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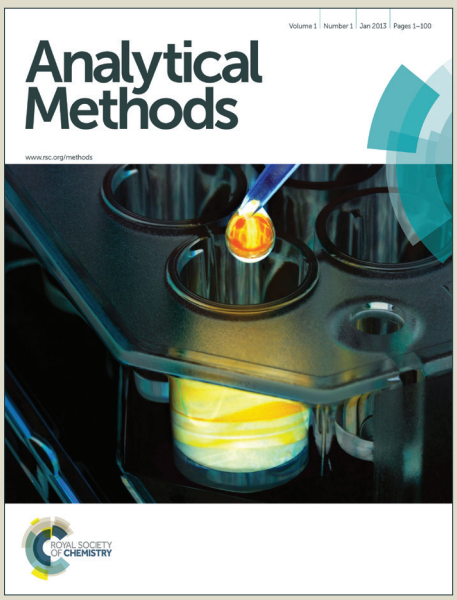

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Generation and characterization of airborne ethyl 2-cyanoacrylate atmospheres in a human whole-body exposure unit

Christian Monsé, Jürgen Bünger, Thomas Brüning, Rolf Merget

Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany

Correspondence to be sent to: Christian Monsé, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany. E-mail: monse@ipa-dguv.de, fon: +49 (0)234 302-4521, fax: +49 (0)234 302-4505.

Abstract

Exposures to air contaminants, such as chemical vapors and particulate matter, pose important health hazards at workplaces. Short-term experimental exposures of human subjects to chemical vapors and particles are a promising attempt to investigate acute effects of such hazards. In the EU, there is an increasing need for regulatory agencies to derive health-based threshold limits based on human inhalation studies.

Ethyl 2-cyanoacrylate (ECA) is the most widely used adhesive, both in industrial and domestic applications. ECA-containing vapors are irritating to the skin, eyes and respiratory system. In Germany, no occupational threshold limit value has been derived for ECA. A necessary prerequisite for inhalation challenge tests is the development of a suitable method to generate and characterize ECA test atmospheres for human whole-body inhalation studies. To address this, we developed a method to evaporate pretreated ECA with the help of a calibration gas generator. Experimental proof of the quantitative evaporation process was attained with a wipe test. The determination of airborne ECA concentrations in our exposure unit was established with chemical ionization real time mass spectroscopy taking into account

temporal stability, different relative humidity and the presence of subjects in the chamber. Finally, we also developed a 5 steps ECA inhalation challenge test.

Keywords: Ethyl 2-cyanoacrylate, human exposure unit, inhalation challenge, occupational asthma

Introduction

Cyanoacrylates (CAs) were first described in 1949, and their potential as adhesives was quickly recognized.[1] Various homologues of CA adhesives have been studied, including methyl- (MCA), ethyl- (ECA), isobutyl-, isohexyl-, and octyl-CA that are used in a wide variety of applications. Clinically, CAs are used as tissue adhesives and sealing materials. Industrial applications of CA glues are widespread and diverse. The broad variety of industrial applications for CAs as adhesives includes the manufacture of lampshades, plastics, electronics, scientific instruments, loudspeakers, shoes, jewellery, sports equipment, cable joining, manicuring (attaching false nails, repairing cracks), dentistry, surgery, and mortuaries. Fingerprint development for police crime scene investigation have also benefitted from the properties of CAs.[2] ECA is the most widely used CA for both industrial and domestic applications; MCA is also used in industry although its use has declined in recent years due to its more pungent odor.[3]

ECA is a highly reactive monomer that undergoes rapid anionic polymerization when a small amount of the monomer is pressed between two surfaces. The polymerization can be catalyzed by the small amount of water vapor normally present in the atmosphere. ECA containing vapors are irritating to the skin, eyes and respiratory system[4], and has the potential to cause contact allergy, either after occupational[5-8] or non-occupational exposure.[6-11] There are only few case reports of possible sensitization of the airways due to ECA and MCA that are almost always a result of occupational exposure to CAs.[12-22] An occupational threshold

limit value for ECA has not yet been established in Germany due to inadequate data.[23] OELs for ECA (time weighted averages (TWA)) were set at 9 µg/L (2 ppm) in Austria, Denmark, Sweden and Switzerland. Belgium, Canada (Ontario) and Spain have all set their OEL at 0.9 µg/L (0.2 ppm)[4], and the American Conference of Industrial Hygienists has also recommended a limit value of 0.9 µg/L (0.2 ppm).[24]

To date, there is only one brief experimental study available in which odor perception and sensory irritation were investigated in humans exposed to MCA vapor in an inhalation exposure unit.[25] However, the study report has several limitations as described by the World Health Organization in 2001.[2] To our knowledge, there are no publications on standardized ECA exposure studies in whole-body inhalation units. Such inhalation studies with ECA could derive an ECA occupational threshold limit value based on findings from different health effect parameters. A respective request was made by the German regulatory agencies. In addition, because ECA may be sensitizing to the airways, exposure experiments may be also used as a tool for the diagnosis of occupational asthma due to ECA.

ECA concentrations have to be produced under standardized exposure conditions and monitored in real-time in the lower ppb-range to perform inhalation studies in an exposure unit. A number of measurement systems to determine airborne ECA have been employed although there are reports of technical difficulties and inaccuracies with many of them. One method involves collecting airborne CA using a bubbler containing diluted sodium hydroxide.[26] CA in the resulting solution is then degraded to produce formaldehyde, which is then derivatized and measured spectrophotometrically. Method No. 55 of the US Occupational Safety and Health Administration involves sampling onto a sorbent tube containing phosphoric acid-coated XAD-7.[27] The tube is subsequently solvent desorbed, and the resulting solution is analyzed by high-pressure liquid chromatography (HPLC). Another

method involves sampling onto Tenax followed by solvent desorption and analysis by gas chromatography equipped with a thermionic-specific detector.[28] A method for measuring airborne CAs that was recently developed and is currently being validated by the Health and Safety Laboratory of the Health and Safety Executive uses various parts of previously published techniques.[29,30] Airborne CAs are collected onto a Tenax sorbent tube as in the Gaind and Jedrzejczak method[28], and are subsequently desorbed with a solvent. The resulting solution is analyzed by HPLC, using the analytical conditions described in OSHA Method No. 55.[27] The main disadvantage of these methods is that all operations are performed offline. Therefore the analytical methods were not suitable for our purpose and we developed an online method that is adapted for our analytical requests.

The aim of this study was to develop a method to evaporate purified and re-stabilized ECA with the help of a calibration gas generator. Target concentrations were in the range between 255 and 2700 ng/L (50 to 600 ppb). In addition, a wipe test was developed to assess the evaporation efficiency of ECA.

Methods

Purification and pretreatment of ECA

To prepare, all instruments made of glass were rinsed with a solution of 100 µL phosphoric acid (H_3PO_4, 85 %, Merck GmbH, Germany) in 100 mL acetone (Merck GmbH, purity 99 %, Germany) to prevent polymerization of ECA monomers. ECA was purchased from Foster and Freeman Ltd., UK (Type Cyanobloom) and was purified by fractional condensation in a glass line in vacuo at $1 * 10^{-4}$ mbar in a series of traps at room temperature, -50°C and -196°C. Before condensation, the trap at -50°C was impinged with 5 µL H_3PO_4 (85 %, Merck GmbH, Germany). 5 mL ECA was condensed in the trap at -50°C as white crystals. ECA melted to a colorless liquid as it warmed to room temperature. The phosphoric acid re-stabilized the ECA,

and the anionic polymerization was inhibited. The solution was stable over a period of several days at room temperature.

NMR characterization of ECA

The molecular structure and purity of freshly condensed and re-stabilized ECA was corroborated by means of ^{1}H- and ^{13}C-NMR spectroscopy. A Bruker Avance DPX apparatus operating at 250 MHz (Bruker BioSpin GmbH, Germany) was used. The spectra were obtained at 25°C in $CDCl_3$ against tetramethylsilane as the internal standard.

Synthesis of polymeric ECA

Polymeric ECA was synthesized via fractional condensation of 5 mL ECA in a trap at -50°C without the use of H_3PO_4. Warming ECA melted to a colorless liquid and was transferred to a 10 mL glass vial. Immediately the polymerization process formed polymeric ECA as a colorless and transparent solid.

Standard solution of polymeric ECA

1.02 mg polymeric ECA was dissolved in 10.0 mL acetone to yield a colorless liquid. The 20 mL glass vial was closed with a septum to prevent evaporation processes associated with possible concentration changes.

Generation of ECA containing atmospheres

The ECA vapor atmospheres were generated in two steps. The process started with a pre-mixture of relatively high gas concentrations in a 300 L glass vessel with the use of a calibration gas generator (HovaCal 521/2-SP, IAS GmbH, Germany). The second step yielded the dilution to the desired concentration in the ExpoLab with the air stream of the air conditioner as described previously.[31]

The calibration gas generators were operated with a total volume flow of 30 L/min (1.8 m^3/h) nitrogen at standard conditions (0°C, 1013 hPa), where a flow of 7.5 L/min was used for the evaporator module and the remaining flow of 22.5 L/min to achieve a stable premixture in the glass vessel. The nitrogen was produced via a nitrogen generator (NGM 22, cmc instruments GmbH, Germany), provided with oil free and dry compressed air. Two independent syringe pumps delivered the purified and pretreated monomeric ECA in a precise, steady flow without variation. While one syringe applied the liquid, the second syringe sucked in liquid. A continuous crossing of one syringe to the other was achieved by positively-controlled rotary slide valves. The capacity of the 12.5 µL syringe pumps was in the range of 1 µL/min to 50 µL/min. The temperature of the evaporator was fixed at 100°C. All parts of the calibration gas generator were pretreated with a solution of 1mL/L H_3PO_4 (85 %, Merck GmbH, Germany) in acetone to prevent polymerization of the ECA.

Measurement of the air exchange rate in the ExpoLab

To calculate the required amounts of liquid ECA the air exchange rate must be exactly determined. For this purpose, we monitored and analyzed a decay curve with the help of an inert gas.[32,33] Propylene (1.75 µg/L; 1.0 ppm) was continuously dosed into the ExpoLab until it reached a stable concentration. The propylene supply was closed while venting the unit with fresh air and the decay was recorded, and a ventilator used to homogenize the continuously diluting gas atmosphere. The software "GraphPad Prism 5.04" (GraphPad Software Inc., USA) was used to calculate the desired air exchange rate. The underlying mathematical model was the analysis of the 1.75 µg/L plateau followed by one phase decay with a least square fit.

Measurement of airborne ECA concentrations

The ECA concentrations in the exposure unit were analyzed with a high performance, real time chemical ionization mass spectrometer (CIMS, Airsense.net, MS4 GmbH, Germany). For more technical details see Hornuss et al.[34] The time resolution was 1 spectrum every 2 s.

Collection of polymeric ECA residue in the dosage system

Experimental proof of the quantitative evaporation process was made with a wipe test. All parts of the dosage system (evaporator, 300 L glass vessel) were rinsed two times with acetone (in total 200 mL), and wiped with an acetone-soaked paper towel. The solvent of the resulting solution (towel inclusive) was evaporated, and the residue was diluted with 30 mL acetone and analyzed with the help of a pyrolysis system (figure 1).

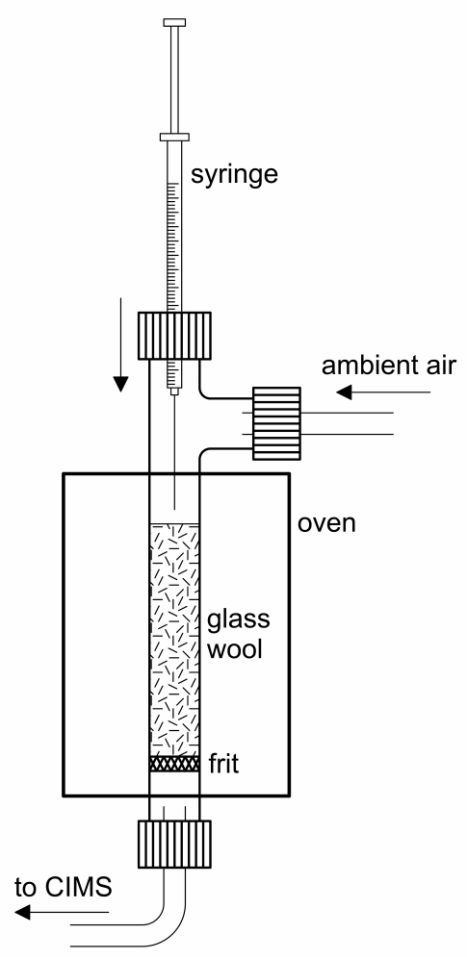
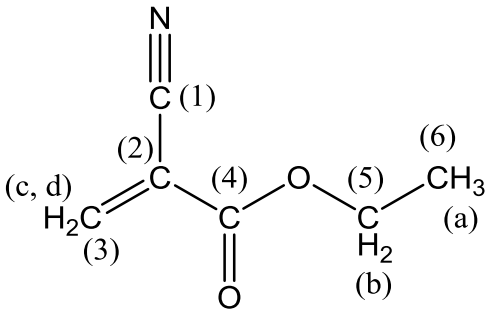


Figure 1: Pictorial schematic of the polymeric ECA pyrolysis system. CIMS: Chemical ionization mass spectrometer.

The acetone solutions (different volumes of the standard solution and the containing polymeric ECA rinse solution) were injected into a glass reactor filled with glass wool that was fixed with a frit at the bottom. The reactor was heated to 220°C. The sample pump of the CIMS analyzer sucked all gaseous pyrolysis products mixed with ambient air at a constant flow rate of 2.5 L/min to determine monomeric ECA concentrations. The time resolution was 1 spectrum every 2 s. The areas under the resulting injection curves were calculated with the software "PeakFit, version 4.12" (SeaSolve Software Inc., USA).

Results and discussion

NMR characterization of re-stabilized ECA



The exact peak positions and intensities are listed below (s = singlet; t = triplet, q = quadruplet; J = coupling constant, Hz): ^{1}H NMR (250 MHz, $CDCl_3$): d: δ = 7.05 ppm (s, 1H); c: δ = 6.61 ppm (s, 1H); b: δ = 4.34 ppm (q, 2H, J = 2.37 Hz); a: δ = 1.37 ppm (t, 3H, J = 2.37 Hz). The peak at δ = 7.24 ppm from the simple hydrogenated impurity in the $CDCl_3$ was used to calibrate the position on different peaks in the solution. $^{13}C\{^{1}H\}$ NMR (62.5 MHz in $CDCl_3$): 4: δ = 160.6 ppm; 3: δ = 143.2 ppm; 1: δ = 117.0 ppm; 2: δ = 114.6 ppm; 5: δ = 63.1 ppm; 6: δ = 14.1 ppm. The signal at δ = 77.1 ppm (t) is caused by $CDCl_3$. The different peaks of the ECA monomer are known from the literature.[35-38] The NMR experiments confirm the presence of ECA of high purity. Other peaks were not observable.

Measurements of airborne ECA

The principle for the measurements was the suction of airborne ECA in the ExpoLab with an internal pump into the mass spectrometer. It operated with a flow rate of 2.5 L/min. The Teflon-tube length was 6 m with an inner diameter of 2 mm. We extended the Teflon-tube with an additional tube (Teflon, length: 4 m, inner diameter: 2 mm) to assess influences of possible desorption effects on tube surfaces by comparing the measurement results with and without extension. Desorption effects were not observable (data not shown). The mass spectrum in figure 2 A was recorded using the quicksilver-mode (Hg-mode) as ionization gas and yielded a molecular ion peak at m/z 125 with relatively small abundance (11 %). Figure 2 B showed no molecular peak because the mass range between m/z 122.5 and 136.5 (grey lines) was skipped. These masses represent the molecular masses of the xenon isotopes that served as chemical ionization gas since spectrum B was recorded with xenon-mode (Xe-mode). All other skipped mass ranges and their corresponding molecules are given in table 1. The highest relative abundance (100 %) was determined at m/z 98 and was clearly identified as a fragment of ECA. The Xe-mode is approximately 1,400 fold more sensitive at m/z 98 in comparison with spectrum A. Very low target concentrations below 4.5 µg/L (1 ppm) in the exposure unit cannot be determined in the Hg-mode. Therefore, we decided to measure ECA in the single ion mode (SIM) with xenon as ionization gas.

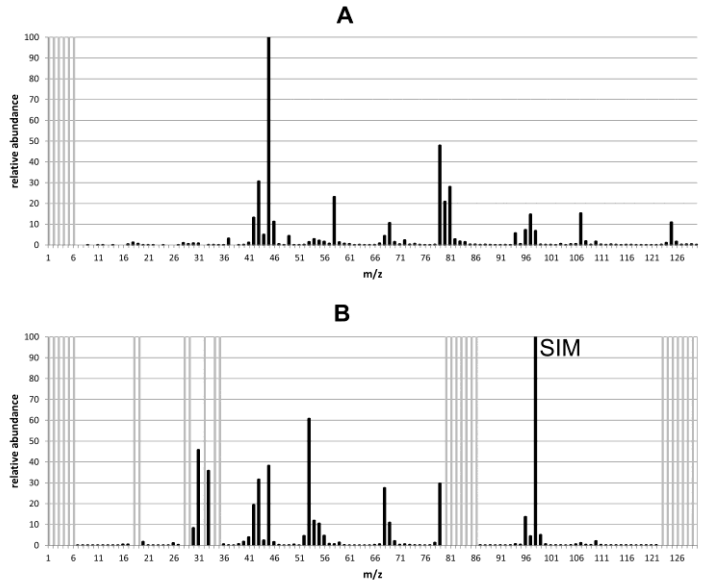


Figure 2: Chemical ionization mass spectra of ECA. **A**: Hg-mode; **B**: Xe-mode; SIM: Adjustment of the analyzer on single ion mode (SIM) with m/z = 98. Grey lines represent skipped masses.

Table 1: Skipped mass ranges and corresponding molecules.

skipped mass ranges [m/z]	corresponding molecule
0 - 6.5	below operating range
17.5 - 19.5	water
27.5 - 29.5	nitrogen
31.5 - 32.5	oxygen ($^{16}O_2$)
33.5 - 35.5	oxygen ($^{18}O^{16}O$)
79. 5 - 86.5	krypton
122.5 - 136.5	xenon

Measurement of the air exchange rate in the ExpoLab

A decay curve was recorded in the ExpoLab performed with propylene after a steady state concentration of 1.75 µg/L (1.0 ppm) at 23°C. The time resolution was 1 spectrum every 2 s.

Calculation of the required amounts of ECA

After recording the decay curve, the exponent *k* was calculated to be 12.21 per hour (equation 1).

$$c(t) = e^{-kt} \quad (1)$$

c(t): concentration dependent on time in µg/L; *k*: air exchange rate per hour; *t*: time in h

The resulting volumetric flow was calculated with an exposure unit volume of V_E =28.567 m^3 (Monsé et al. 2012) and was 348.8 m^3/h (equation 2).[31]

$$V = V_{E*}\, k \quad (2)$$

V: volume flow at measured temperature in m^3/h; V_E: volume of exposure unit in m^3

Conversion into standard volumetric flow at 0°C (equation 3) yielded 321.7 Nm^3/h. An additional value of 1.8 Nm^3/h (nitrogen for the evaporation process in the 300 L glass vessel) has to be considered. Therefore the total volumetric flow at standard conditions is 323.5 Nm^3/h.

$$V_N = V * 273{,}15/(273{,}15 + T) \quad (3)$$

V_N: volume flow at standard condition (T = 0°C) in Nm^3/h; *V*: volume flow at measured temperature in m^3/h; *T*: temperature in °C

A target concentration of 0.5 ppm per m^3 is equivalent to 2.79 mg/m^3 at standard conditions (equation 4).

$$c_{ppm} = V_M/M * c_{mg/m}{}^3 \quad (4)$$

c_{ppm}: concentration in ppm; V_m: molecular gas volume in L = 22.41 (0°C, 1013 hPa); *M*: molecular weight in g/mol; $c_{mg/m}{}^3$: concentration in mg/m^3

The required amounts of 903.2 mg/h (15.05 mg/min) ECA has to be evaporated (equation 5).

$$F = c_{mg/m^3} * V_N \quad (5)$$

F: Liquid ECA flow rate in mg/h

The calibration gas generator is equipped with a digital control software in which the evaporation rate of ECA can be entered with the knowledge of the density and molecular weight of ECA (1.04 g/cm^3; 125.13 g/mol).[4]

Different airborne ECA concentrations in the exposure unit

After reaching a stable concentration in the exposure unit, the ECA signal of the analyzer was adjusted to the calculated concentration of 2.25 µg/L (0.5 ppm). The background calibration of the analyzer was performed with synthetic air from a TOC gas generator (NGG 60, Seraltec, Germany). The limit of detection (LOD) was 45 ng/L (10 ppb). Figure 3 demonstrates the measurement results of a target ECA concentration of 2.25 µg/L (0.5 ppm) without a human subject in the chamber who could confound the concentrations. By decreasing the injection volume of ECA, the corresponding airborne ECA concentration decreased to the same ratio (0.9, 0.45 and 0.225 µg/L; 0.2, 0.1 and 0.05 ppm). A new target concentration was reached after about 30 min. Statistical analysis of the correlation between injection volume and measured ECA concentrations showed a linear correlation with y = 0.9799 x + 0.0056 and r^2 = 0.9997.

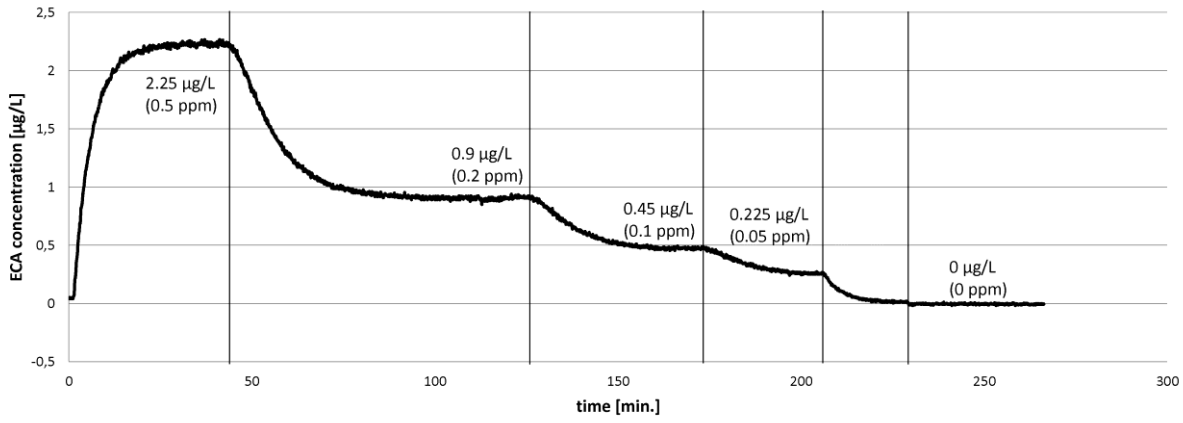


Figure 3: Results of repeated measurements of different ECA concentrations in the exposure unit without human subjects in the exposure unit. LOD: 45 ng/L (10 ppb).

Validation of quantitative evaporation process of ECA

The calculation of the required amounts of ECA needed to reach a particular target concentration assumes a quantitative evaporation process of ECA. The resulting target concentration would be lower than calculated if there was polymerization of significant amounts of gaseous ECA. After performing several ECA exposure scenarios in which in total 2,500 mg ECA were evaporated, we performed a wipe test, described in the section "*Collecting of polymeric ECA residue in the dosage system*". Figure 4 shows the injection peaks of pyrolyzed polymeric ECA. Except for 2 injection peaks marked with *, the system yielded reproducible values. Different injection volumes of the polymeric ECA standard solution were used to calculate a regression curve. Volumes of 10, 30 and 50 µL were pyrolyzed and a zero control was performed with pure acetone (injection volume 10 µL). The LOD was 0.9 µg/L (0.2) ppm related to 10 µL injection volume (equal to 1.26 mg polymeric ECA in 10 mL acetone). The residue of the wipe test, dissolved in 30 mL acetone, was injected in a volume of 10 µL. The calculated regression curve showed a linear correlation with $y = 1.0491\ x - 1.1266$ and $r^2 = 0.9979$ (areas under the curves versus injection volume), and the measured polymeric ECA concentration in the residue solution was determined to be 2.4 mg. Taking into consideration the dilution volume of 30 mL with acetone, the collected amounts of polymeric ECA was 7.2 mg - negligibly small in contrast to the 2,500 mg used for monomeric ECA. The evaporation process was nearly quantitative, where only about 0.3 % of the material was lost due to polymerization. Qualitative wipe tests of the walls in the ExpoLab (25 cm x 65 cm wipe areas) with acetone soaked paper towels yielded no detectable polymeric ECA.

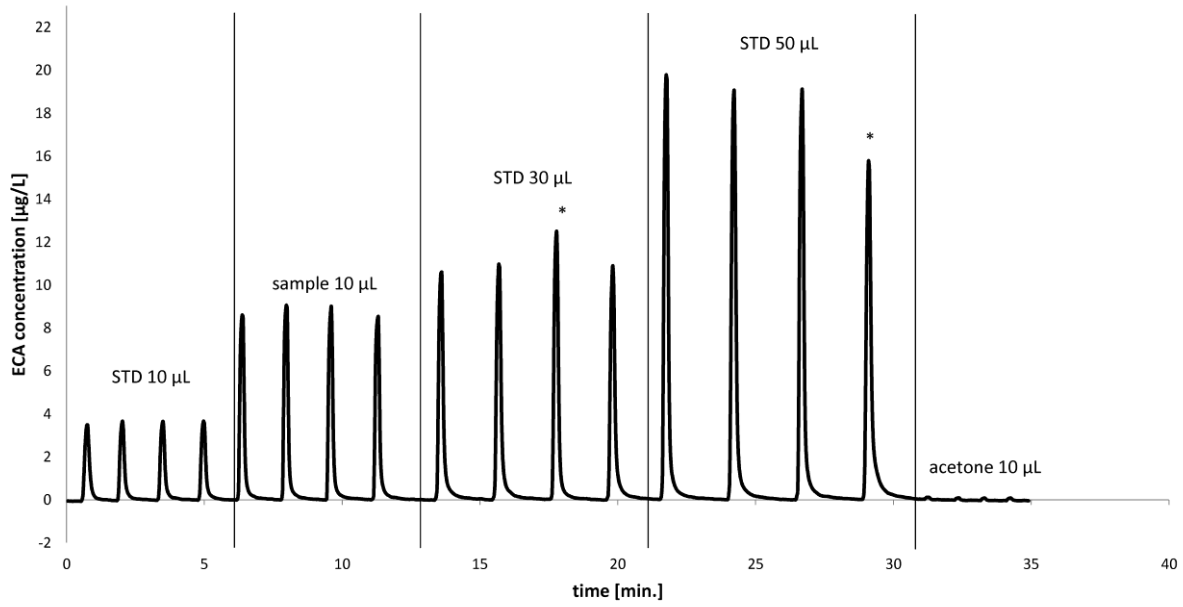


Figure 4: Injection peaks of polymeric ECA. Peaks marked with * were excluded in the calculation of the regression curve. LOD: 0.9 µg/L (0.2 ppm) related to 10 µL injection volume (1.26 mg polymeric ECA in 10 mL acetone).

Validation of the purification and re-stabilization of ECA

The re-stabilization of freshly condensed ECA with 1000 ppm H_3PO_4 inhibits the polymerization process. To check the efficiency of stabilization, 4 mL purified ECA was repeatedly condensed by fractional condensation in a glass line in vacuo at $1 * 10^{-4}$ mbar in a series of traps at room temperature, -50°C and -196°C. The residue in the trap at room temperature was added to 20 mL acetone, and the resulting solution contained 4.7 mg polymeric ECA as determined with the pyrolysis method. That implies an impurity of only 0.1 % and an efficiency of stabilization near 100 %.

Influence of humidity on airborne ECA concentration

The starting climatic parameters were set to 23.0°C and 45 % relative humidity to provide ambient environmental conditions for the subjects. The relative humidity in the air of the exposure unit during a 0.9 µg/L (0.2 ppm) ECA exposure increased to 73 % and represented the limit of technological capabilities. As apparent in figure 5, there is no significant change

in ECA concentration over time. The average ECA concentration was determined to be 0.9 µg/L (0.20 ppm) +/- 1.4 %.

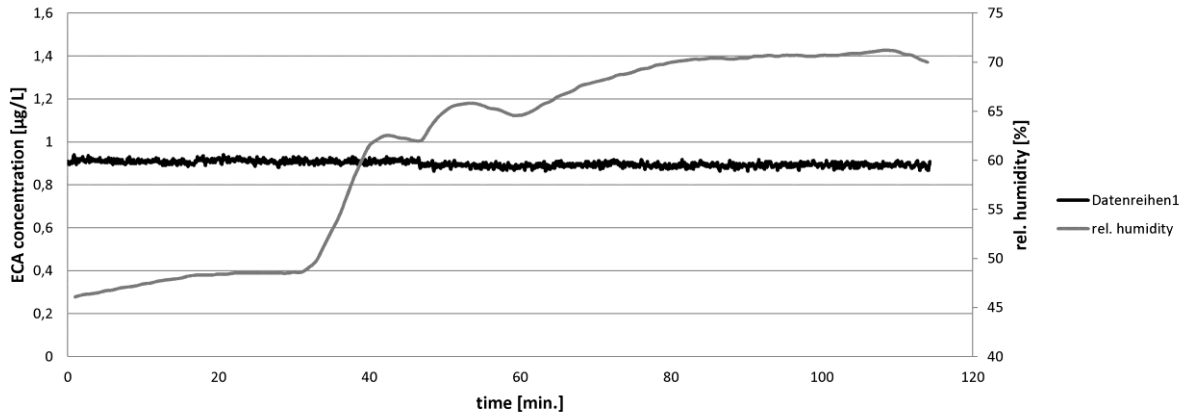


Figure 5: ECA concentration course dependent on relative humidity in the exposure unit.

5-steps ECA challenge

We performed a 5-steps ECA whole body inhalation challenge with a doubling dose model in a patient claiming compensation for occupational asthma due to ECA, starting with the lowest concentration. The test was performed according to the guidelines of the German Society for Occupational and Environmental Medicine.[39] The duration of each concentration step was 20 min. There was a 30 min pause without exposure between the steps. The patient gave written informed consent for the test. As a result, the presence of the patient in the chamber only minimally confounded the temporal stability of airborne ECA concentrations (figure 6). Medical effects were not observed (data not shown).

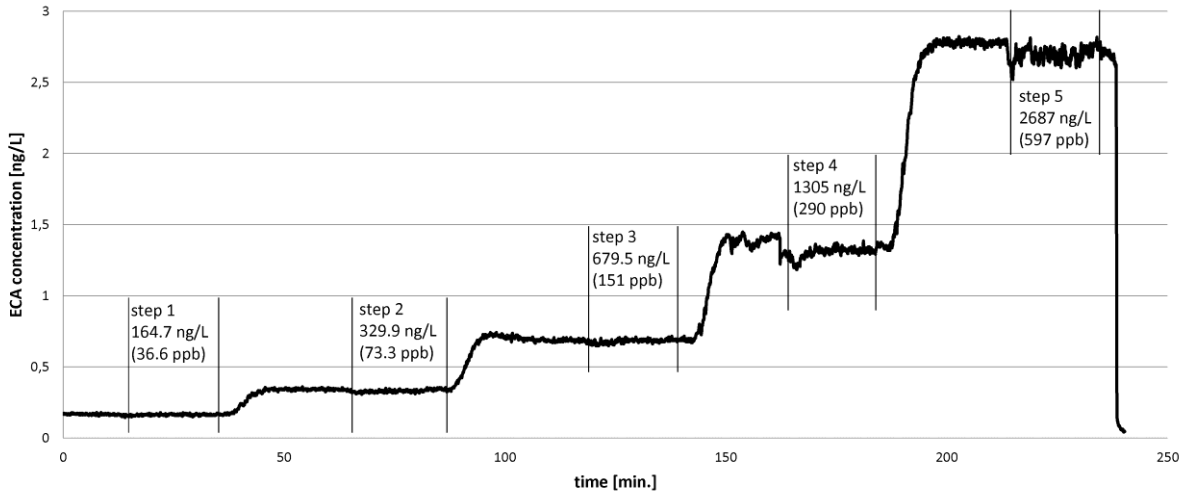


Figure 6: Time course of a 5 steps ECA inhalative challenge with one subject in the ExpoLab.

Table 2 shows the targeted and measured ECA concentrations during the challenge. A linear correlation between targeted and measured ECA concentration is also given ($y = 0.9936\ x - 0.0013$; $r^2 = 0.9997$). The maximum relative standard deviation was recognized at step 1 with 164.7 ng/L (36.6 ppb) +/- 3.5 %. Relative deviations between targeted and measured ECA concentrations were relatively low and lay in the range of -3.3 to +0.7 %. In contrast to figure 6, the relative standard deviations of the measured ECA concentrations without the influence of subjects were in the maximum range of +/- 2.0 %. The subject did not confound the chemical ionization mass spectroscopy measurements. No cross sensitivity was seen at m/z = 98 after the subject had entered the ExpoLab without airborne ECA inside.

Table 2: Targeted and measured ECA concentrations during challenge.

	step 1	step 2	step 3	step 4	step 5
target concentration [ng/L; ppb]	168.8; 37.5	337.5; 75.0	675; 150	1350; 300	2700; 600
averaged concentration [ng/L; ppb]	164.7; 36.6	329.9; 73.3	679.5; 151	1305; 290	2687; 597
standard deviation [%]	3.5	2.6	1.9	2.7	1.8
deviation from target value [%]	- 2.4	- 2.3	+ 0.7	- 3.3	- 0.5

Keen et al. generated dynamic ECA standard atmospheres to develop an analytical method to determine airborne ECA.[30] They used a vapor generator with the help of dry nitrogen. The generator contained an open topped vial of ECA as a diffusion source. The CA-laden nitrogen stream was diluted with clean dry air. The resulting air flow passed into a 5 L glass chamber (at atmospheric pressure) surrounded by a temperature-controlled water jacket. The CA concentration within the chamber's atmosphere was controlled by varying the vapor generator oven temperature, the flow rate of dilution air, or a combination of both. Although the ECA test atmospheres remained stable for long periods, a white deposit gradually accumulated in the pipework of the test atmosphere rig. This was assumed to be polymerized CA, and its presence indicated that it was not possible to calculate the theoretical airborne CA concentration in the standard atmosphere by measuring the weight loss of the CA diffusion source. Other working groups used the same generator type for the development of analytical methods. Therefore, we used a new strategy and decided to treat purchased ECA with fractional condensation. This procedure freed the ECA from possible impurities of polymeric ECA and the added stabilizers of the manufacturers. On addition of phosphoric acid, the condensed product was re-stabilized, and an acid concentration of 1 mL/L was needed to avoid the anionic polymerization. At this concentration, treated ECA cannot be used as an adhesive any more, it is "over-stabilized". NMR spectroscopy of freshly condensed and re-stabilized product confirmed the presence of ECA of high purity. Other peaks were not observable. If no phosphoric acid was added to the freshly condensed ECA, a spontaneous reaction to polymeric ECA was seen. During the evaporation process of the re-stabilized ECA, the H_3PO_4 remained in the oven module of the calibration gas generator and had no potential to confound any medical effect parameters. Validation of the purification and re-stabilization of the ECA showed an excellent efficiency near 100 %. The evaporation was carried out nearly quantitativly, only small amounts of polymeric ECA (in total 7.2 mg, equal to 0.3 % of evaporated total mass) were detectable in the dosage system of the ExpoLab and

no residues were found on the walls inside the unit. Under the prerequisite of a quantitative evaporation and with knowledge of the air exchange rate, determined with a decay curve, the targeted ECA concentrations in the exposure unit can be calculated.

The analytical detection method of ECA was made with the help of chemical ionization mass spectroscopy, using xenon as the ionization gas. This method allowed the detection in the lower ppb-range (LOD: 45 ng/L; 10 ppb) with a temporal resolution of 1 spectrum every 2 s with the mass fragment m/z 98 in a single ion mode. Switching the ionization gas to quicksilver vapor yielded a 1,400 fold weaker response to ECA and was therefore no longer considered. As shown in the section "*Different airborne ECA concentrations in the exposure unit*", a new target concentration was reached after about 30 min. Statistical analysis of the correlation between injection volume and measured ECA concentrations showed a good linear correlation.

Keen et al. validated their method using different relative humidities in the test chamber (20, 50 and 75 %).[30] They found a slight decrease in airborne ECA concentrations at 75 %. Therefore, we increased the relative humidity in the ExpoLab from the starting point of 45 % up to 73 % to assess a possible influence of water vapor. Technical limitations did not allow higher values. We found no significant deviation of the target ECA concentration of 0.9 µg/L (0.2 ppm) and could not confirm the experimental findings of Keen et al.[30] Furthermore this experiment showed the temporal stability of the generating and analyzing devices within a 2 h period.

As expected and shown earlier, the time courses of ECA concentrations were affected in exposure scenarios with human subjects by increasing the standard deviations of the average concentrations.[31] These effects probably occurred due to absorption effects onto the subjects' clothes and were relatively small and negligible.

Our exposure unit can also be used to perform inhalative challenge tests with ECA. We chose a doubling doses model starting with the lowest concentration, comparable with protocols for the diagnosis of occupational diisocyanate-related asthma.[40]

Conclusion

Overall, the described experiments confirm that a safe and constant exposure of ECA can be provided for subjects in the ExpoLab. Thereby, a technical basis is provided to realize a study deriving a threshold limit value for ECA and to perform inhalative challenge tests in the diagnosis of occupational asthma due to ECA.

Declaration of interest

The authors report no conflicts of interest.

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