On-line laser photoacoustic detection of ethene in exhaled air as biomarker of ultraviolet radiation damage of the human skin

F. J. M. Harren,[a] R. Berkelmans,[b),c)] K. Kuiper,[b)] S. te Lintel Hekkert, P. Scheepers,[c)] R. Dekhuijzen,[d)] P. Hollander,[b)] and D. H. Parker
Department of Molecular and Laser Physics, University of Nijmegen, Nijmegen, the Netherlands

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The exhaled air and volatile emission by the skin of human subjects were analyzed for traces of ethene (C_2H_4) by means of CO_2 laser photoacoustic trace gas detection. Due to the extreme sensitivity of the detection system (6 part per trillion volume, $6{:}10^{12}$), these measurements could be performed on-line and noninvasively. Exhaled ethene was used as a biomarker for lipid peroxidation in the skin of human subjects exposed to ultraviolet (UV) radiation from a solarium. A change in the ethene concentration was already observed in the exhaled air after 2 min. Adaptation of the skin to UV exposure and direct skin emission could also be observed. © *1999 American Institute of Physics.* [S0003-6951(99)01312-1]

The composition of exhaled air gives information about a wide variety of processes occurring inside the human body.[1,2] From a medical point of view this is interesting, because trace gas analysis of exhaled air has the potential to monitor processes noninvasively (e.g., Ref. 3) and with a rapid time response.

Under specific physiological conditions, e.g., heat, trauma, radiation, and exercise to excess, the balance between free-radical formation and the normal scavenging capacity of the body is disturbed, and tissues become subjected to oxidative stress.[4] Oxidative stress is the origin or cause of lipid peroxidation and, as a consequence, of a wide variety of pathophysiological processes.[5,6] Lipid peroxidation is the free-radical-induced oxidative degradation of polyunsaturated fatty acids; biomembranes and cells are thereby disrupted, causing cell damage and cell death.[7] As a marker of free-radical-mediated damage in the human body, the measurement of the exhaled volatile hydrocarbons ethene (C_2H_4), ethane (C_2H_6), and pentane (C_5H_{12}) is a good, noninvasive method to monitor lipid peroxidation.[8]

Due to lack of sensitivity, these hydrocarbons need to be concentrated on a solid sorbent and subsequently analyzed by gas chromatography. The choice of the proper sorbent material during the concentration step is of paramount importance; permeability of wall material, adsorption, and contamination with ambient air can make breath samples uninterpretable.[7] Due to their high sensitivity, laser photoacoustic trace gas detectors are able to overcome this delicate and time-consuming concentration step. Recently, this method has proven to be very well suited for the detection of gases emitted from various kinds of biological material.[9–15]

The goal of this study is to see whether ethene can be used in combination with laser-based techniques as an on-line biomarker of lipid peroxidation in humans. For this, the daily or interindividual variation of ethene exhalation at rest must be small. Also, an intervention, which is known to cause lipid peroxidation like ultraviolet (UV) exposure of the human skin, should induce a rise in ethene exhalation, and adaptation to the applied stress factor should be reflected in the reduced exhalation of ethene.[7,16]

A detailed description of the ethene detector has been given elsewhere.[17,18] The detector is a combination of an infrared CO_2 waveguide laser with an intracavity placed photoacoustic cell. This configuration can detect minimum absorptions of $1.8\times10^{-10}\,\mathrm{cm}^{-1}$, which leads to a detection limit of 6 pptv (part per trillion volume, $1{:}10^{12}$).

Within the gas handling system, compressed air flows via a 60 l aluminized buffer bag to the test persons (Fig. 1). Compressed air is used due to the large fluctuation of the ethene concentration in ambient air (1–20 ppbv). From the buffer bag, a three-way valve (Hans–Rudolph type) is used to inhale and exhale. The exhaled air goes to an Oxycon to monitor the exhaled gas volume. Only a small amount of the exhaled air (3 l/h) is sampled and goes toward the detection cell. A reference sampling line is used to monitor the long-

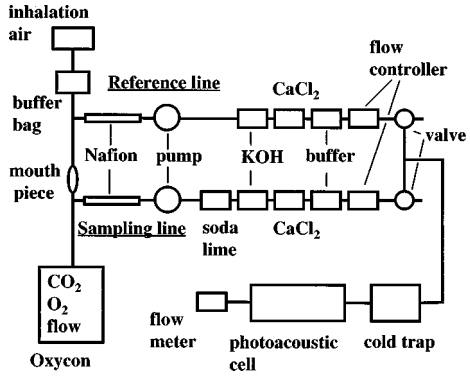

FIG. 1. Sample system for breath analysis. Inhalation air flows via a buffer bag toward the subject which in- and exhales via a three-way (Hans Rudolph) valve. The amount of exhaled air is measured via an Oxycon. Only a small part of the air is directed to the photoacoustic detection system via scrubbers and flow controllers. The reference line is used to monitor the long-term stability of the system.

[a)]Electronic mail: fransh@sci.kun.nl
[b)]Also with the Department of Kinesiology, Free University, Amsterdam, the Netherlands.
[c)]Also with the Department of Toxicology, University of Nijmegen, Nijmegen, the Netherlands.
[d)]Also with the Department of Pulmonary Diseases, Academic Hospital, Nijmegen.

TABLE I. Brand and type of lamps used in the solarium Combi 12/12 used for UV exposure (Dr. Müller, Essen, Germany).

Wavelength	Brand and type	UV-A (315–400 nm)	UV-B (280–315 nm)
Lower bench	Cosmedico		
	Cosmolux UV-A plus 100 W	12×30 W	12×0.21 W
Upper bench	Dr. Müller		
	Power Line 80 W	12×20 W	12×0.32 W
Face browner	Dr. Müller		
	Halogen Metalldampfstrahler 400 W	2×95 W	2×25 W[a]

[a]A filter in front of the face browner removes all UV-B.

term stability of the system. Condensed water droplets could disturb the measurements and are, therefore, extracted with Nafion tubing and $CaCl_2$ grains.[11]

A membrane pump, buffer volume (to suppress pressure fluctuations), and a mass flow controller regulate the transport of exhaled air to the detection cell. Human exhaled air contains around 5% of CO_2 which is removed by using two scrubbers; one for bulk removal (soda lime) and a second (KOH pellets) to lower the concentration below 1 ppmv. To remove other volatiles (e.g., acetone), which can interfere spectroscopically, a cryogenic trap (125 K) is used. All tubing was covered with aluminum foil to avoid UV-induced deterioration of parts of the plastic gas handling system, thereby emitting traces of ethene. Care was taken that all subjects were nonsmokers and did not have chronic illnesses of any kind; both cause elevated levels of ethene.

Daily variation in ethene exhalation was measured from 21 humans at rest. To remove all contamination with volatile hydrocarbons from the ambient air a wash out period of 5 min was used. The exhaled air of the test subjects contained 0.36 ppbv ethene. After correcting for pulmonary volume and body weight this yielded in 1.99 pmol/kg/min with a standard deviation of 0.51 pmol/kg/min (26%), which is low as compared to values reported elsewhere.[7]

For measurements of the ethene emission response under UV, 21 male subjects in underpants were exposed from the neck down to light from a commercial solarium (see Table I and Fig. 2). After having a wash out period the solarium was turned on for a 15 min period (as recommended by the purveyor). Figure 3 shows, for a single person, the ethene exhalation before, during, and after UV radiation. Within 2 min after the solarium is turned on, the ethene concentration in exhaled air starts to rise and keeps increasing until the solarium is turned off. At this point the average ethene exhalation was (21 subjects) 17.2 ± 7.3 pmol/kg/min, while pre-UV exposure levels of these persons were 1.39 ± 0.38 pmol/kg/min, which is a very significant difference ($p < 0.001$).

UV radiation will instantaneously cause the formation of free radicals and ethene production via lipid peroxidation. Assuming that this process occurs within seconds, the shortest route for ethene to be transported from the skin to the lungs will take less than 2 min. During UV irradiation ethene exhalation does not reach a plateau, caused by the buffer capacity of the body tissue.[19] This is also shown in the two-phase decay in ethene emission after exposure (Fig. 3); a fast decay results from wash out by the lungs and blood and a slow long-term decay from the body tissue.

It is generally known that after exposure to sunlight the skin adapts to this applied stress, for example, by higher

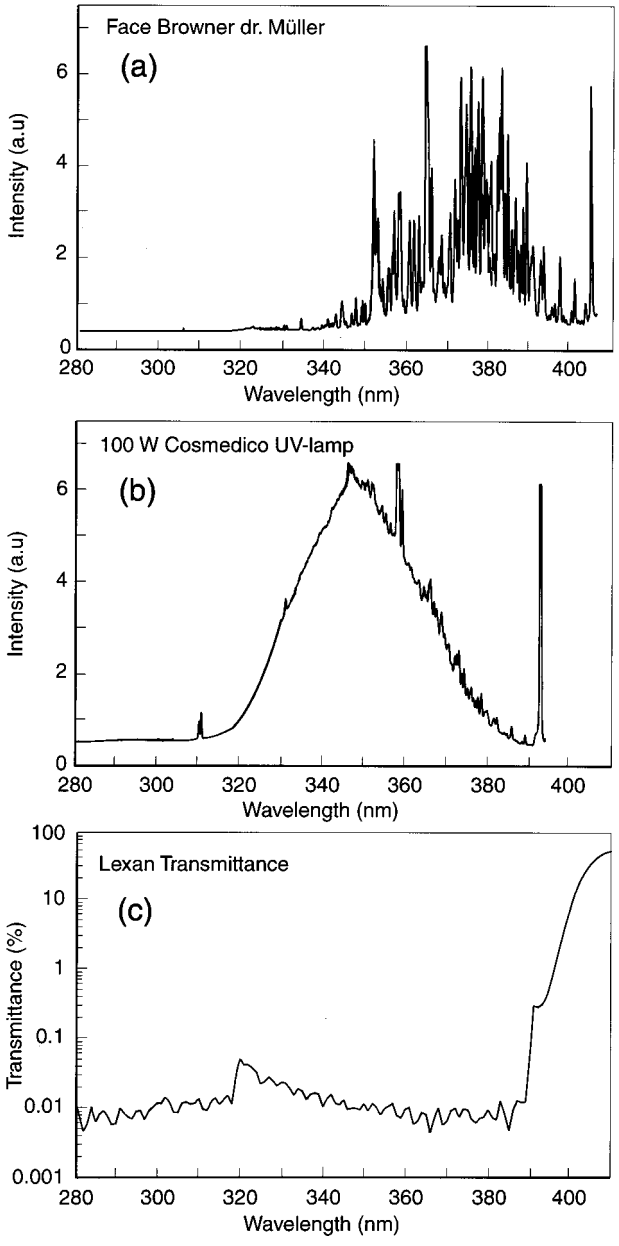

FIG. 2. UV emission spectrum of the face browner (a) and Cosmolux lamp (b) of the solarium (see Table I), and the transmission spectrum of Lexan (c), which was used for shielding.

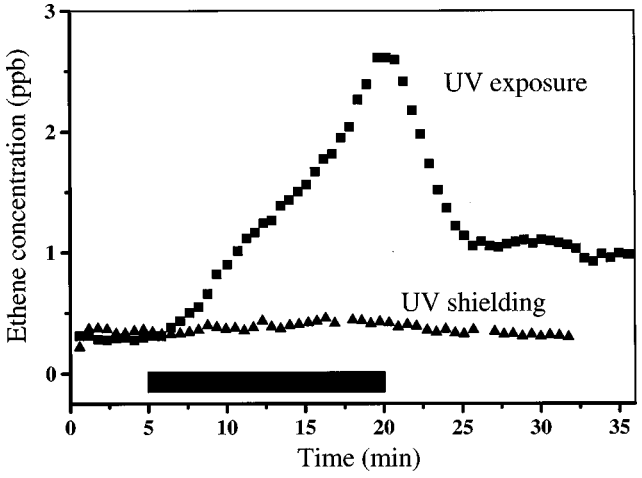

FIG. 3. The on-line excretion of ethene via the breath of a single person due to UV exposure from a solarium. Lower trace shielding of the subject from UV-A and -B radiation by Lexan. Bar indicates exposure to UV.

resistance to erythaemia (reddening of the skin).[16] Here, we observed during a second exposure to UV light (seven subjects) five days after the first UV exposure, an ethene exhalation of 8.6 ± 2.4 pmol/kg/min (same subjects, first exposure 16.0 ± 2.3 pmol/kg/min), a decrease to 54% ($p < 0.05$, see, e.g., Fig. 4).

There are some conceivable points of discussion that may arise. One of these is the nature of the source and the cause of ethene release; is the UV light the real source of ethene production and is the skin the place of origin? A first experiment was done with a subject lying in the solarium inside a Lexan tunnel (diameter 0.7 m, length 2 m, thickness 2 mm), which shielded only UV radiation (Fig. 2). No rise in ethene was observed (Fig. 3). Within a separate experiment the direct ethene emission by the skin was monitored. This direct emission already reaches a plateau after about 2 min of UV exposure (data not shown), indicating that the skin is the origin of the ethene emission and that the slow and persistent rise in exhalation is indeed a result of transport.

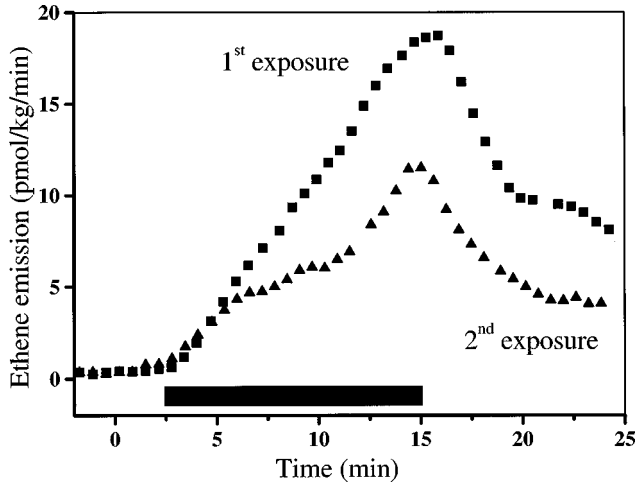

FIG. 4. Adaptation of the skin of a single person to UV exposure via ethene emission; upper trace, first exposure and lower trace, second exposure of the same subject after a five days interval. Bar indicates exposure to UV.

With the use of ethene as a fast, on-line biomarker it will be possible, e.g., to test the wavelength-dependent effects of UV (-A and -B) light on the skin for various skin parts and skin types, the effect of sun cream protection, or to follow the effects of UV treatment of skin diseases, e.g., psoriasis. Besides, it has a potential to be used for diagnostic purposes related to acute or chronic physiological disorders inside the human body, e.g., in inflammatory processes (acute asthma, inflammatory bowel disease)[20,21] or acute myocardial infarction.[22,23] With the recent development of new powerful all-solid-state tunable infrared lasers,[24,25] this method has a high potential in clinical applications.

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