Implementation and Evaluation of the Fluorescent Tracer Technique in Greenhouse Exposure Studies

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Knowledge of the level of exposure is important for health risk estimation and risk management. Recently, the occurrence of dermal exposure in many situations has been recognized and estimated to be relevant for worker health. Dermal exposure measurement techniques are therefore needed and several approaches have been taken to assess this type of exposure. The purpose of the present study was to apply and evaluate the fluorescent tracer technique, being one of the most promising and innovative techniques to estimate dermal exposure. The image acquisition is fully calibrated and validated. The most significant aspects of the image analysis process are validated in laboratory settings. The system is applied in a field study to estimate dermal exposure of operators and harvesters in greenhouses, while chemical analysis of clothing exposure is also performed. For operators, the correlation coefficient between the active substance (propoxur) and the fluorescent compound (Tinopal) was 0.92, and for harvesters 0.85. It is concluded that the variability in the analytical technique used is insignificant with respect to the variability in exposure within and between workers. Instead of improving the measuring technique, one might better lower the variability by measuring, for instance, a larger number of workers and/or by standardizing work procedures. The fluorescent tracer technique, being a fast method to estimate dermal exposure, enables the estimation of larger numbers of individuals. Furthermore, the qualitative use of this technique can lead to a more efficient sampling strategy since the exposed body area to evaluate can easily be visualized and selected. © 1998 British Occupational Hygiene Society. Published by Elsevier Science Ltd.

INTRODUCTION

Agriculture is an example of an industry where the use of pesticides may impose health risks to workers. Both during the application of the pesticides and during re-entry activities in treated crops, for instance harvesting, the workers may be exposed Brouwer et al. (1992a,b). Knowledge of the level of exposure is important for health risk estimation and risk management. The worker can be exposed through different routes, depending on the location where the contaminant is present. To determine the total exposure level with an appropriate measurement strategy, knowledge on the location of the contaminant is very important.

Exposure through the dermal route is recognized to be relevant for worker health. To estimate the contribution of percutaneous absorption to a worker's total exposure, air sampling cannot be used. Dermal exposure measurement techniques are therefore needed and several approaches have been taken to assess this type of exposure. These include quantification of the amount of contaminant on the workers' skin or clothing (direct methods), biological monitoring, and the quantification of the amount of contaminant on the surfaces that the workers come into contact with. A thorough review of dermal exposure measurement methods is presented by McArthur (1992); Fenske (1993) and Van Hemmen and Brouwer (1995).

A fluorescent tracer technique has been adapted for assessing the quantity of material deposited onto the skin during work. Shuresko (1980) and Vo-Dinh (1987) designed a portable optical spotter, which both induced and detected fluorescence, to evaluate deposition of contaminants themselves on the surface of the skin. The use of fluorescent tracers in agriculture had been reported in 1959 by Staniland (Staniland, 1959), mainly to evaluate the performance of application gear, but Fenske (1986) was the first to report the use of a fluorescent tracer (in combination with
an image analysis system) to investigate the dermal exposure of workers to pesticides quantitatively. Archibald et al. (1994a) have further improved the procedure to calibrate this so-called video imaging technique to assess dermal exposure (VITAE). Van Amelsvoort and de Vries (1990) and King and Dobson (1992) applied the technique using photographic prints qualitatively and quantitatively, respectively and concluded that more research was needed before a reliable estimate could be obtained.

Roff (1992) proposed extensive alterations to the illumination equipment to bypass corrections necessary due to non-flat objects. Results using the improved illumination still showed a large variability (Roff, 1997), although the technique corrected the effects due to non-flat objects very well (Roff, 1994). Archibald et al. (1994a) reported an improvement of the calibration procedure originally described by Fenske (1986).

Possible tracers can be selected from a group of compounds known as Fluorescent Whitening Agents (FWA), which are detectable at low concentrations and at low energy levels. These FWAs are used in the paper and cotton industry to create a visual whitening effect (Williamson, 1980). The selection of the tracer depends on the properties of the compounds which it must mimic. The tracer and contaminant should not separate or act independently when a mixture is applied. Of course the tracer itself should not cause any harm to the worker. To be able to apply a fluorescence tracer technique, the tracer must be present at the same locations in the working environment where the compound of interest is deposited. Mixing the tracer with the actual compound before the worker starts work seems the most obvious way to create such a situation. Ideally, the same ratio in the amount of the compound of interest and of the tracer will then be found on the worker's skin and clothing. The deposition of the tracer is visualized using irradiation with ultraviolet light, which activates the emission of fluorescence light by the tracer molecules. The low amount of fluorescence light emitted necessitates exclusion of other light sources during the visualization.

The amount of fluorescent light in an image depends not only on the amount of tracer on the skin, but also on the response of the skin itself. This skin response is closely related to the colour of the skin. The same amount of tracer on a white skin leads to a higher amount of fluorescence light than on black skin. This requires that images of the worker have to be recorded not only after the deposition has occurred but also before deposition. Great care must be taken to match the body parts in both images to be able to estimate the amount of tracer from the amount of fluorescent light and the response of the skin.

The purpose of the present study was to implement and validate the fluorescent tracer technique and its application in a greenhouse situation, to evaluate its performance in relation to known chemical techniques. From the results conclusions can be drawn about the usefulness of this technique in environmental studies to evaluate the exposure of workers.

MATERIALS AND METHODS

The uniform illumination of the body is achieved by two armatures, each holding two TL D-light bulbs (black lights, Philips, Eindhoven, The Netherlands) of 122 cm, with a distance of 50 cm in between. By further modifying the armatures the uniformity of illumination was improved. Figure 1 shows a schematic representation of the illumination set up. This set up results in an illumination (shown in Fig. 2) with a coefficient of variation of less that 5% in the measurement area.

Fig. 1. Drawing of the illumination setup. Visible are the UV-lamps in the modified armatures, the camera and the measuring area in which the person is positioned.

Fig. 2. Schematic drawing of the intensity of illumination in the measuring area.
suring area. The images are taken using a lens to project the subject onto the light sensitive part of the camera. The lens applied (Ernitec, 1:1.2/12.5–75 mm) was set to a fixed position (using f-stop 5.6) and equipped with the necessary barrier filters to prevent disturbance due to reflected ultraviolet light (Wratten 2E, blocks below 410 nm, Kodak) and due to infrared light (BG40, blocks above 700 nm, Schott, Tiel, The Netherlands). The results of experiments indicated that the aberration in our system is 2% at the most. For further work, this small error was considered not significant and not further accounted for.

Images were recorded using a camera (LDH0703/AS, AutoGain switched off, Philips, Eindhoven, The Netherlands) and were digitized with a DT2853 frame grabber (Data Translation, Marlboro, MA, USA) installed in a Personal Computer (AT386). A video camera was chosen because the images are immediate, can be stored without loss of resolution and can be inspected on a monitor for possible errors. Images digitized with this equipment consist of 512 x 512, 8 bit picture elements (pixels). This implies that, with a distance of 1 m between object and camera, each (square) pixel covers an object area of 0.775 mm². Each pixel may have a value from 0 to 255, normally referred to as grey value of the pixel.

The tracer used in the present study was Tinopal CBS-X (Ciba–Geigy, Arnhem, The Netherlands, 4,4'-bis(2-sulfostyryl)biphenyl). This stilbene derivative has an excitation wavelength of 349 nm, an emission wavelength of 424 nm, a molar extinction coefficient of 65,0001/mol.cm and has good water solubility (Gold, 1975). From the toxicological data presented by Burg et al. (1977) a no-adverse-effect level (independent of the route of exposure) of 326 mg per day for a 70 kg human is derived.

Dr. Richard Fenske (University of Washington, Seattle, USA) kindly provided software to acquire and analyse the images. The image acquisition software was adapted to the present particular hardware and demands. Routines to facilitate the matching of the ‘before’ and ‘after’ exposure image and to improve the visibility of the body parts on the monitor were developed additionally. The image analysis is performed within the Scil Image analysis package (TNO–TPD, Delft, The Netherlands) which is implemented on a Silicon Graphics 4D/35 computer (UNIX operating system). Recently, the analysis system has been ported to a PC (Pentium, Windows 95) using Visual C++ and a PC version of the Scil Image package.

The objects under study (primarily arms and hands) are not flat, but the precise shape of the object is not known. Due to the non-flat shape, the surface area and the grey values measured in the image do not reflect the real value of those parameters. To evaluate the arm completely, more than one image needs to be taken. To prevent multiple evaluation of the same sites on the arm, only the centre part (≈ 70%) of the surface of the arm in an image is measured to evaluate the amount of tracer. The deviation from a flat shape of this 70% of a body part is relatively small. Applying the analytical formulae on models of the arm, revealed that the surface area is underestimated by 10%, at the most. With regard to the corrections of the grey value, one can show (using formulae of line source illumination) that the grey value is also underestimated by 10%, at the most. Comparing the error introduced by the shape with the error of using correction factors calculated for an assumed shape led to the conclusion that corrections are not necessary.

To investigate the relationship between the amount of fluorescence and the amount of tracer on human skin, volunteers were spotted using known amounts of tracer, after approval of the protocol by the Medical Ethical Commission. The body parts onto which the tracer was applied were: i) front and back forearms, ii) the side of the upper arms, iii) front and back of lower legs, iv) back and palms of the hands, v) front and back of upper torso.

A total of 6 concentrations of the tracer between 20.9 and 209 mg of Tinopal per litre of distilled water were used. Nineteen volunteers were divided in 6 groups. Each group was spotted with a different combination of the concentrations available. The volunteers were given dark clothing to prevent the masking of the fluorescence of the tracer, and images were taken (pre-images) of the body parts mentioned above. From each concentration, 10 µl was put on an object glass and the tracer on the glass was wiped to the skin (the mean spotsize was 356 mm² (CV 45%)). After application of all the spots, images of the same parts were taken (post-images). While taking a post-image the pre-image was displayed (using red colour) alternately with the live image (using grey values) on a monitor. To improve visibility, logarithmic grey values were used which increase the intensity of lower grey values with respect to the upper grey values. In this way, the volunteers were able to match the post-image part with the pre-image part very well (Note only the display of the images was altered, not the images itself).

The images were corrected for camera-noise, non-uniform illumination and variation of illumination in time, and the mean grey values of the fluorescent spots and of the underlying skin were determined. The relationship calculated was amount of tracer = (spot fluorescence + 0.07–1.92 * skin response)/5.05. The Pearson correlation coefficient of this relationship is r = 0.85, the coefficient of variation of the estimate of the amount of tracer being 73% (n = 420). Using this relationship in a ‘repeated design’ study, for instance to distinguish between exposure using different clothing regimes, a difference of a half standard deviation can be detected using 19 persons. This relationship may only be used if less than 250 ng tracer is deposited per square cm surface area to avoid non-linear responses. The lowest possible amount detectable using this relationship is 9 ng/cm². Detailed descrip-
tion of the relationship is presented in Bierman et al. (1995).

APPLICATION OF THE FLUORESCENT TRACER TECHNIQUE

Operators

In five greenhouses used for the cultivation of carnations, the pesticide propoxur was mixed with the fluorescent tracer Tinopal and applied using a high-volume technique with a spray-boom or spray-pistol. The application rate was 25 g Tinopal CBS-X and 50 g of propoxur per 1000 m². On average, 80 litres of fluid was sprayed over 1000 m², taking approximately 15 minutes. The operators wore cotton monitoring gloves (stretched cotton, 270 g/m², Van de Wee, Tiel, The Netherlands) and cotton monitoring coveralls (240 g/m², KLM, Haaksbergen, The Netherlands) during their work. The gloves and coveralls were pre-washed in a standard way before being provided to the workers. After spraying, the gloves and forearm parts of the coveralls were analysed chemically for pesticide and tracer, using the methods of Brouwer et al. (1997a,b).

Images of hands, arms and head of the operator were taken before and after application. The images were corrected as already described. For each pixel on a body part of interest, any increase in grey value during application was attributed to deposition, and the amount of tracer was calculated from the calibration relationship. The total amount deposited on each body part was calculated by adding together all the pixel contributions.

Harvesters

Harvesters cut carnations from the sprayed crop for one hour, two days after spraying, without gloves and coveralls. The fluorescence technique was used to measure their skin contamination, in the same way as with the operators.

A second group of harvesters wore gloves and coveralls similar to the operators, and these were analysed for Tinopal and propoxur contents. The foliage in the greenhouse also carried methiocarb from earlier applications, and this was also measured in gloves and coveralls. Samples of foliage were analysed for all three substances, and the dislodgeable foliar residues were estimated using the method described by Brouwer et al. (1997a,b).

CALCULATIONS AND DATA ANALYSIS

Using the estimated exposures from the experiment as described above it was possible to address the following questions:

- how does the fluorescent tracer technique compare with chemical analysis (by comparing results of the fluorescent tracer technique on skin with results of chemical analysis of gloves/coveralls for operators, and for both groups of harvesters);
- can Tinopal be used as a tracer for the different chemicals used? (by comparing the results of the chemical analysis of spray liquids, monitoring clothing and foliage);
- what amount of Tinopal penetrates through gloves and coveralls? (by comparing exposure estimates for operators of the monitor clothing with tracer deposition on skin detected by the fluorescent technique);
- what amount of Tinopal is transferred to skin or cotton from foliage? (by comparing two groups of harvesters);
- what amount of propoxur and methiocarb is transferred to cotton from foliage? (by analysis of the harvesters’ gloves, coveralls and foliage).

For the operators, the exposure was expressed in mg per kg of the propoxur applied. For the harvesters, the exposure was expressed in mg per mg/cm² dislodgeable foliar residue.

The statistical analyses were performed using the BMDP—statistical software package on a Vax/VMS 8250 computer. Spearman Rank correlation coefficients were preferred above Pearson's correlation coefficient to avoid assumptions on the normal distribution of data. To compare the exposures of different body parts a Kruskal–Wallis test was used.

RESULTS

Qualitative use of the fluorescent tracer technique

The fluorescent tracer technique proved to be a very simple method to visualize exposure. On the flowers fluorescence was clearly visible in the axilla of the stem until approximately 5 weeks after application. Using hand-held illuminating equipment the distribution of the tracer on the crop could be monitored at night. For the case studied, the material applied by using a hand-held spray-pistol was clearly not homogeneously distributed; the waving movements of the operator could easily be seen. Use of a spray-boom resulted in a more homogeneous distribution of the applied material on the crop.

It became obvious that during mixing and loading, contamination with the spray liquid easily happens, even when the operator did his best to prevent contamination. Deposition on the head was not monitored regularly, neither for operators nor for harvesters. On a few occasions, a spot was seen on, for instance, the cheek of an operator. On hands, forearms and, sometimes, on upper arms, deposition was visible regularly (Fig. 3). Some operators showed also deposition on the lower leg, due to contact with the crop. Normally the hand which held the spray-pistol showed less fluorescence than the other hand, probably due to pulling the contaminated hose. For harvesters, a distinction could be made between the
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Fig 3 These pictures show the deposition of the tracer due to harvesting carnations. On the left-hand side, pictures taken using normal light and on the right-hand side, pictures of the same person but using UV-light. The tracer on the lower arm is clearly visible in the top-right image. On the bottom-left image, the inside of the hands is seen. The hand on the left held the knife while the other hand held the flowers.

Fig 4 illustrates the relationship between the results of the chemical and the fluorescent tracer technique for gloves and hands of harvesters.

Using Tinopal as a tracer

For operators, the correlation coefficient between propoxur and Tinopal (on monitoring clothing, assessed by chemical analysis) was 0.92. The average ratio of the amount of propoxur and Tinopal in the spray liquid was 2:1 and on monitoring clothing of operators, 1.7:1. For harvesters, the correlation coefficients were 0.85 and 0.66, respectively, for propoxur vs. Tinopal and methiocarb vs. Tinopal.

Penetration of gloves and coveralls

The ratio of the amount of Tinopal on skin and on clothing was calculated to be 0.12 for operators. This represents the penetration through the monitoring clothing.

Transfer to cotton of propoxur, methiocarb and Tinopal from foliage

The average ratio propoxur:methiocarb:Tinopal on the foliage of the carnations was 5:16:11, while measured on clothing, this ratio was 5:2:3. All results mentioned are based on chemical analysis. The amount of Tinopal on clothing is twenty times larger than on skin (measured using chemical analysis and fluorescent tracer technique, respectively).

DISCUSSION

Fluorescent tracer technique

Fenske (1986) calculates a regression line (log(amount of fluorescence) vs. log(amount of tracer))
Table 1. Amounts of propoxur, methiocarb and Tinopal measured using chemical analysis of gloves and sleeves, and amount of Tinopal measured using fluorescent tracer technique of hands and forearms of operators and harvesters

<table>
<thead>
<tr>
<th>Body part</th>
<th>Chemical determined</th>
<th>Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Operators: mg chemical per kg chemical in spray liquid (n = 3)</td>
<td>Harvesters: mg per mg/cm² on foliage (n = 9)</td>
</tr>
<tr>
<td>Hands</td>
<td>Propoxur (chemically)</td>
<td>0.60–1.06</td>
</tr>
<tr>
<td></td>
<td>Methiocarb (chemically)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Tinopal (chemically)</td>
<td>0.01–1.23</td>
</tr>
<tr>
<td></td>
<td>Tinopal (fluorescent tracer technique)</td>
<td>0.02–0.24</td>
</tr>
<tr>
<td>Forearm left</td>
<td>Propoxur (chemically)</td>
<td>0.16–2.20</td>
</tr>
<tr>
<td></td>
<td>Methiocarb (chemically)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Tinopal (chemically)</td>
<td>0.05–1.22</td>
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<tr>
<td></td>
<td>Tinopal (fluorescent tracer technique)</td>
<td>0.05–0.24</td>
</tr>
<tr>
<td>Forearm right</td>
<td>Propoxur (chemically)</td>
<td>0.29–5.93</td>
</tr>
<tr>
<td></td>
<td>Methiocarb (chemically)</td>
<td>—</td>
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<tr>
<td></td>
<td>Tinopal (chemically)</td>
<td>0.05–3.26</td>
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<tr>
<td></td>
<td>Tinopal (fluorescent tracer technique)</td>
<td>0.02–0.16</td>
</tr>
</tbody>
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a–c: same characters indicate a significant difference (Kruskal-Wallis, p < 0.05).

per group of values of skin response. The regression coefficients and intercepts of these lines were plotted against the value of the skin response and were used to extract the parameters of the calibration curve of the skin response measured in the practical setting. Archibald et al. (1994a) modified this method and calculated a regression line for each value of skin response. Because of the two-step procedure used in both methods, the real error of the estimate is difficult to calculate. Both calculation methods are an approximation of the method applied in the present paper. Using a multi-regressional analysis between amount of tracer applied, response of the skin and amount of fluorescence, the error of the estimate is found to be rather large. This hampers estimation of exposure of individual persons. The error in the estimate (73% as

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**Fig 4.** Plot of the results of chemical analysis of Tinopal on the gloves and Tinopal at the skin detected by the fluorescent tracer technique. Both in mg chemical per mg chemical/cm² on foliage.
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Transfer of compounds to cotton from foliage

The ratio between the amount of propoxur, methiocarb and Tinopal measured on the clothing does not reflect the ratio of these compounds on the foliage. For harvesters the comparison of the potential exposure as assessed in one subgroup by glove monitoring and actual exposure of the second subgroup assessed by the fluorescent tracer technique is complicated. The moderate correlations observed may reflect the large between-worker variability, as discussed before. The ratio between the amount of Tinopal on cotton monitoring clothing and on skin is high, which may be related to a different process of contact and transfer of the dislodgeable foliar residue from the foliage to cotton and skin. Cotton fabrics tend to have high retention properties compared to the skin surface, since the effect of penetration into and absorption by the fibres is a more efficient process than adhesion of a contaminant to the skin. Fenske et al. (1989) and Davis et al. (1983) compared monitoring gloves and hand rinsing techniques for similar exposure scenarios, and both techniques include chemical analysis of a pesticide designed for use as plant protection product. They reported ratios of 2 and 5, respectively. The magnitude of the ratio (20) observed in the present study deviates much from these ranges, which may be related to the tracer. Tinopal is a compound not specially designed to act as a plant protection product and shows good adhering properties for fibers, which may enhance the retention properties of the cotton compared to the skin.

The observations in this study emphasize the need for a validation of all available methods, i.e., surrogate skin techniques (monitoring gloves), removal techniques (hand washing), and direct monitoring techniques (fluorescent tracer technique) for dermal exposure monitoring in relevant occupational exposure scenarios.

Using Tinopal as a tracer

The ratio between the amount of propoxur and the amount of Tinopal estimated on clothing is in the same range as measured in the spray liquid.

The good correlations between the amount of propoxur and amount of Tinopal measured chemically on clothing indicate that Tinopal is a good tracer for propoxur (ρ = 0.9). In the case of methiocarb, Tinopal is less appropriate (ρ = 0.6), but this could be caused by the fact that this pesticide was not applied at the same time. Methiocarb was not the prime interest in this study and the interval between application and harvesting the crop has not been determined. This may lead to different transfer rates at different intervals.

The correlations between the amount of propoxur and methiocarb on the clothing (estimated chemically) and the amount of Tinopal on the skin (measured using the fluorescent tracer technique) are moderate but similar for both pesticides (ρ = 0.5).
The variability of the estimates

The estimates of the dermal exposure in this study show a large variability, both when using the fluorescent tracer technique and chemical analysis. Quantitative studies reported so far in the occupational literature also show these large variabilities. This concerns not only utilizing fluorescent tracer techniques (e.g., Niven et al., 1993; Mether and Fenske, 1994b; Archibald et al., 1994b), but also hand washing or patch techniques (Fenske et al., 1989) and biological monitoring (Droz, 1989). The qualitative use of the fluorescent tracer technique clearly demonstrates that dermal exposure is generally not caused by uniform deposition of contaminant on all parts of the body but is largely unpremeditated and caused by the variation in work and behaviour, so dermal exposure is highly variable by nature. The within- and between-worker components of variability are dealt with by Kromhout et al. (1993) who concluded that differences between workers are influenced by factors like work style and the mix of tasks involved. Nicas et al. (1991) also showed that the analytical variability is of minor importance compared to the environmental variability. Instead of improving the measuring technique, one might better lower the variability, for instance, by measuring a larger number of workers and/or by standardizing work procedures.

Because the fluorescent tracer technique, once implemented, is a relatively fast method to estimate dermal exposure in contrast to chemical analysis, this enables the estimation of larger numbers of individuals and, moreover, measures exposure of the skin itself and not of a surrogate skin. Furthermore, the qualitative use of this technique can lead to a more efficient sampling strategy since the surface area to evaluate can easily be visualized and selected.

Acknowledgements—The authors wish to thank C. J. M. Van der Wulp for discussing image processing techniques and illumination formulae. The discussions and suggestions—The authors wish to thank C. J. M. Van der Wulp for discussing image processing techniques and illumination formulae. The discussions and suggestions between workers are influenced by factors like work style and the mix of tasks involved. Nicas et al. (1991) also showed that the analytical variability is of minor importance compared to the environmental variability. Instead of improving the measuring technique, one might better lower the variability, for instance, by measuring a larger number of workers and/or by standardizing work procedures.

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