 3a illustrates no exposure, in which there is little discernible change in pixel distribution. Figure 3b shows high exposure, in which most pixels have shifted to higher gray level values, and there is almost no overlap with the pre-exposure pixel distribution. Figure 3c represents an intense exposure pattern, in which the tail of the pixel distribution is extended to gray level values of 40–60, but the number of pixels at these higher levels is small. Figure 3d indicates a diffuse exposure pattern, in which the gray level range for the pre- and postexposure images is identical, but the mode of the pixel distribution has shifted markedly from about gray level 20 to gray level 30. The development of quantitative criteria for image classification is the second issue addressed in the experimental section of this article. QA/QC steps during image classification include adjustment for lens distortion and for differences in pre- and postexposure standard target readings.

Exposure calculation with the VITAE system consists of three steps: (1) anthropometric model adjustment, (2) image histogram subtraction, and (3) exposure quantification with appropriate calibration curves. Anthropometric model adjustment is required to correct for the nonplanar aspect of most skin surfaces. The models employed are geometric approximations of individual body parts and are essentially unchanged from the prototype system. Tests of these models indicated that exposures calculated from nonplanar surfaces were within 10% of identical exposures on a planar surface.

Paired pre- and postexposure images are analyzed by subtracting the number of pixels at each gray level in the pre-exposure image

TABLE I. Operational Procedures for the VITAE System

Sequence of Events	Procedure
System stabilization	30-min system warm-up
Data acquisition	
Pre-exposure session	UV-A intensity reading 1st standard target reading data collection system noise adjustment 2nd standard target reading
Subject exposure	
Postexposure session	UV-A intensity reading 1st standard target reading data collection system noise adjustment 2nd standard target reading
Qualitative exposure evaluation	visual exposure classification
Data collection completed	
Image preparation	
Image outline/overlay	postexposure image outline reference point selection post-pre image overlay
Image classification	
Image comparison	lens distortion adjustment standard target adjustment application of classification criteria
Exposure calculation	
Image analysis	anthropometric model adjustment histogram subtraction calibration curve analysis
Final report	tracer exposure (μg) per body part/view surface area (cm^2) per body part/view

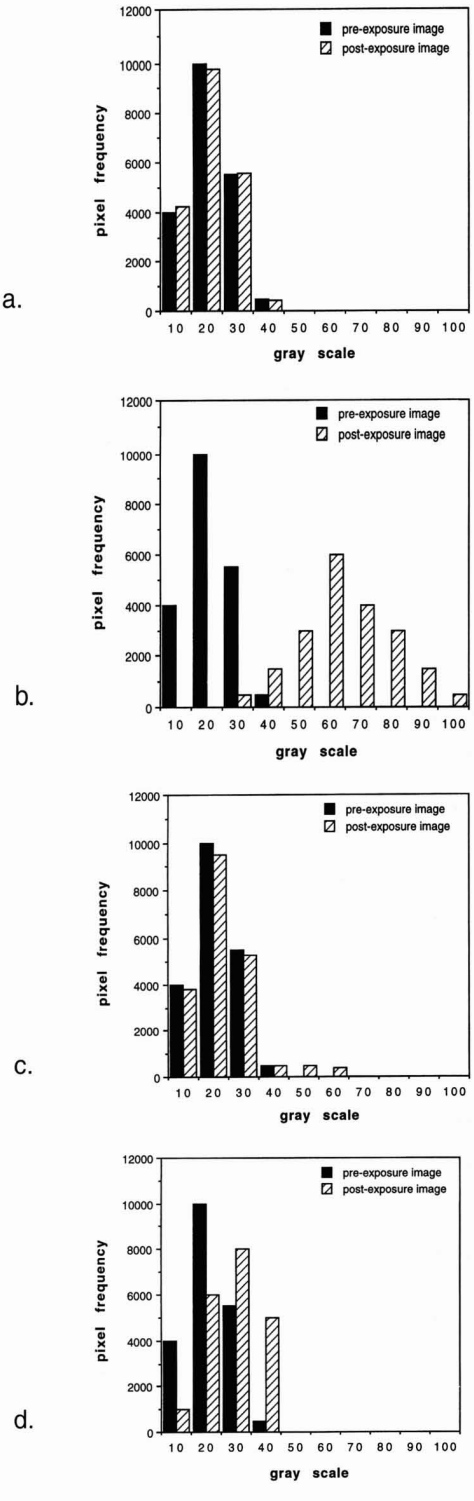

Figure 3. Pixel frequency distribution histograms analyzed by the VITAE system. Each of the histograms compares the distribution of 20,000 pixels from a pre-exposure and a postexposure image across the 256 gray scale (truncated to 100 gray levels here). a: no exposure shows no discernible change in the distribution of pixels; b: high exposure results in an upward shift of pixel distribution and almost no overlap between pre- and postexposure images; c: intense exposure results in the tail of the postexposure distribution exceeding that of the pre-exposure distribution, although most pixels remain in the background range; d: diffuse exposure results in a shift to higher gray levels for most pixels, but the range of pixel distribution is similar for the pre- and postexposure images.

from those in the postexposure image. The resulting pixel distribution across the gray level scale thus includes only those pixels representative of exposed skin. The brightness of these pixels is then translated to mass of tracer on the skin with calibration curves. Current procedures represent a significant modification of those used with the prototype system; this new approach is the third issue addressed in the experimental section.

In summary, the experiments reported here were conducted during the course of the development and deployment of the VITAE system and focus on three questions: (1) What is the variability associated with field deployment, subject positioning, and image outlining procedures? (2) What computer-based criteria can be used to distinguish between exposed and unexposed regions of the body? (3) How can the confounding effects of variable background signal from the skin be minimized during exposure quantitation?

METHODS

Equipment

The design of the current VITAE system is similar to that of the prototype instrument, consisting primarily of a television camera interfaced with a microcomputer with digital imaging capability and custom-designed software. Specific components of the current instrument are as follows: Dell 486 microcomputer with 320 Mbyte hard drive and 4 Mbyte RAM; Cohu 4810 monochrome CCD (charged coupled device) television camera, with a Fujinon TV zoom lens (H6 × 12.5R); DT 2850/2851 image processing boards (Data Translation, Marlboro, Mass.); Trinitron PVM-1342Q color video monitor; 60 Mbyte tape storage system; two custom UV-A lamps, with 4 F40 BLB bulbs (black light) and UV-passing glass filters; custom-designed subject examination frame with 70 × 70 cm interior dimensions. The new system provides increased pixel resolution (512 × 480 pixels) and gray level scale (256 gray levels), as well as vastly increased processing speed. All software programs for operating this system were custom-designed in the C programming language: VITAE-Pic© for image acquisition; VITAE-Map© for image outline/overlay; VITAE-Calc© for exposure evaluation and calculation; VITAE-Tools© for miscellaneous image analysis functions. The programs draw on subroutines provided in the DT-IRIS™ sofware library (Version 1.02, Data Translation) that accompanied the digital imaging board.

Chemicals

Fluorescent tracers were drawn from a class of commercial products known as flourescent whitening agents (FWAs). Initial studies,[6–11] as well as the citrus applicator and greenhouse studies mentioned earlier,[13–15] employed the FWA 4-methyl-diethylaminocoumarin (Calcofluor RWP™; FMC Corp.). Its toxicology has been reviewed previously.[9] The VITAE system limit of detection for this compound, expressed as density of chemical on the skin surface, is 35 ng/cm^2.

When research focus shifted to children's exposure,[16,17] a tracer compound with a more complete toxicological database was sought. The new FWA compound selected was 2,2′-(2,5-Thiophenediyl)-bis (5-tert-butylbenzoxaxole), available commercially as Uvitex OB™ (Ciba-Geigy; CAS 7128-64-5). This compound has been approved by the U.S. Food and Drug Administration as an optical brightener in food wrap,[24] and is

exempted from tolerances by the U.S. Environmental Protection Agency when applied to growing crops.[25] This compound also produces greater fluorescence per unit mass than Calcofluor RWP. The VITAE system limit of detection for Uvitex OB is 7 ng/cm^2.

Operational and Procedural Variability Studies

The stability of the VITAE system during data acquisition was determined by periodic collection of images of a standard target in a mobile laboratory during the central Florida field studies[13–15] over a 6-week period: the orchard study involved 12 workers and 32 exposure evaluation sessions, extending over a 4-week period; the greenhouse studies involved 7 workers, extending over 1 week. The standard target was a 25 cm^2 square of a mildly fluorescent poster board material that produced a median gray level value in the middle of the 0–255 gray scale. The target was positioned in the center of the subject frame, an image collected, and the target area within the image outlined. Target brightness was sampled four times during each worker exposure evaluation: before and after pre-exposure data acquisition, and before and after postexposure data acquisition.

Subject positioning and image outlining are manual tasks that rely on the skill and judgment of the system operator. Laboratory studies were conducted to determine the precision of these activities. One male and one female subject participated in these experiments. The following six body parts/views were sampled for the male: hands/ventral, left forearm/ventral, left upper arm/left side, torso/ventral, left upper leg/ventral, head/ventral. Sampling for the female subject included all body regions except the torso. Each subject was positioned for imaging following the above sequence. When the six images were collected, the sequence was repeated until 10 replicates of each part/view were collected. Overall variability was determined by outlining each of the 10 images for each body part once. Outlining variability was determined by the operator outlining one image of each body part 10 times. Subject positioning variability was calculated as the difference between overall variability and outlining variability. Data for these studies were the number of pixels contained within the outlined area of each image.

Image Classification Studies

Proper classification of images as either exposed or unexposed is most problematic for patterns of intense or diffuse exposure. Two criteria have been developed to determine whether a change in brightness has occurred between pre- and postexposure images, and are defined as follows.

(1) Intense exposure: the pixel distributions of pre- and postexposure images are compared; if the tail of the postexposure pixel distribution exceeds the pre-exposure tail by an operator-assigned number of gray levels (or percentage), then the image is classified as exposed.

(2) Diffuse exposure: the total brightness of the outlined areas in the pre- and postexposure images are compared, where total brightness is the sum of the gray level values of all pixels; if total brightness in the postexposure image exceeds that of the pre-exposure image by an operator-assigned percentage, then the image is classified as exposed.

These classification criteria were applied to sample images collected in the citrus study, greenhouse studies, and lawn care study cited previously,[13–16] with visual classification used as the criterion for "true" classification status. The computer-based criteria were treated as test criteria, using an approach common in evaluation of the accuracy of clinical tests or diagnostic examinations. Three indices of test performance were generated from this comparison: sensitivity, defined as the percentage of those images that were exposed, and were so indicated by the test; specificity, defined as the percentage of those images that were not exposed, and were so indicated by the test; and predictive value, defined as all images indicated as positive by the test, divided by the number all truly positive images.

System Calibration Studies

The prototype VITAE system standard curve was based on random skin spotting of a range of fluorescent tracer concentrations, with fluorescence converted to tracer mass by linear approximations. This approach assumed that the signal produced by the tracer was largely independent of the background signal produced by skin, and measured in pre-exposure images. However, studies initiated in the late 1980s (presented in the Results section) indicated that background brightness from the skin increased with decreasing skin pigmentation, and that, furthermore, the fluorescence of the tracer on skin was augmented as background brightness increased; that is, the greater the brightness produced by unexposed skin, the greater the postexposure fluorescent signal, even after adjustment for background signal. The effect of skin pigmentation was therefore examined in detail in two laboratory studies.

In Study 1 three subjects were selected based on observed differences in skin pigmentation: one subject was African-American, a second was European-American with relatively high pigmentation, and the third was European-American with relatively low pigmentation. Eight concentrations (range of 25–4000 ng/μL) of the fluorescent tracer 4-methy-7-diethyl-aminocoumarin were prepared in acetone. In Study 2, 11 subjects with a wide range of skin pigmentation participated. Eight concentrations (range of 5–750 ng/μL) of the fluorescent tracer 2,2′ (2,5-thiophenediyl) bis (5-tert-butyl benzoxazole) were prepared in acetone.

In each study eight skin-spotting areas were located on the relatively flat portions of the forearms (2), upper arms (2), and upper legs (4). Spotting of specific tracer concentrations was randomized for each subject across these locations. A 25-μL volume of each tracer solution was applied with a positive displacement micropettor to a 5 × 5 cm demarcated skin area. All eight tracer concentrations were applied to each subject in most cases. An image of each skin area was acquired prior to and following application of the fluorescent tracer, with 30 minutes allowed for drying of the acetone prior to postspotting image acquisition. Each postimage was outlined to include the entire spotted skin surface and exclude most unspotted skin. For each pair of pre- and postimages the following values were calculated with the VITAE programs: background gray level, defined as the median gray level of the skin surface in the pre-exposure image (used as a summary statistic for background brightness); exposed gray level, defined as the median gray level of the tracer-spotted area in the postexposure image (used as a summary statistic for exposure); and tracer density, defined as the average mass of tracer deposited per unit area, and expressed as ng/cm^2 or pg/pixel (1 pixel = 0.0114 cm^2 @ 110 cm subject-to-camera distance and 12.5 mm focal length). The natural logarithms of these values were then calculated for use in regression analyses.

Results

Operational and Procedural Variability

Table II presents the mean, range, and standard deviation of the percentage difference between the before and after measurements

for the pre- and postexposure sessions and for the entire worker evaluation session (pre-exposure session compared with postexposure session). The time between standard target sample collection for the first two sessions was normally about 20 minutes, whereas the time interval across a worker evaluation session was either 4–6 hours (orchard study) or 1.5 hours (greenhouse study).

TABLE II. VITAE System Stability During Exposure Assessment Field Studies Based on Standard Target Measurements

Imaging Session[A]	Percent Difference Within Session[B] N	Mean	Range	SD
Orchard study[C]				
Pre-exposure[D]	12	1.79	0–5.1	1.47
Postexposure	32	1.94	0–7.8	1.64
Worker evaluation[E]	12	3.71	0.8–7.3	1.96
Greenhouse study				
Pre-exposure	7	2.10	0.8–3.7	1.19
Postexposure	7	1.59	0–3.9	1.28
Worker evaluation	7	3.42	2.3–5.7	1.16

[A]Pre-exposure session collected background images prior to work; postexposure collected images immediately following work; worker evaluation session is the combination of the pre- and postexposure for each worker.
[B]Percent difference = absolute difference between standard target values divided by the average of the two standard target values times 100.
[C]Orchard study described in reference 13; greenhouse study described in references 14 and 15.
[D]Standard target was measured before and after each pre-exposure and each postexposure evaluation session; time between measurements was approximately 20 min in each case.
[E]Average pre-exposure and postexposure standard target values compared for each worker; time between measurements was 4–6 hours for the orchard study and approximately 1.5 hours for the greenhouse study.

The mean percentage difference over pre- and postexposure sessions was about 2% for both the orchard study (maximum values of 5–8%), and for the greenhouse study (maximum values of about 4%). The mean percentage difference across the entire worker evaluation session was 3–4% for both studies, with maximum values of 5–7%. These data indicate that the system was very stable throughout the data acquisition period for any individual worker. The system was also stable over the course of the entire 22-day orchard study, producing a median gray level value of 140, with a range of 116–167, and a coefficient of variation of 10.2%.

The results of the subject positioning experiments are presented in Table III and are expressed as the average and range of the coefficients of variation produced by the repeated measures. Average overall variability was 3.9%, ranging from 2–6%. Outlining variability averaged 1.2%, ranging from <1–2.5%. The difference in these values was attributed to subject positioning, and averaged 2.7% with a range of <1–5%. Thus, variability in exposure quantification attributable to these two variables rarely exceeded 5%. Subject positioning contributed more to overall variability, accounting for more than two-thirds of the total variability.

TABLE III. Image Outlining and Subject Positioning Precision

Source of Variability	Body Regions[A] (N)	Average Coefficient of Variation[B] (%)	Range of Coefficient of Variation (%)
Positioning + outlining[C]	9	3.91	1.97–5.85
Outlining[D]	11	1.24	0.71–2.51
Positioning[E]	9	2.72	0.93–4.91

[A]Drawn from six body regions of two subjects
[B]Calculated as number of pixels in the outlined region; coefficient of variation (CV) = mean/SD * 100; average and range of these CVs based on 10 replicate outlines
[C]Subjects repositioned and images recorded 10 times for each body region
[D]Each of 11 images outlined 10 times
[E]Calculated by subtracting outlining CV values from positioning + outlining CV values

Image Classification

The criteria for intense and diffuse exposure were applied to 939, 736, and 272 image pairs from the orchard, greenhouse, and lawn care studies, respectively. The criteria were evaluated in regard to their sensitivity, specificity, and predictive value when compared with image classification based on visual observations (Table IV). (The difference between sensitivity and 100% represents the rate of false negatives, whereas the difference between specificity and 100% represents the rate of false positives.) In the first two studies sensitivity was higher for the diffuse exposure criterion compared with the intense criterion, but the converse was true for specificity. This indicated that the diffuse criterion was superior in minimizing false negatives, but that the intense criterion was superior in minimizing false positives. The predictive value of the intense exposure criterion was much higher than the diffuse exposure criterion in the citrus study (91 and 76%, respectively), but the two criteria performed similarly in the greenhouse study (88 and 84%, respectively). Performance of these criteria was improved substantially in the later lawn care studies, with sensitivity and predictive value approaching 100%. These improvements can be attributed primarily to inclusion of a presampling step, in which criteria were tested with a subset of images prior to final criteria definition.

TABLE IV. VITAE System Classification of Images as Exposed or Unexposed Relative to Visual Classification

Study[A]	Exposure Criterion	Criterion Value[B]	N	Sensitivity[C] (%)	Specificity[D] (%)	Predictive Value[E] (%)
Citrus	intense	7	939	54.0	88.2	91.2
	diffuse	10%		76.4	45.6	76.2
Greenhouse	intense	10%	736	76.3	56.2	87.6
	diffuse	10%		83.1	36.3	84.1
Lawn care	intense	6	272	96.6	80.0	99.6
	diffuse	18%		94.8	60.0	99.2

Note: Image classification criteria were treated as tests based on standard approaches used to evaluate the accuracy of clinical tests or diagnostic examinations; visual classifications of image were considered true classifications.
[A]Orchard study;[13] greenhouse study;[14,15] lawn care study[16]
[B]Intense exposure criterion is number of gray levels (or percentage) that tail of postexposure histogram exceeds tail of pre-exposure histogram; diffuse exposure is percentage total brightness by which postexposure histogram exceeds pre-exposure histogram.
[C]Number of images correctly classified as exposed divided by the total number of truly exposed images ×100
[D]Number of images correctly classified as unexposed divided by the total number of truly unexposed images × 100
[E]Total number of truly exposed images divided by total number of images classified as exposed × 100

System Calibration

In Study 1 an average of 18.0 ± 2.25 cm^2 were spotted within the 25 cm^2 skin surface, resulting in a skin loading range of 35–5552 ng/cm^2. Within-subject variability of background gray levels was similar across subjects. Background gray levels overlapped somewhat for Subjects 1 and 2 (ranges of 8–12 and 10–14, respectively), but neither overlapped with Subject 3 (range of 15–20). The effect of skin pigmentation on fluorescence is illustrated in Figure 4 for two of the eight skin loading levels. At a relatively low loading level (140 ng/cm^2) an increase of one background gray level produced an increase of six gray levels in fluorescent response. At a relatively high loading level (1400 ng/cm^2) this effect was more than doubled; that is, 13 gray levels in fluorescent response for each increase in background gray level.

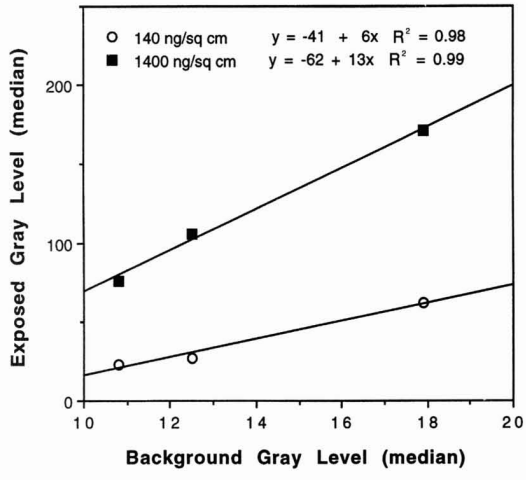

Figure 4. Effect of skin pigmentation on dermal fluorescence. Pigmentation effect represented by median gray level of pre-exposure image (background gray level). Dermal fluorescence is represented by median gray level of postexposure image (exposed gray level).

Data from Study 1 were used to generate linear standard curves ($R^2 > 0.95$), as illustrated in Figure 5. The slopes of these curves did not vary substantially, but the y-intercept decreased with increasing pigmentation, indicating that subjects with relatively high pigmentation produce a greater fluorescent response for the same tracer density. The slope and intercept values from these regression curves were then plotted against the natural logarithm of the average background gray level to construct the linear calibration curves ($R^2 > 0.99$), as illustrated in Figure 6.

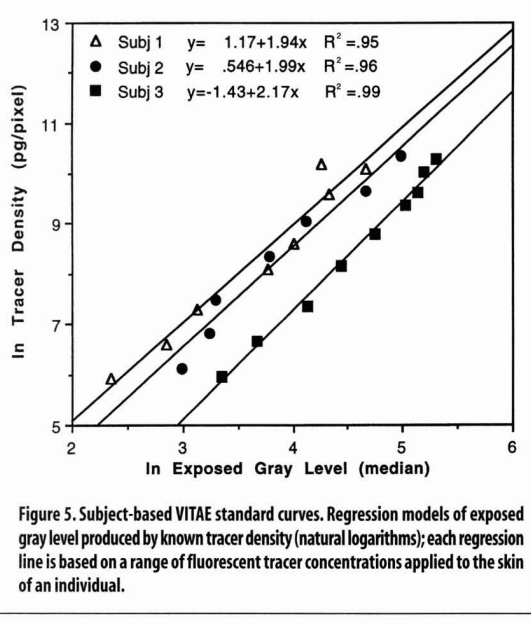

Figure 5. Subject-based VITAE standard curves. Regression models of exposed gray level produced by known tracer density (natural logarithms); each regression line is based on a range of fluorescent tracer concentrations applied to the skin of an individual.

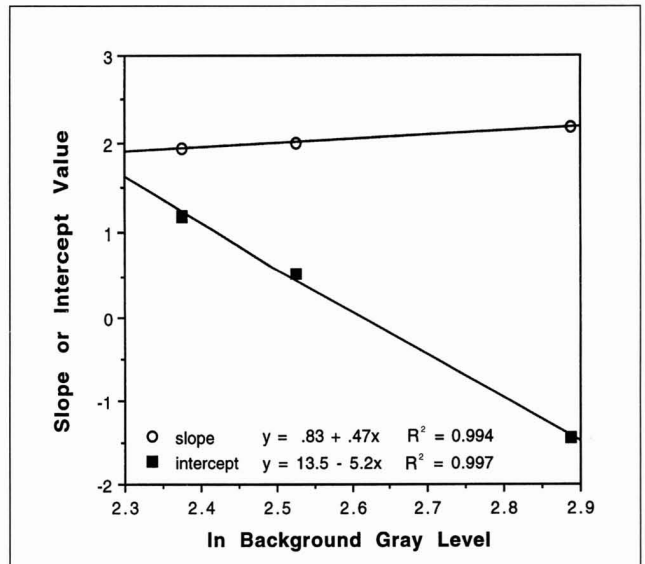

Figure 6. Subject-based slope and intercept calibration curves. Slope and intercept values from Figure 5 are plotted against background gray level (natural logarithm) to produce regression models for image calibration.

In Study 2 background gray level variability across subjects (8–32) was twice that found in Study 1 (8–20). This range of background values represented about 10% of the total gray scale (25 of 256 gray levels). Within-subject variability was also higher; that is, five subjects exhibited ranges of more than 7 gray levels across the 8 spotted skin surfaces; the most extreme case had background gray levels ranging from 16–29. Preliminary analysis of these data indicated that background gray level variability could be reduced if exposed skin surfaces were grouped in a subject-independent manner, an approach previously demonstrated to be feasible.[16] Thus, the data were classified into five background gray level categories (8–11, 13–16, 17–19, 20–24, 25–32) and analyzed according to the procedures described above.

Regression analysis produced five standard curves, as indicated in Table V. Sample sizes were uneven because the background gray levels of these subjects were not known a *priori*. Slopes increased consistently while intercepts decreased consistently with increasing background brightness. R^2 values ranged from 0.89–0.95 for these curves. The slope and intercept calibration curves generated from these regression analyses are also presented in Table V and are illustrated in Figure 7. The fit of the regression model to the intercept data was much better than to the slope data (R^2 of 0.99 and 0.90, respectively). It is also evident that the slope was much less influenced by changes in background gray level than was the intercept. Slope values for the five curves increased only about 7% (2.14 to 2.30), whereas intercept values decreased approximately 30-fold. Studies 1 and 2 were consistent in demonstrating that changes in fluorescent response due to differential skin pigmentation appear mathematically as a shift in the standard curve intercept.

TABLE V. VITAE Standard Curves and Resulting Slope and Intercept Calibration Curves Based on Background Gray Level Grouping

Background Gray Level	Sample Size	Slope	Intercept	R^2 Value
Standard curves				
8–11	16	2.14	0.099	0.892
13–16	16	2.19	−1.20	0.932
17–19	23	2.23	−1.68	0.954
20–24	19	2.31	−2.45	0.949
25–32	10	2.30	−2.88	0.947
Calibration curves				
Slope	5	0.175	1.73	0.903
Intercept	5	2.87	6.58	0.989

DISCUSSION

The second generation imaging system described here employs new technology, software, and calibration procedures to improve the reliability of fluorescent tracer measurements for dermal exposure assessment. Imaging analysis systems with greater resolution are commercially available, but they require proportionately greater data processing capability. The current VITAE system represents a balance between image/gray scale resolution and data management and has proven adequate for the types of quantitative analyses reported here. Rapid advances in computer technology almost ensure that a system such as this will be outdated by the time it is installed and tested. For example, the DT-IRIS™ software subroutine version used in the system is no longer supported by its manufacturer. Investigators interested in constructing a similar system should consider new technological capabilities, such as optical disks for data collection and storage.

The new system demonstrated high stability during operation in a mobile laboratory over an extended field sampling period. The conditions of use in Florida approached worst case from the perspective of environmental and data collection conditions; that is, outdoor temperature normally exceeded 32°C (90°F) and 90% humidity at midday, and the mobile laboratory was moved to a new work site each day.

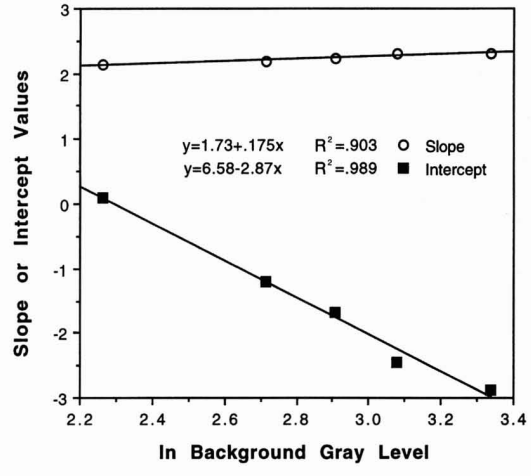

FIGURE 7. Background-based slope and intercept calibration curves. Slope and intercept values from Table V are used to produce regression models. Each exposed image is assigned unique calibration parameters from these regression models based on background gray level (natural logarithm).

Subject positioning and image outlining do not appear to be significant contributors to variability in quantification of skin exposure patterns, although it has been the authors' experience that a small number of subjects find the positioning instructions difficult to follow. Classification of images by the VITAE system remains problematic. In current field studies visual classification remains the gold standard by which VITAE classification is judged. Further investigations are required to refine criteria that minimize false positive and false negative classification.

The major improvement in quantitative procedures results from a recognition of the significant role of variable skin pigmentation, and the development of calibration curve algorithms for adjustment on an image-by-image basis. The findings from these calibration studies were incorporated into the VITAE system software in 1990[12] and are an integral part of the exposure calculation algorithm. The VITAE-Calc program requires four parameters from these calibration curves: slope and intercept of the slope curve, and slope and intercept of the intercept curve (see Table V). With these inputs the program determines an appropriate slope and intercept for each data image based on the image's background gray level, thereby producing a unique standard curve for each image (as in Figure 7). This procedure appears to substantially correct exposure values for the effect of differential skin pigmentation.

The problem of background effects can be attributed in part to the optical properties of human skin and the effect of melanin on UV-A absorption and reflectance, discussed in detail elsewhere.[26] The need for background correction during skin exposure evaluation was also addressed in the development of a luminoscope for coal tar exposures,[27] but these studies were limited to mouse skin and did not address the issue of variable background effects in human populations. The new calibration procedures presented in this article are consistent with the earlier findings of Black[16] that background adjustments can be accomplished in a person-independent fashion; that is, it is not necessary to develop individualized calibration data for each subject in a study.

Researchers at the University of Guelph adopted the second generation VITAE system, including its software and standard operating procedures, for evaluation of pesticide applicators in greenhouses. Their initial study[18] employed the background gray level grouping technique described here, and used the authors' procedures for creating slope and intercept calibration curves (see Figure 7). The novel aspect of this work was their ability to restrict each standard curve to a single background gray level rather than using a range of such levels (see Table V). They accomplished this refinement by generating a larger number of standard skin spotting surfaces, and by working with a relatively limited range of background gray levels (range of 15 gray levels compared to 25 gray levels in the work reported here). Their subsequent study of 9 greenhouse applicators demonstrated a high correlation between VITAE skin exposure measurements and urinary pesticide metabolites.[19] These findings confirmed those of an earlier study in which measurements with the prototype VITAE system were highly correlated with malathion urinary metabolites in 21 citrus orchard mixers and applicators.[11]

It appears from the work reported here, and from the independent work at the University of Guelph, that current calibration procedures adjust adequately for the effect of differential background response due to skin pigmentation. However, more recent work[28] suggests that the accuracy of VITAE measurements may also be affected by the distributional characteristics of tracer on the

skin; that is, calibration based on relatively uniform spotting of small skin surfaces under controlled conditions may not adequately characterize more complex exposure patterns. Further evaluation of these procedures under more variable conditions is merited.

CONCLUSIONS

Imaging analysis of fluorescent tracer deposition on skin provides a useful means of quantifying dermal exposure to hazardous chemicals. The second generation VITAE system exhibits stable performance in the field, even under extreme conditions, and the key operational procedures, such as subject positioning and skin area outlining, can be performed with relatively high precision. Visual classification of skin surfaces as exposed or unexposed remains more reliable than computer-based criteria, but presampling of images can significantly increase the sensitivity and predictive value of such criteria. Calibration procedures that adjust for the effect of differential skin pigmentation on exposure quantitation can improve substantially the accuracy of exposure measurements.

The VITAE methodology is technically challenging, and such systems have not yet progressed to the stage of routine environmental monitoring. However, the method clearly has value in the documentation of dermal exposure patterns, in the elucidation of exposure pathways, and in the development of strategies to prevent or minimize dermal exposure to hazardous chemicals.

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