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Bioluminescent liquid light guide pad biosensor for indoor air toxicity monitoring

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ABSTRACT: Indoor air pollution became a recent concern oftentimes worse than outdoor air quality. We developed a tool that is cheap, simple and enables continuous monitoring of air toxicity. It is a biosensor with both a non-disposable (monitor) and disposable (calcium alginate pads with immobilized bacteria) elements. Various parameters to enhance its signal have been tested (including the effect of the pad's orientation, it's exposure to either temperature or time with the air toxicant analyte and various concentrations thereof. Lastly, the sensor has demonstrated its ability to sense the presence of chemicals in a real, indoor environment. This is the first step in the creation of a sensitive and simple operative tool that may be used in different indoor environments.

KEYWORDS: Air pollution, Bioluminescence, Biosensor, Pad, Bioreporter

1. Introduction

The World Health Organization estimates that nearly two million people die prematurely each year from indoor air pollution[1].

Indoor air pollutants include: particulate matter, as in the case of dust arising simply from ventilation[2] or in the severe case of dust from solid combustibles (which has become major source of indoor air pollution in China[3]; combustion gases, such as nitrogen dioxide, carbon monoxide and sulfur dioxide; microbial products; cigarette smoke; volatile organic compounds from carpets, upholstery and toiletries; and pollutants from ambient air[4]. The increased use of air conditioning in many countries aggravates the exposure to indoor pollutants[5].

Of interest would be to evaluate the effect of these pollutants on human health and tools in their estimation would be welcome. One such device would be one that would monitor continuously and real-time, indoor air pollution. Conventional methods allow highly accurate and sensitive determination of the tested air samples, however, they exhibit limitatins such as the need to collect air samples on-site and thereafter transport them to a laboratory for analysis with gas chromatography (GC), fluorometry or high-performance liquid chromatography (HPLC)[6-8]. These are time-consuming (days to weeks to obtain the results) and provide only the detection of a single compound or a group of structurally related compounds at any given time. In addition, all these techniques require skilled personnel and expensive equipment. Smart gas sensing systems, i.e. electronic noses, comprising an array of gas sensors[9-11], quartz-crystal resonators[12] and other sensors are being developed to fill in the indoor air monitoring niche. However, the main disadvantage of all these applications is their inability to measure the biological effects of tested air pollutants for various types of toxicities (e.g. cyto-, neuro, .geno-toxicities or endocrine disrupting effects).

Therefore, a cheap, simple and continuous monitoring methodology for a wide range of pollutants would be welcome. Biosensors offer some advantages for air toxicity analysis as they may include living organisms and offer the possibility of miniaturization and portability, which enable their use as field devices for on-site monitoring[13].

Bioluminescent bioreporter assays have gained increased attention thanks to advances in genetic manipulation techniques, which offer the possibility to change non-emitting organisms, isolated from different habitats, into both luminescent and, more specifically, responsive eporting ones[14]. The advantages offered by whole-cell applications include high sensitivity, low cost, large test populations, rapid responses and, most importantly, the capability to program bacteria to respond in a specific manner to particular classes of compounds[15]. The bioluminescence assay based on reporter genes from *Vibrio fischeri* bacteria was found to be the most sensitive to a wide range of chemical pollutants, compared to many other methodologies[14]. It also showed good correlation with other toxicological bioassays, such as fish and algae etc., while providing a much faster response. These said bacterial reporter organisms may become integrated into a biosensor system [16,17]. For this these must be immobilized in some way in intimacy with an inorganic transducer.

Different immobilization methodologies have been employed (e.g. covalent [18,19], agarose [20,21], antibody modified surfaces [22] and sol-gel [23-27]) to fix whole cells to various surfaces. Alginates are a very commonly used class of hydrogel immobilization matrices [28]. Alginates may spontaneously form gels in a single-step process in the presence of divalent ions (e.g. calcium [29], strontium [30]). The high porosity provided by the open lattice structure and gentle environment provided by the gels makes the alginates the optimal choice for cell entrapment, encapsulation while enabling analyte diffusion and metabolite secretion [31].

A field-operable system for directly measure air quality with bacteria immobilized to a fiber optic used a TV1061 *Escherichia coli* strain immobilized in a calcium alginate hydrogel set onto optical fibers, for detecting air pollutants [32]. Using this original concept, a new approach for monitoring air toxicity is here proposed: a biosensor with both non-disposable (monitor) and disposable (calcium alginate pads with immobilized bacteria) elements. Various parameters enhancing the signal obtained have been tested here including the effect of the pad`s position, exposure to temperature and time with the air toxicant analyte and various concentrations thereof. Lastly, the sensor has demonstrated its capability to sense the presence of chemicals in a real, indoor environment. This is the first step in the creation of a sensitive and simple operative tool that may be used in different indoor environments.

2. Materials and methods

2.1 Materials

LB Agar Difco (244520) was purchased from Becton Dickinson. Chloroform (03080521), methanol (13680521), diethyl ether (05280501) and toluene (203635) were purchased from Bio Lab Ltd. Kanamycin (K1377), alginate (A2158), formaldehyde (F1635), N-methyl-2-pyrrolidone (328634), 2-mercaptoethanol (M6250), octanol (O4500), tetrahydrofuran (401757), ammonium hydroxide (A6899), N,N-diisopropylethylamine (550043), benzyl alcohol (402834) and calcium chloride (C5670) were of analytical grade and were purchased from Sigma (Rehovot, Israel). Triphenyl phosphate (T84654), tetraethylorthosilicate (86578), epichlorohydrin (E1055), N-methylmorpholine (407704) and (3-glycidyloxypropyl)trimethoxysilane (440167) were purchased from Sigma Aldrich (Rehovot, Israel). Dimethylformamide (103053) and diethylene glycol (803128) were purchased from Merck (Bellirica, USA). Piperidine (80645) was purchased from Fluka (Rehovot, Israel) and pyridine (33553) was purchased from Riedel-de Haën . Glycerin (2355519000024) was purchased from Frutarom (Haifa, Israel). Acetone (70-508201) was purchased from

CARLO ERBA (Paris, France). All stock solutions were diluted with double distilled water (ddW) and stored at temperatures suggested by the manufacturers.

2.2 Bacterial strains

The *Escherichia coli* strain used in this study, TV1061 [33], was obtained as a gift from S. Belkin (Hebrew University, Jerusalem, Israel). This strain harbors plasmid-borne fusions of the specific *grpE* [34] promoter sensitive to metabolic changes, such as with cytotoxic substances. All these promoters are plasmids integrated to the *lux* CDABE reporter operon, which has five promoterless structural genes. These are responsible for both the heterodimeric luciferase units (lux A and B) and the synthesis of the luciferase substrate, tetradecanal, by an ATP- and NADPH-dependent multi-enzyme complex composed of fatty acid reductase, transferase, and a synthetase (*lux* C, D and E) [35]. Strain stocks were stored at −80 °C with 20% (v/v) of glycerol as a cell cryoprotectant additive [36]. The bioreporter strains from stock solution were placed on Luria-Bertani (LB)-agar plates (NaCl 5 g/L, yeast extract 5 g/L, tryptone 10 g/L, agar 15 g/L) supplemented with 50 µg/mL kanamycin and, after an additional overnight growth at 37 °C in an incubator (Gerhardt, Germany), were stored at 4 °C for future experiments.

2.3. Growth conditions

Bacterial cultivation prior to measurements was performed in 10 mL LB-medium (NaCl 5 g/L, yeast extract 5 g/L, tryptone 10 g/L) [37] supplemented with 50 µg/mL kanamycin. Cells were grown overnight at 37 °C in a rotary thermo-shaker (Gerhardt, Germany) at 120 rpm in the presence of the kanamycin. Cultures were then diluted to approximately 10^7 cells/mL and re-grown in 25 mL LB at 26 °C, without shaking or antibiotics, until an early exponential phase (OD_{600nm0} of 0.2) as determined by an Ultrospec 2100 Pro spectrophotometer (Amersham, England).

2.4. Immobilization procedures

The harvested cells were mixed at a 1:1 ratio with a filter-sterilized 2% (w/v) low viscosity sodium alginate solution. 30 µL of 0.5 mM $CaCl_2$ solution were dripped into a cylinder with a diameter of 0.6 mm and 50 µL of a mixture of alginate and bacterial bioreporter cells was dripped above it to create a pad.

2.5. Instrument setup

A field-operable fiber-optic photodetector device was previously designed [29]. In order to monitor air toxicity, the device was modified by Neobionics Ltd (Israel) so as to include the components allowing for such a feature (Fig. 1). The original instrument set-up was placed in a light-tight box. The output signal, in analog measurements, was the mean value of the signals that included AC components (pulses) generated after multi-anode magnification in the photomultiplier tube. A Hamamatsu HC135-01 PMT Sensor Module was used for bioluminescence measurements (490 nm), combining the sensitivity of a photomultiplier tube with the intelligence of a microcontroller (Fig. 1).

The detector was optimized to the blue light region and included a 21 mm diameter active area convenient to gather light radiation without any optical focusing elements [29]. Several components exhibiting various features were then added to this device as follows. Bacteria were immobilized within calcium alginate pads, placed at the proximal end of a liquid light guide (53694, Edmund optic, USA) and the near end was placed in front of the PMT module (Figure 1). To receive and treat data, a specific driver was developed using LabView (version 3.1, National Instruments Corporation), which allowed monitoring of the bioluminescent signal and data handling in real-time.

2.5 Pad optimization

2.5.1 Pad position

In order to examine the effect of the pad position on bioluminescence response, pads (prepared as described above) were placed in $20*10^{-6}$ m³ vials. Two pads were placed in each vial, one in an the erect position and the other in a supine position. 1 µL of chloroform was dripped near the pads without direct contact between chemical and the bacteria and the vial was closed and incubated at 30 °C for 30 minutes. The liquid pollutant was expected to evaporate during the exposure time and penetrate via diffusion into the pad matrix thereby reaching the immobilized bacteria. The pads were then taken out of the vials and inserted into 96-well microtiter plates (Dynatech). Bioluminescence activity was measured every 5 minutes using a Luminoskan Ascent luminometer (Thermo Fisher Scientific, United States). During the measurement, the temperature of the samples was maintained at 26 °C. Measured luminescence values are presented in relative light units (RLU).

2.5.2 Effect of temperature and exposure time

Immobilized cells were exposed to different chloroform concentrations (e.g. 12.7, 63.7, 318.5 and 637 ppb) in $20*10^{-6}$ m³ vials and incubated for 0.5, 1 and 2 hours at 4 °C, 26 °C, 30 °C and 37 °C.

2.6 Exposure to confined toxins

Immobilized TV1061 strain pads were exposed (but kept separate) to different liquid toxicants (Table 1) placed in a hermetically closed chamber. The liquid pollutant evaporated during exposure time and was assumed to diffuse through the alginate matrix to the immobilized bacteria. Toxicants used were glue spray, bleach, oil stain remover, Tipex, paint, fuel and weed killer so was, the effect of cigarette smoke tested. Bacteria were exposed to 5, 10, 15, 20, 40, 60 and 120 seconds of passive smoke and incubated for 1 hour at 30 °C.

2.7 Office exposure to a toxic environment

In order to determine the capability of the sensor to sense indoor environment, the device was operated (in the dark) in an office. With continuous measurement, bacteria were exposed to either 5 or 10 mL of chloroform as well as 2 mL of acetone, in a mock spike nearby the biosensor within a radius of 1 meter from the immobilized bacteria.

2.9 Data analysis

The bioluminescence signal of bacterial response to chemicals was expressed as the induction factor, calculated using the following formula: Induction factor = Bi/BC, where Bi is the value of the bioluminescence signal for the tested toxin and BC is the value for the control.

2.10 Safety considerations

All chemicals used in this research are toxic and measuring procedures required special precautions. All experiments were carried out in chemical hoods and with very low toxicant concentrations to protect laboratory personnel. During office air toxicity measurments, the room was ventilated for 24 hours after toxicant exposure.

3. Results

3.1 Understanding pad optimization

3.1.1 Pad position

Two different pad orientations were tested: erect (i.e. two interfaces of the alginate matrix were exposed to the toxic environment) and supine (i.e. one side was exposed directly to the contaminated air) and their respective toxicity responses recorded (Figure 2). Cells placed in the standing mode showed a much higher inhibitory effect (after five hours more than 500 times) (Figure 2), therefore achieving an expending higher diffusion rate of the chemical into the matrix.

3.1. Temperature and chemical exposure time

The effect of temperature on bacterial response was evaluated by exposure of the immobilized bioreporter bacteria to different chloroform concentrations at four different temperatures (4 °C, RT, 30 °C and 37 °C) (Figure 3A) and three different (30 min, 1 hr, 2hrs) exposure times (Figure 3B). Figure 3 demonstrates the effect of temperature on diffusion rate, but must take into account that cell metabolism is also affected by temperature. Increased incubation temperatures increased cell response. Whilst at 30 °C there was inhibition of bacterial response, at 37 °C there was induction. At lower testing temperature modes (e.g. 4 °C and RT) light intensity hardly changed over time (Figure 3A), Figure 3B represents the effect of two different parameters (exposure time to the air toxicant and effect of the temperature) on bacterial response to different concentrations of chloroform. In general, increasing the toxicant concentration in air increased cell response. At lower chloroform concentrations, cells incubated in 30 °C and 37 °C have shown the highest induction factor values. On the other hand, increasing toxicant concentration in air induced bacterial response even at lower incubation temperatures (Figure 3B).

3.2 Exposure to confined pollutants

The immobilized bacteria were exposed to various chemicals in a confined environment,and they responded in a dose-dependent manner (Figure 4). Increasing the chemical concentration in air induced (as in case of methanol or toluene) or inhibited (in case of formaldehyde and 2-mercaptoethanol) the bacterial bioluminescent bioreporting responses. When exposed to chemicals found in indoor environments (e.g. acetone, bleach, glue, paint, etc.) the exposed bacteria responded in a dose-dependent manner (Figure 4B). For example, fuel and acetone induced, while cigarette smoke and weed killer inhibited cellular response.

3.3 Exposure to a chemical exposed in an office room

The sensor was exposed to the presence of contaminants indoors while in continuous operation with either acetone (2 mL) or chloroform (5 and 10 mL) added as spike spills in the office room (Figure 5). There was an increase in bacterial luminescence (four fold) in the case of acetone and (25,000 fold) in the case of chloroform after the “incident”.

4. Discussion

Inhalation of gases and vapors in indoor environments may cause a wide range of adverse health effects, ranging from simple irritation to debilitating systemic diseases. Although biosensors and traditional analytical methodologies are able to determine the presence albeit in no simple way the type of the toxicant in an air sample, there remain many problems and issues to be solved. Some of these methods are expensive, some require special laboratory equipment and some are not amenable to real-time monitoring of air toxicity. However, the biggest problem for all of these technologies is the requirement for skilled, expensive personnel, which will slow down (or prevent) their wide usage as toxicity-monitoring tools. Previously, a fiber optic biosensor based on bioluminescent bacteria was developed and enabled real-time monitoring of confirmed air toxicity[32]. This paper describes the next step in the creation of a tool for air toxicity monitoring.

4.1 Optimization of the biosensor characteristics

A liquid light guide based bacterial system for online monitoring of toxic pollutants in air was developed (Figure 1). This biosensor consists of two parts: a photodetector that will measure cell response and the replaceable, disposable pads with immobilized bacteria, sensitive to various toxicants (e.g. heavy metals, EDCs, antibiotics [17] or whole water toxicity) that can be stored in a refrigerator. A one-step action will remove the pads from the device and a second, one-step procedure will place a new one at the device endface. The simplicity of this procedure will enable personnel without any technical or scientific experience to use the device

In studying this new system three variables were tested. The first was to determine the effect of the orientation of the immobilization matrix on toxicant diffusion rates and on cell response. To determine this, two different orientations were tested (supine and erect). The main difference in these orientations is the (almost) doubling of the surface area exposed to the contaminated air. Thus enabling a faster diffusion rate of material brought to the bacteria, which was seen as greater inhibition of cells (Figure 2). However, despite these results the device was designed to hold the pads in the supine position while still being useful in being able to monitor the presence of air pollutants at low concentrations.

Thereafter, we determined the effect of incubation temperature on biosensor sensitivity. Immobilized bacteria were incubated in the presence of airborne toxicants at different temperatures (Figure 3). Investigation of the effects of temperature is a very important step in the creation of whole cell-based biosensors. On the one hand, increasing temperature is a powerful instrument for accelerating diffusion and increasing the concentration of the chemicals inside the matrix but on the other hand, high temperatures may affect the functionality of the sensor. For example it may inhibit luciferase (*V. fischeri*) activity due to its instability in *E. coli* at temperatures in excess of 30 °C [38], or temperatures higher than 37 °C may turn on heat-shock repair mechanisms and induce false responses of the bioreporter bacteria [33]. Figure 3A demonstrates that increasing exposure temperature increased bacterial responses. Higher cell responses were observed at 37 °C; at this step only the effect of the incubation/exposure temperature was evaluated. The measurement temperature was maintained at 26 °C, thus there was no longer the effect of heat on cell responses after the exposure step.

We then determined the cumulative effect of the toxicant exposure time and incubation temperature on sensor activities. Immobilized cells were exposed to different concentrations of chloroform for different time periods (e.g. 0.5, 1 and 2 hrs.) and temperatures (Figures 3B). Generally, the induction effect was increased with increases in the exposure time, incubation temperature or the concentration of the toxic chemical in air. At lower temperatures at various chloroform concentrations, increasing the exposure time increased cell response. At these temperatures, diffusion and metabolic rates are slower. Thus, it takes time for the pollutant to reach the cells and for the microorganisms to react. A similar effect was found previously with incubation of the TV1061 strain with different concentrations of atrazine [39]. It was then found that cold incubation reduced the effect of the bacteria's own metabolism or, more likely, allowed the accumulation of the toxicant inside the cell during a slowing of the overall metabolism. Consequently, this enhanced the capability of the bacteria to sense the effect of chloroform at very low concentrations (4 ºC) (10 fg/mL) [39]. At higher concentrations it showed a strong inducing effect.

4.2 Response of the biosensor to various air borne pollutants

Sensors based on enzymatic [40] and whole cell methodologies [41-43] have been developed and have shown differing capability to monitor air toxicants. The simplicity of maintaining, and sensitivity of bacterial-based biosensors make them worth exploring [15,32,44-46]. In this study, immobilized bacteria of *E.coli* TV1061 strain, sensitive to cytotoxic stresses, were exposed to various air toxicants (Figure 4) at multiple concentrations (Table 1) and these were seen to respond differently. In some cases they were highly induced (ammonium hydroxide or dimethylformamide) or inhibited (formaldehyde or piperidine). However, in all tested compounds the immobilized cells responsed differently and in a dose-dependent manner. These differences in bacterial responses (strength of the induction/inhibition at different concentrations) will allow us in the future not only to determine the presence of the toxicant in the air but also to create specific fingerprints for them. This concept has already been used for the determination of the presence of antibacterial compounds in water [47]. Addition of other bacterial strains to the sensor (sensitive to different stresses) and their simultaneous measurement of the air condition will allow for the creation of more sensitive and precise tools for air quality control.

Next, the toxic effect of some commercial chemicals that may be found in an indoor environment was evaluated. For all strong responses (induction or inhibition) were measured. The highly toxic effect of passive smoke is well known and documented [48-50]. Bioreporter bacteria used in this study showed highly negative responses (i.e. strong inhibition) for all tested cigarette smoke concentrations (Figure 4B). A highly inhibitory effect was achieved also from paint that may contain different toxic chemicals (e.g.

formaldehyde [51-53], heavy metals [54] and brominated flame retardants [55-57]) and from weed killer, which may penetrate houses from fields nearby to our homes [58].

The strongest response was to acetone, which is a common air pollutant in homes and public buildings worldwide, as it is frequently used as a solvent in the printing industry and in analytical laboratories. It is a major constituent of many common household chemicals [59] and it has been identified as a component of environmental tobacco smoke [60]. Furthermore, acetone was also found to be an abundant carbonyl among selected industrial sectors and chemical manufacturers' workplaces in China and Hong Kong [61]. The pattern of bacterial responses in this case varied with different toxicants, suggesting the need for fingerprints in the future.

4.3 Exposure to chemical "spill" interior

The main goal of this study was not only to show the capability of the device to determine toxicants in air within confined testing chambers but also to monitor real, indoor spaces. A biosensor was placed in an office room and, during continuous operation, chemicals were intentionally spilled in the room. Figure 5 shows the response of the bacteria to two chosen toxicants (chloroform and acetone). There was a response to both pollutants; however, cells were induced much more strongly in the presence of chloroform in air. The American Conference of Governmental Industrial Hygienists (ACGIH) suspects chloroform to be a human carcinogen (A2 substance) [62]. Chloroform may be released into indoor air by vaporization from different sources, such as: chlorinated water, bleach products as well as office and household products manufactured using chloroform as a solvent [63].

In summary, the response of the sensor to the "spills" showed the capability of the device to respond to the presence of the toxicants in indoor environment. This is a first step in the creation of a sensitive, user-friendly and portable device for air quality control.

5. Conclusions

A portable biosensor for real-time air quality monitoring was developed and tested. The biosensor is built from two parts, a non-disposable (PMT and liquid light guide) and a consumable (bioluminescence bacteria immobilized in calcium alginate pads) component. The effects of different conditions (e.g. pad orientation, temperature and time of exposure) on sensor responses were evaluated. It was found that increasing the temperature and exposure time induced cell responses. Later, bacteria were exposed to different chemicals (e.g. organic solvents), including commercially available compounds that may be found in an indoor environment (e.g. cigarette smoke, acetone, paints, etc.). The presence of all of these chemicals in the air not only stimulated cell response (e.g. bioluminescence induction or inhibition) in a dose-dependent fashion but also in a different manner. This may allow in the future, with additional bacterial strains sensitive to the various stresses (specific or general), the creation of fingerprints for all tested chemicals. This prototype is the first step towards the creation of a simple, sensitive and user-friendly device that will allow continuous air toxicity monitoring. Further miniaturization of the setup, increased measuring periods and the addition of wireless capabilities will allow creation of a real commercial remote product.

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Table 1: Conversion of the volumes of chemicals used in this study to the ppb.

Figure 1: Schematic representation of the biosensor for air toxicity monitoring.

Figure 2: Effect of pads orientation on bacterial responses.

Figure 3: Effect of the different conditions (e.g. exposure time and temperature) on cellular responses to the various concentrations of chloroform. A. Kinetical response of the *E.coli* TV1061 strain to the 318.5 ppb chloroform. B. Effect of the exposure time to different chloroform (63.7, 318.5 and 637 ppb) concentrations on cells bioluminescence.

Figure 4: Response of the immobilized bacteria to various concentrations of chemicals (A) and to commercial available compounds (B) that may be found in indoor air environment.

Figure 5: Response of the biosensor to the "spilling accidents" of 2 mL acetone (A) and 5 mL and 10 mL chloroform (B) in real office room.

Figure 1: Schematic representation of the biosensor for air toxicity monitoring.

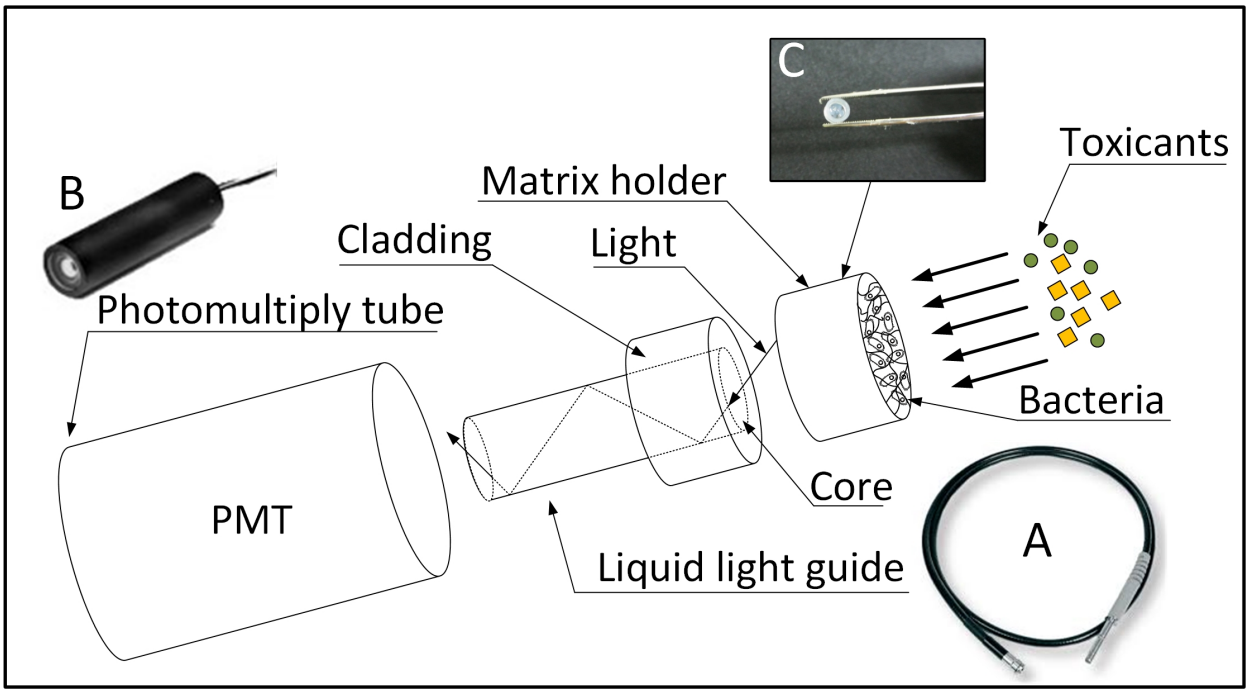


Figure 2: Effect of pads orientation on bacterial responses.

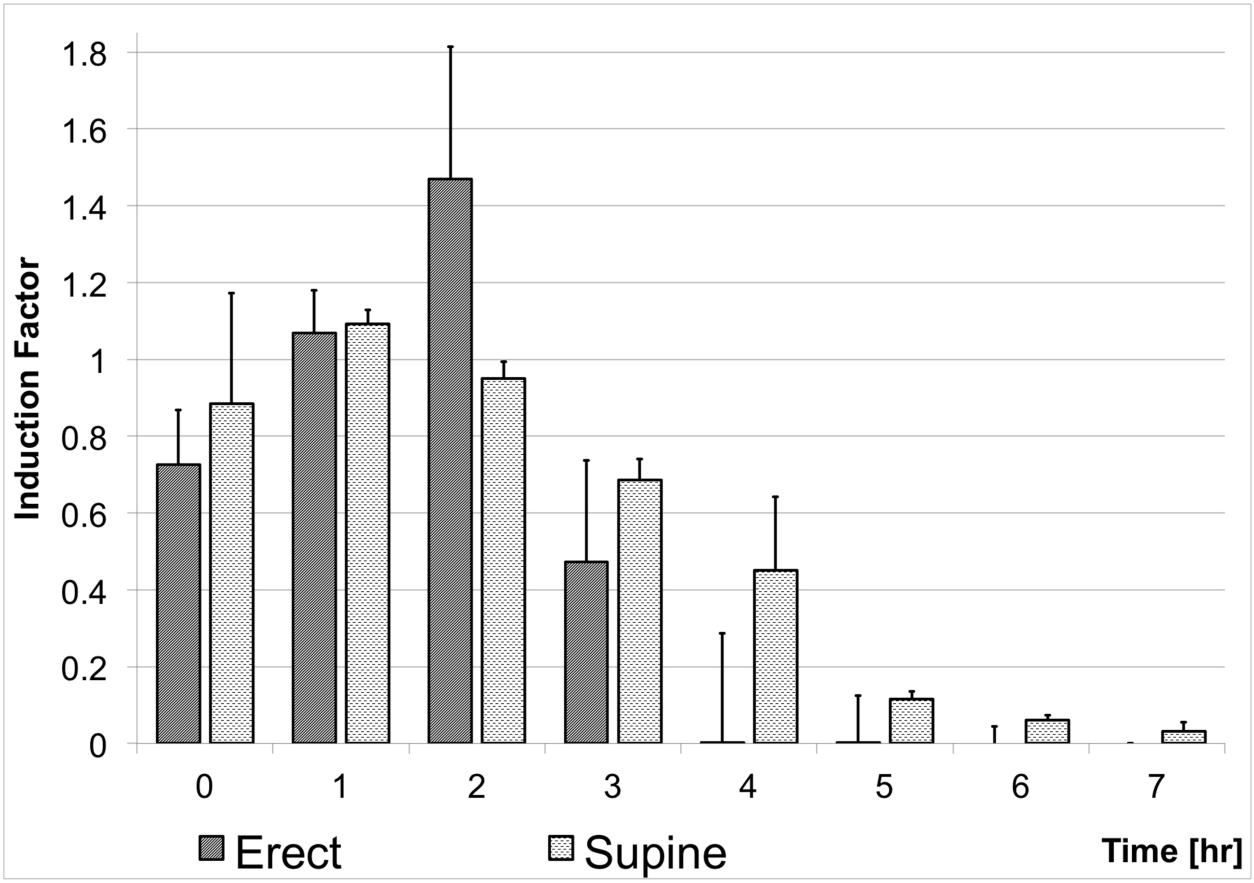


Figure 3: Effect of the different conditions (e.g. exposure time and temperature) on cellular responses to the various concentrations of chloroform.

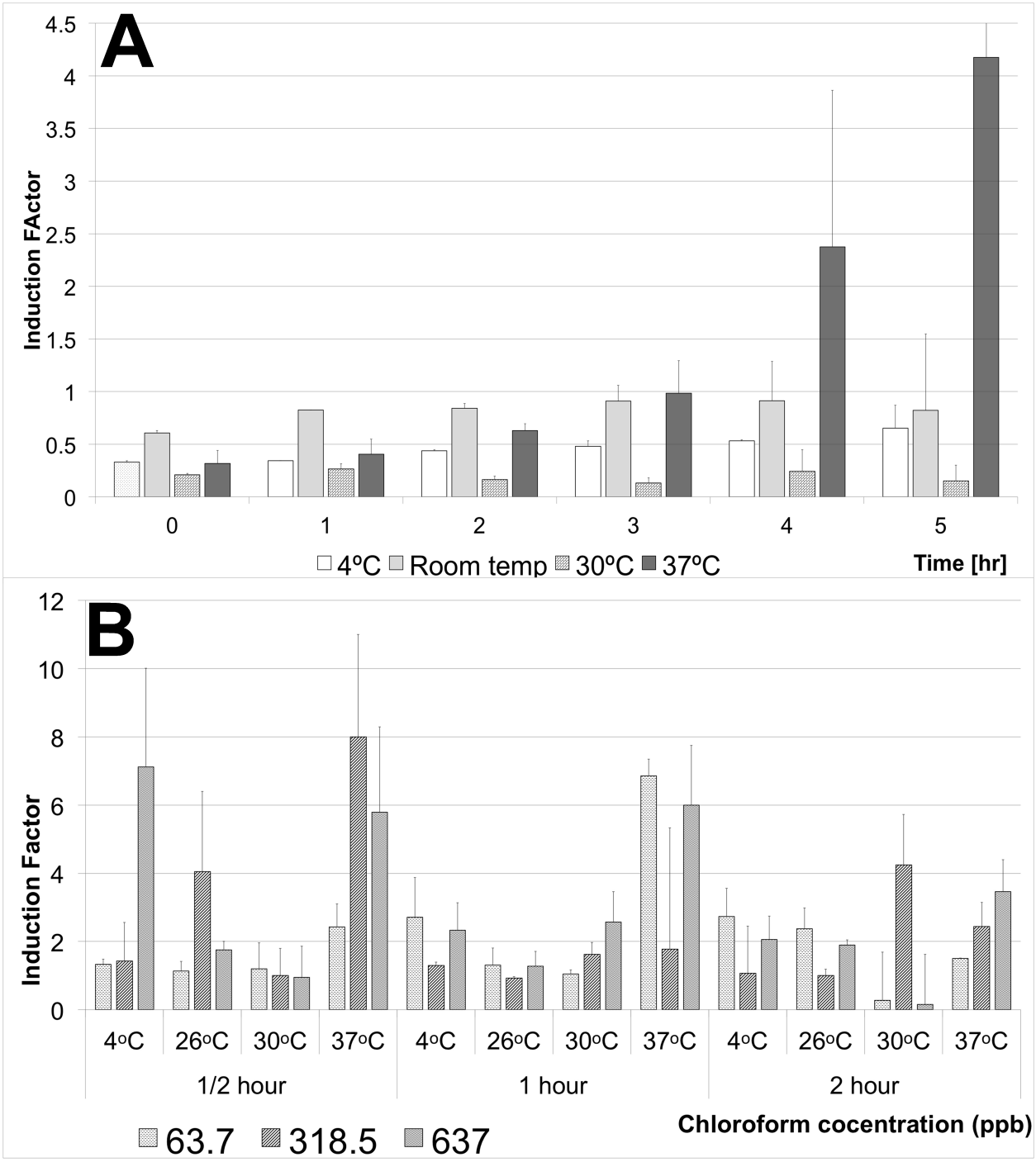


Figure 4: Response of the immobilized bacteria to various concentrations of chemicals (A) and to commercial available compounds (B) that may be found in indoor air environment.

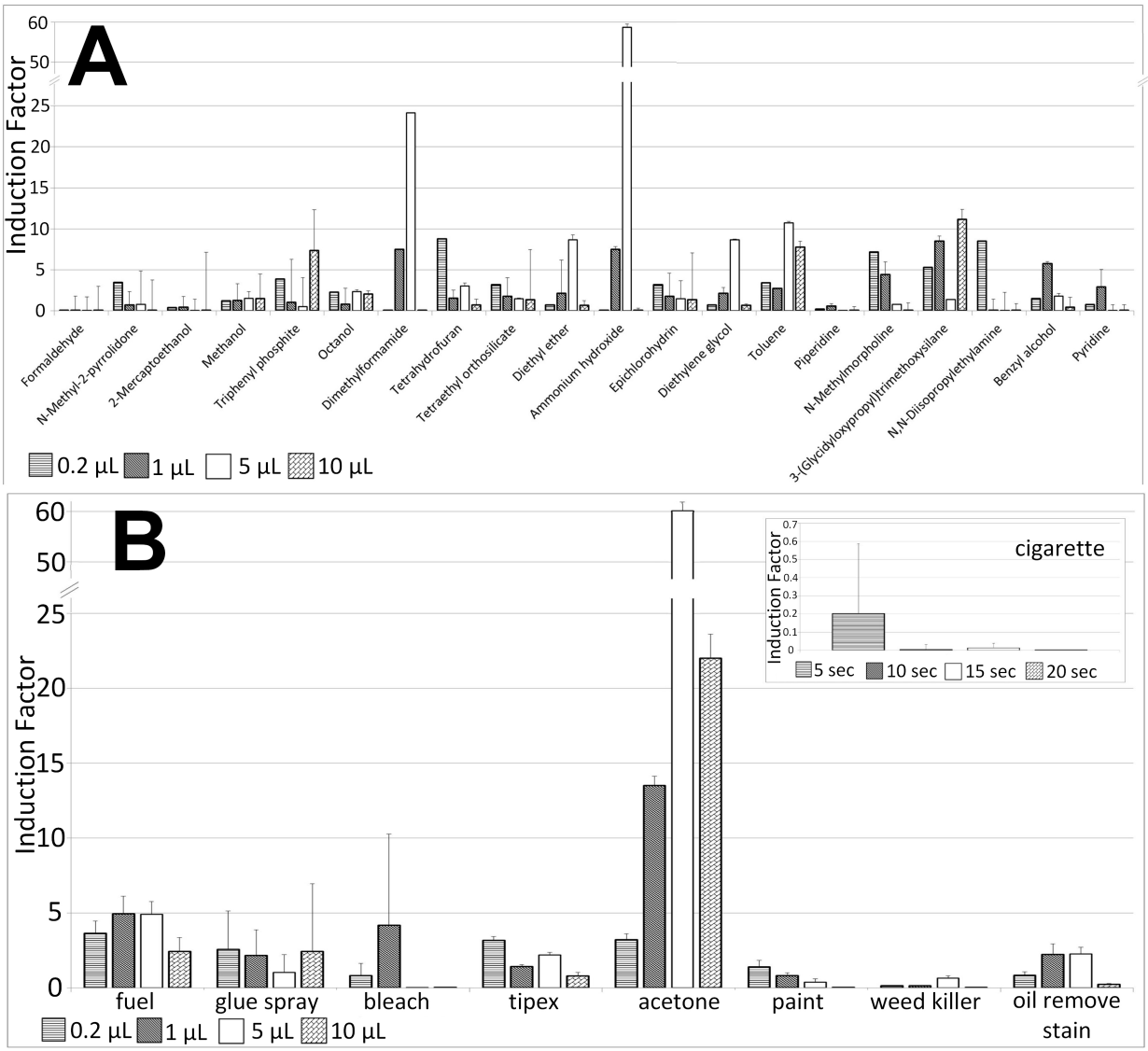


Figure 5: Response of the biosensor to the "spilling accidents" of 2 mL acetone (A) and 5 mL and 10 mL chloroform (B) in real office room.

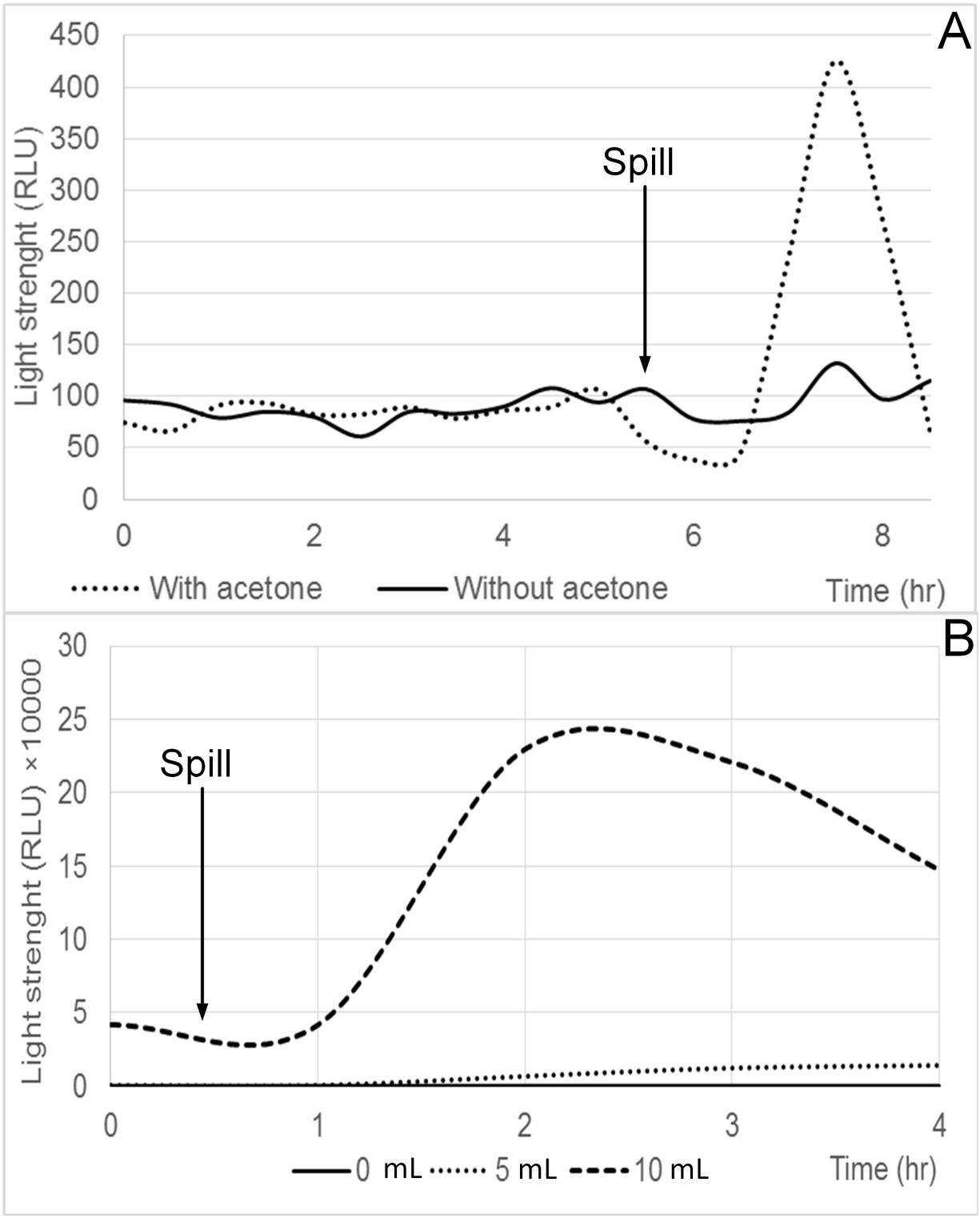
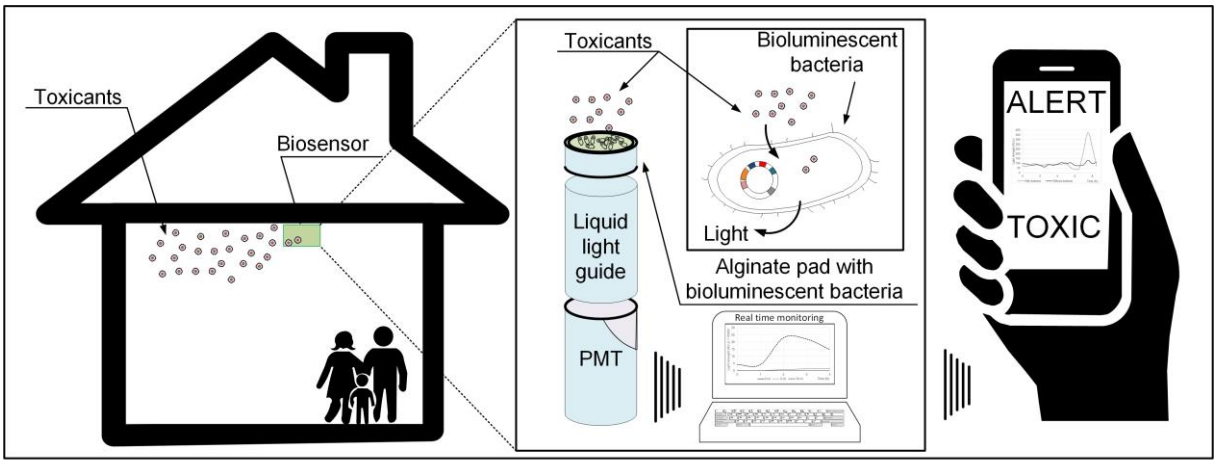


Table 1: Conversion of the volumes of chemicals used in this study to the ppb.

Chemical	Tested concentrations			
	ppb [0.2µL]	ppb [1µL]	ppb [5µL]	ppb [10µL]
(3-Glycidyloxypropyl)trimethoxysilane	9.2	46	229.8	459.6
2-Mercaptoethanol	9.6	47.9	239.3	478.5
Ammonium hydroxide	7.6	37.8	189	378
Benzyl alcohol	9	44.8	224.1	448.2
Chloroform	12.7	63.7	318.5	637
Diethyl ether	6.1	30.6	153.2	306.4
Diethylene glycol	9.6	48	240.1	480.2
Dimethylformamide	8.1	40.7	203.6	407.2
Epichlorohydrin	10.1	50.7	253.3	506.6
Formaldehyde	8	39.9	199.5	399
Methanol	6.8	34	170.1	340.1
N,N-Diisopropylethylamine	6.4	31.9	159.4	318.7
N-Methyl-2-pyrrolidone	8.8	44.2	220.8	441.5
N-Methylmorpholine	7.9	39.5	197.6	395.2
Octanol	7.1	35.5	177.3	354.7
Piperidine	7.4	37	185.1	370.3
Pyridine	8.4	42.2	210.9	421.8
Tetraethyl orthosilicate	8	40.1	200.4	400.8
Tetrahydrofuran	7.6	38.2	191	382
Toluene	7.5	37.4	186.9	373.7
Triphenyl phosphite	10.1	50.6	253.1	506.2

For TOC Only


Toxicants
Biosensor
Toxicants
Bioluminescent bacteria
Liquid light guide
Light
Alginate pad with bioluminescent bacteria
PMT
Real time monitoring
ALERT
TOXIC