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Vapor Phase Hydrogen Peroxide – Method for Decontamination of Surfaces and Working Areas from Organic Pollutants

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1. Introduction

Decontamination, i.e. cleaning by removal of chemicals or germs, is a term commonly used for the process of treating devices, instruments and surfaces in order to ensure their safe operation. It is of exceptional importance in healthcare, food and pharmaceutical industry as well as in the areas of army and public defense. The decontamination process covers several steps like simple washing with water and soap and final disinfection or sterilization. Sterilization is a process which reduces the microbial contamination by 6 logs and can thus prevent the spread of infectious diseases in medical centers, where it forms a part of daily cleaning routines. Disinfection is a very similar process to sterilization, but the reduction rate of microbial contamination is only 5 logs. (Favero & Bond, 1991; Sagripanti & Bonifacino, 1996)

Decontamination is not only elimination of biological pollution but it also comprises detoxification and removal of dangerous chemical compounds. It should be applied whenever a real threat of microbial or chemical contamination exists.

2. History

While the sophisticated ("scientific") ways of decontamination only started to emerge about 150 years ago, essentially similar process are already mentioned in the Bible, in the works of the poet Homer and in the files of Aristotle. An important milestone concerning decontamination was the year 1438 when Sanitary council in Venice was found to provide fumigation of cargo delivered to the port. This institution represented the fundamental prevention and active defense against infectious diseases and parasites. Maturity of Italian health service was demonstrated in the works of poet, philosopher and physician Girolam Fracastor (1478-1553). He first promoted an idea that epidemics had been caused by very small particles which can be transferred among people by three different ways: by contact with infected patients, by contact with the contaminated staff (medicinal staff) and by air transport. Although this great man's work can be considered a huge milestone in the fight

with infectious diseases, in his time it was forgotten and only rediscovered in the 20th century (1930 - W. C. Wright, 1960 - W. Bulloch). In 1676, a chemical (vinegar) was used to kill germs for the first time by Antonie van Leeuwenhoek who observed them with his microscope, calling them „animalcules". However, the breakpoint in this topic came in the second half of the 18th century with the discovery of chlorine (1774, C. W. Scheele) and hypochlorites (1789, C. L. Berthollet). These compounds quickly found their application in deadhouses, sewers, hospitals areas, ships, prisons and mainly in drinking water treatment. In 1810 Nicolas Appert discovered the modern food sterilization method by temperature conservation. Shortly after this, the founder of microbiology Louis Pasteur discovered the sterilization effect of overheated steam. This further inspired Charles Chamberland to construct the first steam autoclave (1879). Parallel with the development of this excellent technique, in 1877 A. Downes and T. P. Blunt discovered the antimicrobial effects of ultraviolet light and M. Wald (1892) continued in this work describing the relation of light wavelength and its germicidal effect (blue light is more effective than red one). Another famous man connected with the decontamination process was Robert Koch who in his book "On Disinfection" (1881) described the potential of 70 chemical compounds at different concentrations, temperatures and various mixtures to eliminate the spores of anthrax. In 1897 B. Krönig and T. Paul developed the grounds of chemical disinfection and these principles were applied in the famous "phenol coefficient method" to test the effectiveness of disinfection compounds. The following 20th century meant a great improvement in chemistry – mainly organic – which lead to the discovery of many disinfection compounds (Block, 1991; Fraise, 2004).

2.1 Reasons for decontamination

The need of decontamination already appeared during the army operations in the Antiquity. Aristotle (384 – 332 B.C.) revealed the danger of infectious diseases and recommended preventive measures for the army troops of Alexander the Great. The progress of medicine and the study of infectious diseases lead to formulation of strict rules to prevent infections. During epidemic periods, the application of protective means was becoming common, e.g. application of antiseptic compounds against gangrene (1750 J. Pringle), using of hypochlorite solutions before surgeries (O. W. Holmese - 1843 a I. P. Semmelweise - 1861), treatment of surgery instruments with flame, sterilization of bandage by heat (L. Pasteur) and many others. Despite all these advances, more soldiers died during the Second World War due to infections and diseases than as a consequence of fight injuries (Block, 1991).

Decontamination is an important part of the whole modern medicine system and is based on strict rules and application of several procedures like cleaning, disinfection, sterilization etc. Nonetheless, nowadays a real danger of pandemic (epidemic spreading in parallel in several states or continents) also exists, which was evident in the case of the two recent pandemics of flu (bird flu, pig flu) that proceeded very fast. Therefore, hospital decontamination is based on proper decontamination equipment (built-in or mobile), protective items and educated staff.

Another very important reason for the progress of decontamination is chemical war as a new military strategy. In the 19th and 20th centuries, the great progress of chemistry led to the development of several poisonous substances (weapons of mass destruction) (Duffy, 2009). A warfare agent of such kind was used for the first time during the WWI when the

Germans used chlorine (April 22, 1915). Later, phosgene, benzyl bromide and others were used (Duffy, 2009). The destructive force of these weapons was improved by new stable, more potent and easily spreading compounds. From the beginning of the WWI to the end of WWII, blistering agents were developed such as yperite and even more dangerous nerve agents like somane, sarine or tabune. Fortunately, they have never been used in war. After the WWII, research was focused on the development of nerve agents and effective defense against them. The new types of V-agents were developed in 1995 and represent the most toxic compounds ever synthesized.

Although the application of chemical weapons is currently considered a war crime (1993 Paris convention) (International Committee of the Red Cross, 2005), they are used by terrorists against civilian population. For instance, one can mention the sarine terroristic attacks in Japan (1995 Tokyo subway, 1994 Matsumoto town) (Okumura et al., 1998, 2003) or the bio-terrorist attacks by Anthrax spores delivered by mail service in the USA in 2001 (24 buildings were contaminated and the remedies cost as much as 200 million USD) (Jernigan et al., 2002). These incidents showed global unpreparedness for large contamination and improper decontamination methods for such spaces.

Decontamination is therefore highly important, both in the defense of an individual person and the defense of a country mainly against the pandemics or terroristic attacks. Beside this, the huge amounts of toxic compounds which are daily manufactured, modified and transported need to be considered. In case of an accident or improper manipulation, these substances can endanger the safety of a particular person or the whole environment. As a few examples of the 20th century, we would like to mention the outflow of toxic dioxin in Italian town Seveso in 1976 (initiated two prevention guidelines SEVESO I and SEVESO II), the nuclear accident in Chernobyl (1986) and the biggest industrial accident in the town Bhopal, India, (1984) where approximately 20,000 people died (Sharma, 2005).

For the sake of global prevention, it is extremely desirable to develop novel effective decontamination methods appropriate for application in large areas like rooms, buildings, airplanes, subways or airports. Despite the fact that decontamination of such premises represents an issue of foremost importance, it is not solved satisfactorily at present. An example of such solution is the patent of the United Technologies Corporation (Watkins, 2006) dealing with easy distribution of hydrogen peroxide aerosol or gas to large areas. A list of important methods usable for large and closed areas can be found in the Compilation of Available Data on Building Decontamination Alternatives issued by the U.S. Environmental Protection Agency (EPA) in 2005 (U.S. Environmental Protection Agency, 2005). In the last three decades, chemical contamination has most frequently been caused by pollution with toxic and usually carcinogenic pesticides or industrial intermediates while accidents or terrorist attacks have only formed a minority of cases. These compounds are mainly characterized by greater stability and usually persist in the environment for a long time. A second important group of polluting compounds are pharmaceuticals, diagnostics, flavoring substances and other bioactive compounds. Presently, the production, distribution and application of bioactive substances indeed represent a fast-growing branch of industry. These compounds and their bioactive metabolites are mainly concentrated in wastewaters, which are subsequently drained into the environment. Although their concentration is rather limited (ng l^{-1} – ug l^{-1}, corresponds to ppt – ppb) and they do not seem to be an actual danger, their final influence can be very dangerous. The active pharmaceutical substances are designed for a very specific effect, but their final side effects can often be unpredictable.

Lists of pharmaceuticals which contaminate the environment can be found in the literature. These compounds come either in the unchanged form or in the form of their biotransformation products (metabolites, which can be more active than the original substances (Daughton & Ternes, 1999)). The lists include for example analgesics, antidepressants, antiepileptics, antihypertensives, antiseptics, cytostatics, hormones, cholesterol reducing substances, radiocontrast substances, steroids, tranquilizers and others. (Daughton & Ternes, 1999; Lopez et al., 2003; Pereira et al., 2007). Big sources of these substances are of course pharmaceutical companies, research laboratories, hospitals, pharmacies and households in which these compounds are used.

National Institute for Occupational Safety and Health, NIOSH, estimates that 5.5 million of workers can be indirectly exposed to dangerous pharmaceutical substances which are commonly labeled "cytotoxic". These data are based on evaluation between 1996-1998 in the USA (National Institute for Occupational Safety and Health, 2004) among research staff, pharmacists, physicians, nurses and other supporting staff. Staff from pharmaceutical factories were not considered because of the strict rules for clean premises which reduced the risk of contamination to minimum. On the contrary, in hospitals the staff and the patient relatives can be in touch with hazardous pharmaceutical substances like cytostatics, antivirotics, hormones etc. (National Institute for Occupational Safety and Health, 2004). These substances can cause undesirable effects (Castegnaro et al., 1997) by transport into their bodies through skin absorption, inhalation of aerosols, syringe needles or open strokes. The danger of indirect contamination with pharmaceutical substances on the inner surfaces of 14 hospitals in Germany is described in the work of Schmaus (Schmaus et al., 2002). Better results were naturally achieved in those hospitals which strictly followed the safety measures and where their staff worked properly with cytotoxic substances.

Methods of protection against dangerous cytotoxic substances are nowadays undergoing a fast development. The most important measure is to strictly follow proper working rules to avoid contact of staff with these compounds, which means e.g. working in isolators with intrinsic decontamination system, proper waste management, safe storage or periodic evaluation of the level of contamination. (Fisher & Caputo, 2004; National Institute for Occupational Safety and Health, 2004) Currently, the development is focused on new decontamination methods to provide perfect cleanup and inactivation of dangerous substances in various kinds of waste material packaging and biological liquids before their disposal (Cazin & Gosselin, 1999; Hansel et al., 1997).

2.2 State of the art of decontamination techniques

Up to now, a lot of decontamination methods to inactivate biological pollution on different surfaces have been developed. Surface decontamination of chemical (mainly cytotoxic) substances was studied less intensively but nowadays it is also becoming a priority. Decontamination can be carried out by several ways depending on the contaminant, environment, area size or target (people, tools, indoor or outdoor space). Among the main factors affecting the right selection of suitable decontamination techniques are: the method of distribution of the decontamination substance (washing, wiping, spraying, foaming, using aerosol, fumigation etc.); the operation range (selectivity to the microorganism or chemical pollutants); the influence of working conditions (temperature, humidity, presence of other compounds); the operation time (time necessary for proper reaction – minutes or hours) and the influence of the decontaminant upon target materials (possibility of damage).

It is possible to categorize the decontamination methods according to their principle of action to mechanical, chemical, physical and physicochemical. The most widespread are chemical decontamination procedures (an action of a chemical agent to a decontaminated item) that can be applied in two different ways – the wet approach, which uses water or other solution of active agent and the dry method which uses gas or vapor phase of active substance.

Application of the aforementioned methods is connected with serious danger because of toxicity, carcinogenicity, flammability or explosiveness of agents, irradiation or burn caused by rays, and potential toxic residues which are harmful to the environment. A big disadvantage of liquid agents' application is a non-uniform distribution on the surfaces in all target areas. This disadvantage was solved by spraying of the agents or using fumigation (vapor or gas of the active agent). It is evident (see Table 1), that chemical agents are a very heterogeneous group with different mechanisms of action. It is also important that several chemical agents can be mixed with detergents, which can support the deactivation process, or other compounds that improve their properties (anticorrosives, aromatic additives and others). These additives can e.g. substantially reduce the surveillance of germs (bacteria, viruses or fungi). According to their influence, these agents can be divided into two types:

- (Bacteria) -cide- meaning permanent dispatch
- (Bacteria) -static- meaning temporary loss of any ability, i.e. multiplication or growing.

Type of decontamination		Active agent
Mechanical ways		Sucking, washing, wiping etc.
Chemical ways	*Wet*	Water solution of ClO_2, CH_3COOOH, H_2O_2, NaOCl, liquid detergents (presence of quaternary ammonia salts), alcohols, aldehydes, phenol derivatives, iodoform, Fenton agent and others.
	Vapor or gas phase	Ethylene oxide, formaldehyde, ClO_2, O_3, CH_3COOOH, H_2O_2, propylene oxide, β-propiolactone, methylene bromide and others.
Physical ways		X-ray, gamma ray, microwave and UV radiation, heat (dry, wet – water steam), freezing, plasma, photochemical reaction, hydrostatic pressure and others.
Physicochemical ways		Heat or radiation combined with chemical agents

Table 1. A list of basic decontamination methods (Kuzma et al., 2008; McDonnell, 2004; McDonnell & Russell, 1999; Rogers et al., 2005; Russell, 1990, 1991).

It is necessary to regularly change the decontamination agent with respect to a different active substance in order to avoid the potential resistance of the microorganisms to the agent used. An ideal procedure for the cleanup of chemical contaminants using chemical agents should consist of their physical removal from the surface followed by effective degradation to nontoxic or at least less toxic compounds. Not a single decontamination agent with such a

wide spectrum of reactivity exists, which could be used for the decontamination of all biologically active substances. Their degradation can even lead to formation of products which are more toxic than the original substance. Identification of these products is very difficult. Therefore, it is preferred to apply "one use" surfaces during the operation with biologically active substances. These surfaces are sequentially washed but their decontamination is quite risky due to the removal of highly toxic contaminants like cytostatics or immunosuppresives. Moreover, they can be drained to the environment which is dangerous not only for the staff but, more importantly, to the whole population. It was already mentioned that these substances are strictly designed for a specific application but they can have numerous side effects on people. Thus, the contact with these compounds has to be avoided as much as possible (Roberts et al., 2006).

NIOSH recommends to decontaminate all surfaces which have been in touch with cytotoxics according a protocol that includes appropriate agent able to deactivate or remove chemical or biological contaminants (National Institute for Occupational Safety and Health, 2004). The basic question to be solved in the field of chemical decontamination is the criterion determining the level at which the contaminant can be considered deactivated. In the case of biologically active substances like warfare agents or pharmaceuticals, this criterion represents the loss of their biological activity, i.e. changes in the chemical structure of the contaminant leading to biological inactivity. An ideal degradation leads to gaseous, nontoxic products which can be easily exhausted – oxides of elements commonly contained in organic molecules (CO_2, H_2O, NO_x). Since they are products of oxidative reactions, it is appropriate that strong oxidative agents be used. Even though the application of $KMnO_4$ seems to be very effective, due to safety reasons it is not acceptable in common places like hospitals (Barek et al., 1998). Other well-known oxidative substances like $Ca(OCl)_2$, NaOCl or H_2O_2 are widely applicable mainly in the liquid form and thus they can only be used in local areas. Unfortunately, the methods described above are inapplicable for treatment of large areas in routine application. In the development of novel decontamination technologies, it is necessary to approach the "ideal decontamination agent" (Rutala & Weber, 1999).

3. Ideal decontamination agents

Based on the above-mentioned facts, the "ideal decontamination agent" can be defined. It should possess high activity against a wide spectrum of biological and chemical contaminants, quick start of action and long-lasting effect. It should be nontoxic to humans and the environment, compatible with a variety of materials, resistant to organic materials, have a non-limited disposal and long term stability during storage. It should also be easily detectable, have a pleasant or no smell and a reasonable cost. Its handling (application and storage) should be easy and safe. Another useful property of the ideal agent can be e.g. applicability to large areas or whole buildings mainly in case of pandemics, chemical accidents or terrorist attacks. The contaminants can be found in places difficult to clean like cracks in walls, carpets, woods, ventilation pipelines and air conditioning units. The common methods are inapplicable in large areas where the only solution is application of a gaseous decontamination agent because it provides easy distribution and penetration in broken surfaces. However, the most commonly known gas phase decontamination agents are connected with a number of disadvantages such as toxicity, material incompatibility, concentration requirements, time of exposition and aeration time (Rogers et al., 2005). From

the aforementioned chemical agents, hydrogen peroxide looks like an ideal agent due to the non-toxic products of its decomposition – water and oxygen. The well-described antimicrobial activity and strong oxidative potential are also a good precondition for its wide application mainly against biological and chemical contaminants.

4. Vapor phase hydrogen peroxide

Very close to "the ideal decontamination agent" seems to be Vapor Phase Hydrogen Peroxide (VPHP). It is a relatively new but very progressive method with many advantages:

- approved sterilization of a wide range of microorganisms
- higher germicidal activity than what can be achieved with a liquid solution of hydrogen peroxide
- environmental friendliness – the decomposition products are water and oxygen and leave no toxic residues on the surfaces
- possibility of its usage under common conditions - atmospheric pressure, laboratory temperature
- applicability in larger areas and rugged surfaces.

For its excellent antimicrobial activity and nontoxic decomposition residues, the VPHP process tends to replace especially toxic, carcinogenic and potentially explosive formaldehyde and ethylene oxide used for sterilization of heat-sensitive materials (Block, 1991; Heckert et al., 1997).

VPHP is typically generated from a water solution of hydrogen peroxide (35% w/w). A common way of vapor generation is controlled heating of the solution under proper conditions avoiding decomposition of the VPHP. Like all other decontamination agents in vapor phase, also VPHP is decomposed during the operation (in fact, the rate of decomposition of hydrogen peroxide is higher than the decomposition rate of ClO_2) and it is thus necessary to refill "fresh" VPHP to the target area. The dose of new VPHP keeps the required concentration during the whole process. At the end of decontamination, the generator is switched off and the rest of hydrogen peroxide vapor is ventilated out by aseptic air. This exhaust goes through the catalyst to decompose hydrogen peroxide (U.S. Environmental Protection Agency, 2005).

The sterilization properties of VPHP were for the first time mentioned in the 70's of the 20th century, but the modern concept is dated 1989 when this method was used for quick sterilization of rugged dental instruments (Block, 1991). In the same year, EPA approved the usage of VPHP in closed premises like isolators, closed rooms or operation boxes (U.S. Environmental Protection Agency, 2005; McDonnell et al., 2007). Since then, fast-growing application of this agent has started, focusing mainly on bio-decontamination in pharmaceutical industry, health service and food industry (Block, 1991; Kahnert et al., 2005; Klapes & Vesley, 1990). In 2001, VPHP was used for the first time for decontamination of two post office buildings (the General Services Administration's Buildings 410 in Washington, D.C. and the U.S. State Department Mail Facility in Sterling, Virginia; contaminated space 30 000 – 60 000 m^3). These buildings were contaminated by Anthrax spores released from "Anthrax letters" by terroristic attacks in the USA (U.S. Environmental Protection Agency, 2005).

In the literature (Block, 1991; Heckert et al., 1997; Johnston et al., 2005; Klapes & Vesley, 1990; Roberts et al., 2006), there are numerous applications of VPHP in the decontamination of fermenters, dialysers, incubators, isolators (Fisher & Caputo, 2004; Lysfjord & Porter,

1998), glove boxes, hazard boxes (Hall et al., 2007), animal houses (Kahnert et al., 2005; Krause et al., 2001), hospital wards (French et al., 2004; Hardy et al., 2007), inner space of airplanes (Krieger & Mielnik, 2005; Shaffstall et al., 2011), ambulances, various large spaces (Krause et al., 2001), lyophylisators (Johnson et al., 1992), ultra centrifuges, sterilization tests (Kokubo et al., 1998), product and pipe lines (Hatanaka & Schibauchi, 1989), dental and surgery instruments (catheters, endoscopes, etc.) (Bathina et al., 1998), contact lenses, hardware space systems (Chung et al., 2008), and food commodities (Forney et al., 1991; Gruhn et al., 1995; Sapers et al., 2003; Simmons et al., 1997). It is a method by which a high selectivity of the process can be achieved due to very precise control of the sterilization conditions (concentration of VPHP, temperature, time of sterilization) and thus only pathogenic microorganisms are destroyed leaving normal living cells unharmed. Therefore, this method is feasible for the decontamination of the surface of living cell cultures. The world-known companies like Tetra Pak International, PepsiCo Inc. or Tetra Laval Holding & Finance are dealing with research and application of in-line sterilization of food packaging by VPHP, which clearly demonstrates how important this technology is in this branch. An interesting and effective application of this method is bleaching of textile materials, which allows to decrease the operating temperature and thus to increase the economics of the whole process. The main leaders in bio-decontamination of surfaces by VPHP are at present companies Steris (Mentor, USA) and Bioquell (Andover, UK). Beside them, several other companies work on VPHP technology, e.g. Pharmaceutical Systems (Franklin Lakes, USA), American Sterilizer Company (Mentor, USA), Johnson & Johnson and division Ethicon (New Brunswick, New Jersey, USA) or Surgikos (New Brunswick, USA).

5. VPHP – Mechanism of action

Information about the mechanism of VPHP action is actually very limited as the process is still in the focus of basic research (Klapes & Vesley, 1990). Current literature data (Chung et al., 2008; Unger-Bimczok et al., 2008; U.S. Environmental Protection Agency, 2005) indicate that the VPHP process is a multi-parameter problem, the effectiveness of which is mainly influenced by the concentration of gaseous hydrogen peroxide, temperature, relative humidity, and condensation of hydrogen peroxide on the decontaminated surfaces. Similar behavior was found for gaseous formaldehyde as a decontamination agent which has been described in detail (Hoffman & Spiner, 1970). Unger et al. (Unger et al., 2007) for first time studied the influence of all these conditions on the sporicidal effect of VPHP. The results suggest that the main parameter for microbial deactivation is the molecular distribution of water and hydrogen peroxide on the surface while the concentration of hydrogen peroxide only plays a secondary role. It was also found that the decontamination cycle using a relatively lower concentration of hydrogen peroxide and higher relative humidity gave very similar results as an experiment with higher concentration of hydrogen peroxide and lower relative humidity. This confirms the possibility of conducting the VPHP process in two ways: “wet” or “dry”.

6. VPHP – Operational conditions

Decontamination of closed areas by VPHP is carried out in 4 consecutive steps (Fisher & Caputo, 2004; Heckert et al., 1997; Roberts et al., 2006; Watling et al., 2002). The first phase is dehumidification, i.e. reduction of humidity to an acceptable level, and also temperature

stabilization of the VPHP generator. The second phase is conditioning which includes the transport of evaporated hydrogen peroxide by the carrying medium (air) to the decontaminated area and achievement of the required hydrogen peroxide concentration. The third phase is the decontamination itself – that means steady evaporation of hydrogen peroxide and its transport to the decontaminated area to maintain a constant concentration during the whole process. Aeration represents the final phase which consists in feeding of aseptic air in the decontaminated area to exhaust hydrogen peroxide vapor and to keep its concentration at a safe level. The process is illustrated in Figure 1.

Duration of the whole decontamination cycle depends on many parameters. The main ones are the size of area, the profile of surfaces, the way of VPHP generation, endurance of the contaminant against VPHP and the method of space aeration. The whole decontamination cycle should not exceed 10 hours as this is a limit of application of this chemical compound as a sterilization agent (Gurevich, 1991). The decontamination cycle based on biocidal properties of VPHP meets this requirement. There are two different ideas about how to carry out the surface decontamination with VPHP (Fisher & Caputo, 2004; Unger-Bimczok et al., 2008; Watling et al., 2002). The traditional one prefers to perform the decontamination under "dry" conditions without condensation of hydrogen peroxide or water (preferred by Steris (Fisher & Caputo, 2004)). Condensation is unwanted because of corrosion of many materials and prolonged aeration time. The process is not under control and thus in case of condensation the decontamination is not homogeneous (Unger-Bimczok et al., 2008; Watling et al., 2002). In case of the "dry" way of the VPHP process, decontamination of closed indoor spaces and surfaces or quick inactivation of contaminants due to high concentration of hydrogen peroxide vapors is quickly achieved. However, the atmosphere in a defined space can absorb only a limited amount of water and hydrogen peroxide, so it is necessary to remove humidity out of this space by desiccators to avoid condensation (Fisher & Caputo, 2004). On the contrary, the second popular opinion says that the hydrogen peroxide vapors are stable and condensation is necessary. In this view, also condensation is the primary reason of the VPHP decontamination and thus condensation is necessary in order to carry out surface decontamination by hydrogen peroxide vapors (Watling et al., 2002). This theory is supported by theoretical and experimental analysis which clearly show that condensation, and mainly "microcondensation" (i.e. non-visible condensation in small amounts), are key and critical parameters for quick and reproducible inactivation of microorganisms by VPHP (Unger-Bimczok et al., 2008). Condensation (preferred by Bioquel (Fisher & Caputo, 2004)) in case of the "wet" VPHP process is the basic requirement of the technology. A thin condensation film is formed on the decontaminated surfaces. It is necessary to control the condensation level of decontamination agents during the whole process because it inhibits the process (Sheth & Upchurch, 1996). Determination of the vapor mixture dew point (i.e. hydrogen peroxide and water vapours) is very difficult. Its value depends on the temperature and pressure and cannot be predicted easily. This dependence is well described by the Raoult's law which requires the input knowledge of activity coefficients that are also dependent on temperature and concentration. For better understanding of the difference between "wet" and "dry" processes, it is good to study the thermodynamics of the hydrogen peroxide-water solution and its behavior during evaporation and condensation (Manatt & Manatt, 2004; Scatchard et al., 1952). The pressure of saturated vapor of water and hydrogen peroxide is below atmospheric so both compounds start evaporating under atmospheric conditions. Lower pressure of saturated vapor of hydrogen peroxide causes

the higher boiling point of hydrogen peroxide and slower rate of evaporation compared to water. Binary system hydrogen peroxide and water has a reduced dew point because of H-bonds (i.e. reduced total vapor pressure) and thus saturation and eventual condensation can occur at a level of relative humidity lower than 100%.

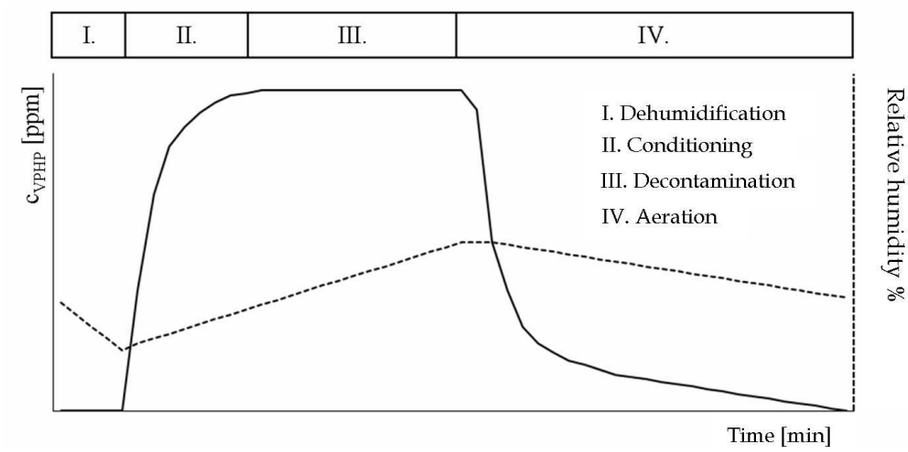

Fig. 1. Time dependence of the VPHP concentration with respect to the decontamination phases.

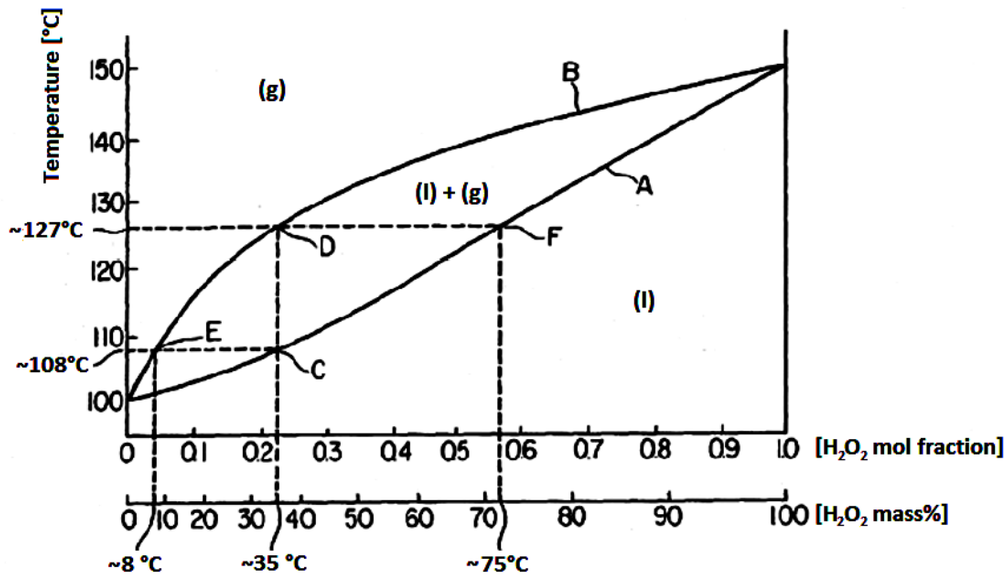

Fig. 2. Isobaric equilibrium liquid-vapor (T,x,y diagram) for the real mixture of hydrogen peroxide and water (Hatanaka & Schibauchi, 1989)

The graphic illustration (U.S. Peroxide, 2009) of the liquid-vapor equilibrium clearly shows the differences in hydrogen peroxide concentrations on the decontaminated surfaces during "wet" and "dry" processes. The differences are caused by different condensation speed of both compounds which also has an influence on the decontamination rate – the higher the concentration of hydrogen peroxide, the faster the decontamination.

Concentration H_2O_2 at 25 °C [mass. %]	
Vapor	**Liquid**
1,9	32,1
8,0	55,7
24,1	73,8
35,0	77,8
58,4	88,3

Table 2. The equilibrium concentration of vapor and liquid phase of H_2O_2 reached by evaporation (Hultman et al., 2007)

The hydrogen peroxide vapor can be generated in two ways – by controlled or flash evaporation. If the liquid solution of hydrogen peroxide evaporates in a dry closed space at 25°C (normal conditions), the concentration of hydrogen peroxide in the gas phase is much lower than in the liquid phase because of faster water evaporation from the hydrogen peroxide solution. For example, by evaporation of 35% (w/w) hydrogen peroxide solution, the final gas phase contains 2.15 % (w/w) H_2O_2 and 65% of H_2O (w/w) (Hultman et al., 2007). Saturation is a state when no more hydrogen peroxide and water vapor can be absorbed and thus condensation occurs.

A different situation occurs when water and hydrogen peroxide vapor condense at 25 °C. In Table 2, it is shown that the equilibrium condensate concentration formed from the vapor above 35% (w/w) hydrogen peroxide solution is 77.8 %, which is about two times higher than in the parent solution. Hydrogen peroxide in the vapor phase condenses preferentially over water. If the system contains vapors of hydrogen peroxide, its condensation occurs and thus its concentration in the vapor phase decreases.

The rate of evaporation can be increased by supplying heat. However, this must be done with a particular care because of safety reasons – hydrogen peroxide is extremely unstable at higher temperatures. Heat supply to the concentrated evaporated hydrogen peroxide slightly above normal conditions is safe and can be used for proper evaporation of concentrated hydrogen peroxide. The condensate is a highly concentrated solution that is not compatible with a wide range of materials and can cause corrosion.

Flash evaporation is another kind of a process related to hydrogen peroxide evaporation. The solution of hydrogen peroxide can be directly applied on a heated surface and thus evaporated. During the flash evaporation, hydrogen peroxide and water are evaporated from the solution simultaneously, so the concentration in the vapor phase is approximately the same as the concentration of the starting solution (decomposition of hydrogen peroxide is not considered). Thus, the concentration in the condensate is the same as in the parent solution.

In case of the "wet" VPHP process, the high concentration of hydrogen peroxide in the condensate can have a positive effect with respect to faster microbial decontamination, but only if the condensate covers the entire surface homogeneously. This is, however, nearly impossible to ensure, owing to different surface profiles of materials. In large rooms, temperature differences and different circulation of the atmosphere also play an important role. These factors, together with surface properties like wettability, sorption and catalytic activity, lead to formation of heterogeneous condensate in the form of drops or a thin film (depends on the wettability).

The application of VPHP in the solely "dry" process is advantageous because gas has a uniform contact with all exposed surfaces. All types of surfaces can thus be decontaminated to the same degree, including those with complex geometry – horizontal, vertical, cracks and curved surfaces. Moreover, it is possible to quickly remove the gaseous hydrogen peroxide from the area at the end of decontamination and thus save time of the whole cycle.
A theoretical model of decontamination by VPHP was presented (Watling et al., 2002) and it described the concentration profile of hydrogen peroxide vapor in a closed space during all four phases of the decontamination cycle and compared it with experimental results. The goal of this work was to create the model that could predict the main parameters of the decontamination process (concentration of VPHP, dew point, etc.) on the basis of operation conditions and other parameters (space dimensions), and thus control and conduct this process under optimal conditions with the highest efficiency.

7. VPHP – An excellent biocidal agent

Similarly as in its liquid solution, hydrogen peroxide also has sterilization properties in the vapor phase against vegetative bacteria and highly resistant bacteria endospores (Block, 1991; French et al., 2004; Hall et al., 2007; Johnston et al., 2005; Kahnert et al., 2005; Klapes & Vesley, 1990; Kokubo et al., 1998; Rogers et al., 2005; Sapers et al., 2003; Unger-Bimczok et al., 2008), viruses (Heckert et al., 1997), fungi (Forney et al., 1991), yeast, amoebae, infective proteins and other microorganisms (Fichet et al., 2004; Klapes & Vesley, 1990; Vassal et al., 1998). As a model organism for the validation, *Bacillus stearothermophilus* (*Geobacillus stearothermophilus*) which is mainly deposited on stainless steel is commonly used (Block, 1991; Bounoure et al., 2006; Chung et al., 2008, Fisher & Caputo, 2004; Johnston et al., 2005; Klapes & Vesley, 1990; Unger et al., 2007). This microorganism is very resistive against VPHP and serves as a surrogate of anthrax (*Bacillus anthracis*) because of their very similar behavior. The VPHP process is considered successful when all these microorganisms are deactivated. A wide range of commercial biological indicators designed for VPHP (spores of *Bacillus subtilis*) exist that are often used for VPHP validation (Klapes & Vesley, 1990; Kokubo et al., 1998).
Sporicidal efficiency of chemical decontamination agents is often expressed as the D-value, which represents the time (minutes) necessary to kill 90 % of the starting amount of microorganisms (or logarithms of the amount) at a constant temperature (Gould, 2004; Unger-Bimczok et al., 2008). In the next table, D-values of selected bacterial spores are compared and evaluated by liquid hydrogen peroxide and VPHP decontamination (U.S. Environmental Protection Agency, 2005). These results (Table 3) show that, in order to kill selected microorganisms, a 200-fold concentrated solution of hydrogen peroxide is necessary to get comparable results as in vapor phase.
Although it is well known that the bactericidal activity of liquid hydrogen peroxide solution grows with its increasing concentration, the linear dependence of its vapor-phase concentration on the killing activity of selected microorganisms is still a widely discussed topic. Some authors say that the antimicrobial activity grows with higher concentration of gaseous hydrogen peroxide, others express a completely opposite opinion and prefer the microbial deactivation at lower concentrations (Unger-Bimczok et al., 2008). In Table 4, all microorganisms tested for VPHP inactivation are summarized (Forney et al., 1991; Hall et al., 2007; Heckert et al., 1997; Johnston et al., 2005; Klapes & Vesley, 1990; Kokubo et al.,

1998; Reich & Caputo, 2004; Simmons et al., 1997). The table clearly shows that the application of VPHP as a biocidal agent is a well mapped topic.

Tested microorganism (Spores)	**D-value [min]**	
	Liquid solution of H_2O_2	**VPHP**
	$c(H_2O_2)$ = 370 mg l^{-1} T = 24 - 25 °C	$c(H_2O_2)$ = 1 - 2 mg l^{-1} T = 24 - 25 °C
Bacillus	1.5	1 - 2
Bacillus subtilis	2.0 - 7.3	0.5 - 1
Clostridium sporogenes	0.8	0.5 - 1

Table 3. Comparison of sporicidal effect of liquid and gaseous hydrogen peroxide (VPHP)

Bacteria + spores
Aeromonas sp.; Acholeplasma laidlawii; Acinetobacter baumannii; Acinetobacter calcoaceticus; Anaerobic cocci; Asperigillus spores; Bacillus anthracis (antrax illness); *Bacillus alvei; Bacillus cereus; Bacillus circulans; Bacillus firmus; Bacillus licheniformis; Bacillus megaterium; Bacillus pumilus; Bacillus sphaericus; Bacillus (resp. Geobacillus) stearothermophilus; Bacillus subtilis; Bacillus thuringiensis; Bacteroides fragilis; Campylobacter sp.; Clostridium botulinum; Clostridium difficile; Clostridium perfringens; Clostridium piliforme; Clostridium sporogenes; Clostridium tetani; Deinococcus radiodurans; Enterobacter cloacae; Enterococcus faecium/faecalis; Escherichia coli; Fusobacterium sp.; Lactobacillus caesei; Legionella pneumoniae; Listeria monocytogenes; Klebsiella pneumoniae; Methicillin-resistant Staphylococcus aureus* (MRSA); *Micrococcus sp.; Moroxelia osloensis; Mycobacterium bovis; Mycobacterium chelonei; Mycobacterium smegmatis; Mycobacterium tuberculosis; Pseudomonas aeruginosa; Pseudomonas cepacia; Salmonella choleraesuis; Salmonella typhimurium; Shigella sp.; Staphylococcus*
Viruses (family: type of virus)
Adenoviridae: Adenovirus, Canine adenovirus; Caliciviridae: Feline calicivirus, Vesicular exanthema virus; *Coronaviridae: Infectious bronchitis virus*; *Flaviviridae*: *Dengue virus*; *Hog cholera virus*; *Herpesviridae: Herpes simplex Type 1, Pseudorabies virus*; *Iridoviridae: African swine fever virus*; *Orthomyxoviridae: Influenza A2, Avian infuenza virus*; *Paramyxoviridae: Newcastle disease virus*; *Parvoviridae: Parvovirus, Canine parvovirus, Feline parvovirus*; *Picornaviridae: Rhinovirus 14, Polio type 1, Swine vesicular disease*; *Poxviridae: Vaccinia; Reoviridae: Bluetongue virus*; *Rhabdoviridae: Vesicular stomatitis virus*
Fungi
Alternaria; Aspergillus niger; Aspergillus sp., Blastomyces dermatitidis; Botrytis cinerea; Candida albicans; Candida parapsilosis; Coccidioides immitis; Histoplasma capsulatum; Penicillium sp.; Trichophyton mentagrophytes
Other microorganisms
Caenrohabditis elegans; Cryptosporidium parvum, Lactococcal bacteriophage; Syphacia muris

Table 4. List of microorganisms which have been inactivated by VPHP

8. Synergism of VPHP and related chemicophysical factors

Most of the works concerning the synergism of hydrogen peroxide were focused on liquid-phase reactions (water disposal treatment) and the synergism of VPHP is a considerably less studied topic.

In order to boost up the effects of hydrogen peroxide vapor, its ionization by plasma can be performed (Bathina et al., 1998; U.S. Environmental Protection Agency, 2005; Vassal et al., 1998). Commonly, plasma is generated from gases (argon, helium, nitrogen, etc.) by an electric pulse, radiofrequency or microwave irradiation. It can be formed at atmospheric pressure and higher temperature (105 °C) or at reduced pressure (~ 40 Pa) and substantially lower temperature (55 – 60 °C) which is called low-temperature plasma (Crow & Smith, 1995). It consists of free radicals (mainly hydroxyl or hydroperoxyl radicals), ions, neutral particles and excited atoms or molecules which show high activity in deactivation of contaminants (U.S. Environmental Protection Agency, 2005). The Johnson & Johnson Medical company and its division Surgikos Inc. patented for the first time a decontamination device in the form of a vacuum chamber where the contaminated instruments were treated with hydrogen peroxide plasma (Parisi & Young, 1991). This kind of decontamination, marked as „STERAD sterilization system", is recommended by the Food and Drug Administration (FDA) as an advanced sterilization technique for enclosed spaces (Crow & Smith, 1995). Although the systems using VPHP together with plasma are very useful for temperature- or water-sensitive materials, their big disadvantage is that they can only be applied in small closed spaces because of the high vacuum or temperature required in the chamber (Adams et al., 1998).

Other synergistic effect can be observed by combination of VPHP with UV irradiation (Klapes & Vesley, 1990). Application of UV irradiation alone requires a relatively long time and thus its combination with VPHP can significantly shorten the operations. The UV/H_2O_2 combination (photo-oxidation) is very effective in destroying microorganisms and heavy decomposable organic pollutants, i.e. volatile organic compounds (VOC) like benzene, toluene, phenol, *tert*-butyl methyl ether, halogenated compounds, pharmaceutical substances and pesticides, mainly in water (Esplugas et al., 2002; Kang & Lee, 1997; Lopez et al., 2003; Pereira et al., 2007; Prousek, 1996). It is known that hydrogen peroxide absorbs UV light within the range of 185 - 400 nm (Esplugas et al., 2002). The radiation energy at these wavelengths is sufficient to provoke photo excitation of the hydrogen peroxide molecule and a subsequent cleavage (photolysis) of the –O–O– peroxide bond, forming hydroxyl radicals which can initiate radical chain reactions of contaminants. The homolytic splitting of the hydrogen peroxide molecule leads to generation of hydroxyl radicals due to the absorption of a photon (Dionysiou et al., 2004). Hydroxyl radicals can be formed by the wide range of low wavelength ultraviolet radiation within 200-280 nm (Lopez et al., 2003). This interval of wavelengths is called UV-C and because of its germicidal properties it is commonly used for water and air sterilization and also for the degradation of photo unstable organic pollutants (Pereira et al., 2007). The UV radiation not only consists of UV-C (100-280 nm) but also contains the UV-B (280-315 nm) and UV-A (315-400 nm) ranges. These two last radiation areas are not used for the activation of hydrogen peroxide. The most common source (Lopez et al., 2003) of the UV-C radiation are low-pressure mercury lamps with the emission maximum at 254 nm since at this wavelength, the quantum yield of hydroxyl radicals equals to 1. The synergistic combination of liquid hydrogen peroxide and UV-C at the 254 nm emission maximum (indirect photolysis) used for the degradation of

organic pollutants shows that it is highly effective and that the rate of reaction is higher compared to individual application of hydrogen peroxide or UV-C irradiation (Esplugas et al., 2002; Lopez et al., 2003; Pereira et al., 2007). The total rate of chemical degradation of contaminants by UV-C and hydrogen peroxide is dependent on the mechanism of the reaction of OH radicals with the contaminants, on the reaction rate of direct photolysis of contaminant (absorptivity of substrate), on the absorptivity of by-products and other absorbents of UV-C radiation at 254 nm (competitive absorption), on the intensity of the UV-C source and on the concentration of hydrogen peroxide (Kang & Lee, 1997). Degradation of organic substances by this effective combination proceeds by a radical oxidative reaction (Ray, 2000).

There are several practical applications of the VPHP decontamination process employing the synergistic effect of UV-C (VPHP/UV-C) for the sterilization of continual filing processes of food in liquid form (milk, water, juice). VPHP is applied in the first step of decontamination to treat surface and then the UV-C radiation treatment follows. It is also possible to combine the above-mentioned VPHP/UV-C system with other oxidative agents such as ozone, or the catalytic properties of TiO_2 can be used. Decontamination processes combining O_3/UV-C/VPHP show the most effective contaminant degradation and allow complete mineralization of pollutants (Esplugas et al., 2002). In the USA, the Department of Energy patented a portable device for surface chemical and biological decontamination which uses ozone as the main agent, reinforced by UV radiation and hydrogen peroxide added to reach higher reactivity (O'Neill & Brubaker, 2003).

In 1972, Fujishima and Honda (Fujishima & Honda, 1972) discovered the photocatalytic properties of nanocrystalline TiO_2 and predicted its possible application in chemical decontamination (Linsebigler et al., 1995; Wold, 1993). The TiO_2/UV system was described in several studies (Mills & Hunte, 1997; Peral et al., 1997; Rauf & Ashraf, 2009; Tschirch et al., 2008; Zhao et al., 2005), mainly with regard to a higher efficiency of degradation of chemical pollutants in water and air. Total mineralization of pollutants was observed, producing only CO_2, H_2O and inorganic ions (Zhao et al., 2005). Thus, the process is highly environmentally friendly. Its reasonable price, compared to other Advanced Oxidative Processes (Rauf & Ashraf, 2009), is also highly important.

The decontamination by TiO_2/UV/H_2O_2 is studied mainly in the liquid phase, preferentially in water (Domínguez et al., 2005), and thus there is no information on a VPHP-based modification of this system. The addition of a small amount of hydrogen peroxide shows a synergistic effect, i.e. a notable speedup of the photocatalytic TiO_2/UV/H_2O_2 degradation of pollutants (Tschirch et al., 2008). This positive effect is due to increased formation of hydroxyl radicals as a result of direct photolysis of hydrogen peroxide and its interaction with the active TiO_2 surface. However, higher concentrations of hydrogen peroxide inhibit the degradation because under these conditions, recombination of hydroxyl radicals is preferred (Dionysiou et al., 2004; Esplugas et al., 2002). Thus, it is necessary to work with optimal concentration of hydrogen peroxide to achieve the highest efficiency of contaminant degradation in the TiO_2/UV/H_2O_2 system (Elmolla & Chaudhuri, 2010). Although similar properties as TiO_2 are also provided by other semiconductor materials as ZnO, ZnS, CdS, Fe_2O_3, WO_3, these materials do not show the efficiency of degradation as high as TiO_2 does (Ray, 2000).

There are also other possibilities to promote VPHP process, e.g. by the addition of other oxidative agents like ozone, peracetic acid, or concentrated solution of hydrogen peroxide before evaporation. The main problem of hydrogen peroxide evaporation is water which

can condensate on rugged surfaces like medical instruments and it impedes the surface penetration by the hydrogen peroxide. Hydrogen peroxide is able to form anhydrous complexes with a wide range of organic compounds: polyvinylpyrrolidone, urea, glycine anhydride-peroxide complex, and inorganic compounds: $Na_4P_2O_7 \cdot 3H_2O_2$, $KH_2PO_4 \cdot H_2O_2$, which can be prepared easily using known procedures based on their crystallization from solutions. In these complexes, the hydrogen peroxide moiety only binds to the electronegative atom of the other molecule via two H-bonds, which greatly facilitates its release from these substances. For example, thermal decomposition or vacuum can be used to perform this. The vapors of hydrogen peroxide formed this way can be generated directly in the decontaminated area or in another place, in which case they can be transported by a pipeline to the decontaminated space. Another interesting kind of hydrogen peroxide potentiation is the addition of metals (Ag, Al, Ca, Ce, Cu Mg, Sr, Sn, Ti, Zn) (Carnes et al., 2004), oxides or hydroxides, the particles of which (must) have a relatively high surface (at least 15 $m^2\ g^{-1}$). The application of transition metals for hydrogen peroxide activation (i.e. for the formation of OH or other radicals) is mainly used in the liquid phase, like Fenton oxidation (Fenton, 1894) or photo-Fenton oxidation (Prousek, 1996), where Fe^{3+} ions (Xu, 2001) or Cu complexes are used (Martínez et al., 2008). These systems are mainly used for the degradation of resistant organic contaminants like dyes. Another (and also very interesting) combination is mixing of VPHP with volatile basic compounds like ammonia. This method seems to be very promising in the area of deactivation of warfare agents (Wagner et al., 2007).

9. VPHP decontamination of chemicals – Molecular structure effects of decontaminants

There is only limited information concerning the effects of the contaminant chemical structure upon decontamination by VPHP (McVey et al., 2006; Roberts et al., 2006). Several functional groups which are sensitive to VPHP have been found (Švrček, 2010), namely the aldehyde group, aliphatic tertiary nitrogen and the sulfide group (thioethers). Since all of the tested substances contain one of these groups, they were successfully degraded by VPHP. Other VPHP-sensitive compounds seem to be phenols, out of which mainly their hydroxy- and amino-derivatives. It can be expected that the decontamination of more complicated structures (pharmaceutical substances) will be sufficient if these compounds contain one or more such reactive groups in their structure.

9.1 Aldehyde group

Substances containing the aldehyde group undergo preferentially an oxidation process leading to carboxylic acid but other reactions can also take place, for example Dakin reaction (Pan et al., 1999), decarbonylation, substitution, or cracking.

The mechanism of Dakin reaction is illustrated in Figure 3 on an example of vanillin degradation, which in principle proceeds by insertion of an oxygen atom as a result of ketone oxidation (Baeyer-Villiger oxidation). In the first step, an OH radical attacks the carbonyl group (1) and an alkoxy radical (2) is generated, which is then transformed to an unstable alkylhydroperoxide (3) that is rearranged into a more stable product. A subsequent hydrogen shift (a) leads to the final product – carboxylic acid (4). In some cases, migration of a different group can occur, e.g. the aryl group that forms a formate (5) as a key intermediate in the above-mentioned Dakin reaction (b). These compounds are very often

unstable and are subject to hydrolysis (cleavage of formic acid) or decarboxylation (giving CO_2) and formation of a phenol derivative – in case of vanillin it is 2-methoxyhydroquinone (6). The ability of different groups to migrate can be sorted in the following order: tertiary alkyl > secondary alkyl, aryl > primary alkyl > methyl. Electron-donating substituents on the benzene ring of vanillin increase migration ability of this aryl group by the hydroperoxide rearrangement. Electron-withdrawing substituents have opposite effects and impede migration. Therefore, vanillin can easily undergo Dakin reaction that leads to 4-hydroxy-3-methoxyfenyl formate (5). The key factor of Dakin reaction is the presence of strong electron-donating substituents (-OH or -NH_2) in the structure of aromatic aldehydes in the *ortho-* or *para-* position to the –CHO group (Švrček, 2010).

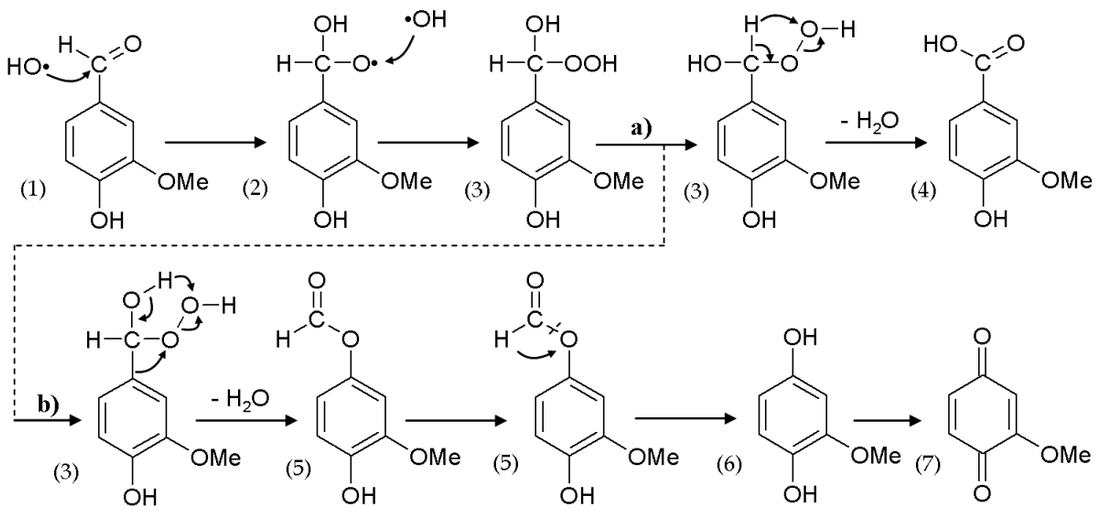

Fig. 3. Expected mechanism of the VPHP-induced degradation of vanillin

9.2 Aliphatic tertiary nitrogen

Other compounds which are highly sensitive to VPHP seem to be those containing an aliphatic tertiary nitrogen atom. All tested substances (Švrček, 2010) were decomposed by VPHP, which could be deduced from notable color changes, decrease in the sample weight, and detected products of degradation. However, the high volatility of starting compounds or degradation products prevented proper analysis by GC-MS and NMR. It is known that in the presence of H_2O_2, tertiary amines are converted to *N*-oxides that can be decomposed by the Cope elimination reaction leading to an alkene and *N*-hydroxylamine. This elimination is mainly carried out at high temperature but we can assume that it can proceed under laboratory conditions (Cope et al., 1949).

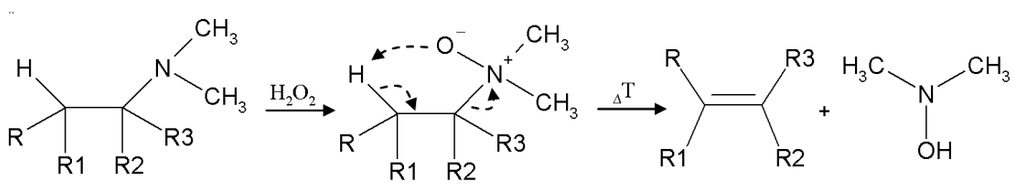

Fig. 4. Mechanism of Cope elimination

9.3 Sulfidic sulphur

In the presence of hydrogen peroxide, oxidation of sulfidic compounds (thioethers) proceeds and more stable higher oxidative compounds are formed (sulfoxides and sulfones) in a rate depending on the concentration of the oxidative agent.

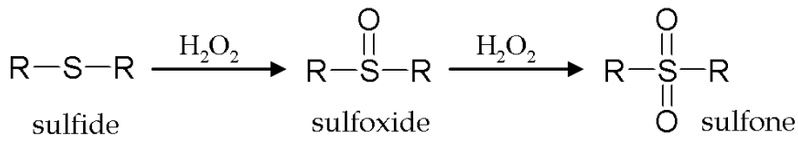

Fig. 5. Mechanism of oxidation of sulfidic compounds (thioethers)

The molecules containing sulfidic sulphur are oxidized by VPHP to both products. In case of thioanisol (phenyl methyl sulfide), after a VPHP degradation process only phenyl methyl sulfoxide was detected by MS. The final oxidative product (phenyl methyl sulfone) was not detected because of its high volatility and thus quick evaporation during the decontamination test, but we can assume its formation. Oxidation of dimethyl sulfoxide to dimethyl sulfone is driven by VPHP to a total conversion (Švrček, 2010).

9.4 Phenol derivatives

VPHP can also decompose phenol derivatives substituted in the *para*- position with electron-donating groups – mainly hydroxyl and amino. On the contrary, electron-withdrawing substituents (-CN, -NO_2, -COOH) make the contaminants intact to the VPHP atmosphere (Švrček, 2010).

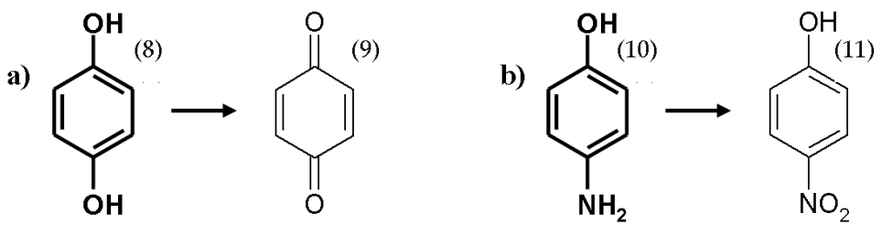

Fig. 6. Oxidation of hydroquinone and *p*-aminophenol by VPHP vapors

Hydroquinone (8) is oxidized by VPHP to 1,4-benzoquinone (9) which was also true for vanillin degradation. In case of *p*-aminophenol (10), a solid, weak and soluble mixture of degradation products was formed in the VPHP atmosphere. One of the products was *p*-nitrophenol (11) and the other compounds were products of oligo- and polymerization reactions between the starting compound and the products of degradation.

9.5 Molecular structure effects promoting the VPHP-induced degradation

A group of model chemical substances with similar structures (approximately 30 benzaldehyde and phenol derivatives bearing different types, numbers and position of substituents on the aromatic ring) was tested to study the influence of structure on the sensitivity to the VPHP process and reaction schemes were evaluated (Švrček, 2010). It is clear that the presence of electron-donating group on the benzaldehyde skeleton in many

cases leads to an increase in its sensitivity to degradation by VPHP. The type, number and position of substituents on the aromatic ring with respect to the –CHO group play an important role. The highest sensitivity is shown with substances bearing high electro-donating substituents (like -NR_2) located in the *para*- position to –CHO. With the decreasing number of substituents, the time necessary for the VPHP degradation also decreased. The lowest sensitivity was found with benzaldehyde derivatives containing a halogen atom, an electron-withdrawing substituent or sterically demanding substituents.

10. VPHP as an agent for decontamination of biologically active compounds

In 1985, the International Agency for Research on Cancer (IARC) issued recommendations on the application of oxidative processes for the decontamination of cytotoxic compounds in waste waters (Roberts et al., 2006). Following studies (Barek et al., 1998; Hansel et al., 1997) tried to find the ideal oxidative agent that could be used in hospitals for the purposes described above. Three systems were tested: water solution of hydrogen peroxide (< 30 %, w/w), NaOCl and Fenton reagent for the degradation of selected cytostatics. Hydrogen peroxide showed the lowest efficiency of degradation compares to other oxidative systems, but it still appeared to be a promising agent.

The efficiency of 35% (w/w) liquid solution of hydrogen peroxide, VPHP and eight liquid detergents containing NaOCl for the deactivation of cytotoxic compounds present on the inner surface of pharmaceutical isolators was compared (McVey et al., 2006; Roberts et al., 2006). The contaminants tested were cyclophosphamide, doxorubicin and 5-fluorouracil. The liquid detergents removed all substances from the surfaces except for doxorubicin that showed high resistance to alkaline detergents. However, the contaminants were only washed away from the surface and their bioactivity was not eliminated. Cyclophosphamide and 5-fluorouracil were completely intact after a one-hour exposure to concentrated solutions of detergents which means that they preserved their full biological activity. Degradation of doxorubicin only occurred in a strong alkaline detergent (pH = 13.2, 80% conversion over 1 hour) and it remained unchanged in the water solution of hydrogen peroxide over 1 hour. The results from similar studies (Castegnaro et al., 1997; Hansel et al., 1997) showed that the application of 30% (w/w) hydrogen peroxide solution led to slow degradation of doxorubicin (60% conversion after 24 hours) and total degradation of cyclophosphamide after 1 hour. The VPHP "dry" process at temperature below 30 °C and reaction time of 1 hour exhibited slight degradation of 5-fluorouracil and significant degradation of doxorubicin (44 – 92 %). Unfortunately, cyclophosphamide remained intact. The mechanism was not studied (Roberts et al., 2006).

On the contrary, the application of VPHP as a deactivator of warfare agents is much more studied. A main leader on this topic is the STERIS company that found a quick and safe means of decontamination of large polluted areas (McVey et al., 2006). Their method seems to be very promising mainly because of a danger of terrorist attacks. The application of hydrogen peroxide to deactivate chemical weapons of mass destruction was studied (Wagner & Yang, 2001) which resulted in creation of a universal liquid deactivation agent called "Decon Green". This agent is composed of a hydrogen peroxide water solution, alcohol (ethanol, isopropyl alcohol, *tert*-butanol, polypropylene glycol) and carbonates, bicarbonates or monoperoxocarbonate - Na_2CO_3, K_2CO_3, Li_2CO_3, NH_4HCO_3). „Decon Green" provides high efficiency for the degradation of nerve agents and blistering agent like sarine (GB), soman (GD), sulfide yperite (HD) and VX

compounds. The addition of carbonate to the liquid solution of peroxide leads to the formation of an OOH^- anion that is highly reactive, mainly with nerve agents. It speeds up the per-hydrolysis of VX and G compound families. The addition of peroxocarbonate, which is highly active for the oxidation of HD substances, leads to the formation of nontoxic products (Wagner & Yang, 2001). The addition of alcohol serves as the nonfreezing part of this mixture.

Carbonates are very good activators of hydrogen peroxide but they cannot be used in the VPHP process as they are not volatile. The special arrangement can distribute carbonates in the gaseous phase which supports vapor-phase decontamination. A pilot work (McVey et al., 2006) studied the application of VPHP to the degradation of warfare agents. It was shown that the VPHP process was highly potent in the degradation of HD and VX compounds even without any activator present. The G type agents are very stable under the VPHP conditions. It is known that they can be decomposed easily under alkaline conditions (Wagner et al., 2007). Hydrogen peroxide is relatively unstable in alkaline solutions but it has been shown (McVey et al., 2006; Wagner et al., 2007) that under these conditions the GD compounds can be decomposed to nontoxic products. Furthermore, the combination of VPHP and a base also accelerated the degradation of VX and HD compounds compared with VPHP alone (McVey et al., 2006; Wagner et al., 2007). The degradation started at an ammonia level that was below the safety limit of 25 ppm. It was observed that the rate of degradation was increasing with the increasing ammonia concentration (Wagner et al., 2007). A detailed study on the degradation of GD compounds using a mixture of VPHP/NH_3 shows a different mechanism compared with liquid "Decon Green". In case of application of the VPHP/NH_3 mixture to HD and VX, higher reaction rate was observed together with some differences in the decomposition mechanism (Wagner et al., 2007). It is interesting that the commercial decontamination agents containing ammonia are only active for the degradation of G but not for VX and HD.

The higher efficiency of VPHP for the degradation of warfare agents compared to water solution of hydrogen peroxide is explained as follows. The acidic products formed by per-hydrolysis are volatile and are ventilated out. On the contrary, in the water solution the acidic products are concentrated and thus the pH value decreases under a level which is necessary for the formation of peroxide anions and the reaction is thus stopped. Higher efficiency of VPHP is also explained by a model simulating the distribution of contaminants on contaminated surfaces.

Hydrogen peroxide is due to its properties closer to organic solvents than to water and thus it is selectively adsorbed and concentrated inside drops or film of the contaminant. Molecular distribution results in a higher local concentration of H_2O_2 inside the contaminant and exclusion of water vapor out of the contaminant organic layer (Wagner et al., 2007). When using the liquid phase, competitive adsorption of water and hydrogen peroxide to contaminant molecules occurs which slows down its degradation noticeably.

It was showed (Wagner et al., 2007) that the exposition time necessary to decontaminate inner premises of buildings contaminated by the warfare agents using VPHP/NH_3 is 24 hours. These results clearly show that such modified VPHP process (VPHP/ NH_3) is the right choice for quick and effective decontamination of large areas and objects (military vehicles, airplanes) contaminated by dangerous compounds.

11. VPHP – Material compatibility

The extent of contaminant elimination by hydrogen peroxide in vapor phase is mainly influenced by the concentration of VPHP, exposure time, temperature, humidity, condensation and also the physical and chemical properties of materials (Chung et al., 2008). The influence of the contaminated surface upon the efficiency of VPHP-induced inactivation of the spores of *Bacillus stearothermophilus*, which serves as a biological indicator, was studied (Unger et al., 2007). Among the notable influences are the chemical composition of the selected material, its potential catalytic activity, absorption of gaseous hydrogen peroxide in the material and, last but not least, the differences in the surface production or its treatment. The relationships between the deactivation rate and porosity and wettability are documented (Unger et al., 2007) – VPHP is mainly effective for the sterilization of smooth surfaces and therefore stainless steel and glass coupons are used for the evaluation of the process efficiency (Bounoure et al., 2006; Unger et al., 2007). Spores on a porous surface can be hidden in cavities and in such a case the penetration of hydrogen peroxide to the material plays an important role in decontamination (Bounoure et al., 2006). Some materials have demonstrated an inhibition effect to the microorganisms without any decontamination agent, such as ethylene-propylene-diene rubbers. All knowledge mentioned above was implemented by FDA in the Guidelines for industry. It is recommended to choose suitable materials for the design of any aseptic process. They have to provide proper compatibility with the chemicals used and thus can be easily cleaned and decontaminated. It is highly important to choose construction materials with appropriate texture and porosity by the build-up of the aseptic process mainly when the validation of decontamination process is required (Unger et al., 2007). The VPHP decontamination was tested on various surfaces but the toxicological evaluation reports on surfaces treated in such a way are very limited. Hydrogen peroxide is characterized by high cytotoxicity (i.e. harmfulness to cells) so its residual amounts in the materials can cause irritation of eyes, skin, mucous membrane and also acute lung dropsy in cases of long-time inhalation. Therefore, the construction materials have to be selected not only due to their compatibility with the VPHP process but also with respect to the efficiency of residual hydrogen peroxide aeration out of these materials. Up to now, the studies have mainly focused on polymeric materials because of their popularity in the pharmaceutical industry. The permeation of gases through plastic materials occurs in two steps: the first one consists in dissolution of gas in the thin surface layer and the second is its diffusion to the material. It was found that polyethylene and polypropylene can easily release hydrogen peroxide because of its limited migration in these materials. In contrast, polystyrene, polyurethane, poly(methyl methacrylate) (PMMA), poly(2-hydroxyethylmethacrylate) (HEMA), fluorosilicone acrylate and mixture of polyurethane and silicone demonstrated strong cytotoxicity after standard aeration (Ikarashi et al., 1995). The PMMA and HEMA materials are used for the production of contact lenses and thus, after their sterilization by VPHP, the attention needs to be paid to residual hydrogen peroxide. Hydrogen peroxide can easily penetrate through polyolefins in general, out of which PVC is used for packaging of infusion solutions. Therefore, the VPHP process has to be applied very carefully in order to avoid the contact of residual hydrogen peroxide with the infusion solution due to resulting oxidative reactions (Ikarashi et al., 1995). Nonetheless, information on the resistance of other construction materials is very limited. Although there have been reported interactions of several materials with VPHP

which can lead to their damaging (Hultman et al., 2007), the process is still considered non-corrosive for surface decontamination (U.S. Environmental Protection Agency, 2005). Several works tested the resistivity of commonly-used devices to VPHP but the results were evaluated only visually and by testing of functionality of the devices. Generally, it was concluded that VPHP was an acceptable sterilization method for the devices (Hall et al., 2007; Heckert et al., 1997).

In global, materials can be divided into four groups according to their tolerance to VPHP. Group 1 represents such materials that can be in the contact with hydrogen peroxide for a long time, like pure aluminum, tin, borosilicate glass or Teflon. In contrast to this group stands Group 4 which comprises materials that cannot be in any contact with hydrogen peroxide because of their fast decomposition or formation of explosive mixtures (copper, iron, carbon steel, magnesium alloys). The concentration of 45% (w/w) hydrogen peroxide is considered critical (Hultman et al., 2007) because, when exceeded, undesirable interaction or damage of different materials can occur. For chemical decontamination, higher-concentrated peroxide is necessary and in a proper arrangement it can be a safe system. It can be expected that during the "wet" VPHP process, the concentration of the condensate is above this critical concentration and thus the materials can be damaged. However, this concentration can also be exceeded during the "dry" VPHP process in the aeration phase when the absorbed hydrogen peroxide is concentrated on the surfaces. If the damage is not visible, it does not mean that the material has not been damaged. Microscopic lesions can appear and the macroscopic ones can become evident only after a long-time exposure to VPHP. The VPHP process was also tested for the interiors of airplanes and ambulances (Krieger & Mielnik, 2005; Shaffstall et al., 2006). As these vehicles are frequently in the contact with infection, quick and effective decontamination is important to avoid the propagation of infection (SARS, bird flu). Application of VPHP in these facilities is risky because several sensitive devices which are vital for their proper function can be damaged. The Federal Aviation Administration (FAA) studied the influence of the VPHP process on textile materials of airplane interiors and found significant changes. The leader in the VPHP technology, Bioquell, tried to map the influence of long-time application of VPHP to the hospital equipment. The company tried to fill in the information gap of the VPHP material compatibility. Some materials were shown to be highly resistant to VPHP (called „VPHP-resistant"), some were degradable by VPHP and some absorbed hydrogen peroxide substantially. There are also materials unsuitable for VPHP like untreated aluminum, copper, soft steels, coated steels and generally materials that have similar behavior in the liquid state. Materials containing cellulose tend to absorb hydrogen peroxide and are further degraded; therefore, they are also unsuitable for VPHP (von Woedtke et al., 2004). Furthermore, EPA studied the VPHP compatibility of common construction or decorative materials (U.S. Environmental Protection Agency, 2008). The visual inspection did not reveal any significant changes of the tested materials but the tensile strength was reduced, which was caused by changes in their inner structure.

Low-temperature H_2O_2 plasma material compatibility is actually a much more studied topic. The Johnson & Johnson company tested a wide range of materials and devices which were in a periodic contact with hydrogen peroxide plasma. It was found that the materials containing amines in their structure were unsuitable because of their oxidation that damaged the structure. Also the S–S bond in the materials was proven to be incompatible with this process.

It is necessary to select such materials that are fully compatible with VPHP by the design of the device or the process where it is expected to use VPHP for decontamination. In such case, it needs to be known how the materials interact with hydrogen peroxide, namely with respect to absorption and the rate of hydrogen peroxide decomposition. It is also important to know the aeration time required to vent out hydrogen peroxide in order to avoid undesirable cytotoxic effects (Ikarashi et al., 1995). It is necessary to evaluate the resistivity to hydrogen peroxide and the amount of residual hydrogen peroxide left in the material. There are several analytical methods for the determination of hydrogen peroxide such as reduction by SnCl2 (Egerton et al., 1954), thiocyanate method (Egerton et al., 1954; Ikarashi et al., 1995), enzymatic method of dimerization of p-hydroxyphenolic acid (Christensen, 2000), color changes of $Ti_2(SO_4)_3$ or $TiCl_4$ by spectrophotometric detection (Egerton et al., 1954), UV spectroscopy, titration of $KMnO_4$, I_3^-, or $Ce(SO_4)_2$ (Klassen et al., 1994), electrochemical, polarographic and other methods (Higashi et al., 2005).

12. Glory and pitfalls of VPHP

The VPHP technology is a very progressive method of sterilization and chemical decontamination. Its popularity was mainly achieved due to low toxicity of hydrogen peroxide and its non-toxic decomposition products, environmentally friendly behavior, relative flexibility and a wide spectrum of applications. Its high efficiency against a wide range of microorganisms makes this method universal in terms of bio-decontamination and thus it is very popular in medical and pharmaceutical industry. This application is widely studied and described. The absence of theoretical knowledge is the only drawback of this method. Proper understanding of the sterilization principle would allow to optimize the method and to make it exceptionally powerful. On the other hand, the chemical decontamination by VPHP is still a growing area with a wide potential of application. Mainly the combination and potentiation of VPHP by other chemicals or physical phenomena should improve it and make it a very powerful tool for the decontamination of dangerous chemicals. Also in this case, the absence of proper theoretical knowledge limits its application.

13. Future perspective of VPHP

As already mentioned above, the highly efficient VPHP process has found a great deal of new applications in bio and chemical decontamination. The first challenge seems to be theoretical understanding of its mechanism and thus obtaining the basics for finding new applications or improving existing processes. The next challenge is optimization of existing processes by application of new approaches and knowledge as a product of undergoing research and development. Another very important challenge is to discover novel possibilities of VPHP potentiation, mainly with respect to chemical decontamination. There is a great need for the decontamination of a wide range of chemical pollutants. The VPHP process can play a very important role in the case of homeland security as it can be easily implemented to the defense system, which provides greater security for the state and citizens against terrorists, spreading of infections, chemical and biological accidents, etc.

14. Conclusion

At the present time, a broad spectrum of decontamination techniques is acknowledged to be utilized to remove biological and chemical contaminants from different surfaces. Nevertheless, new physical and chemical processes are continuously being developed. The main reasons for the development of new decontamination methods are negative properties of many decontamination agents, especially their toxicity or toxicity of residues formed after their application. This review summarizes the recent findings concerning a new promising decontamination agent Vapor Phase Hydrogen Peroxide (VPHP) whose properties were observed to be very close to an ideal decontamination agent. VPHP has become the method of choice by meeting many bio-decontamination requirements in the pharmaceutical, biomedical and healthcare sectors for it is reliability, rapidness, the fact that it leaves no residues (breaks down into water and oxygen) and the advantage that it can be validated. It is also a decontamination method of choice for chemically and biologically active compounds. The application of VPHP as a potential decontamination agent is apparently still in its infancy. Therefore, it comes as no surprise that the knowledge of the cidal action mechanism(s) and the influential factors is yet to be completed.

15. Acknowledgement

The project is supported by the grant of Ministry of Defense of the Czech Republic (OVVSCHT200901).

16. References

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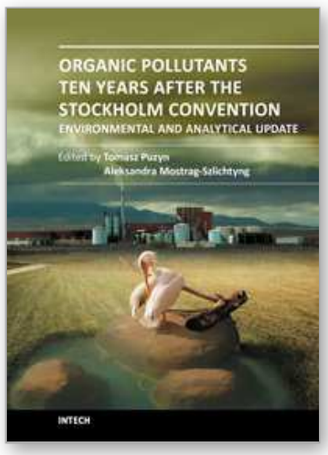

Organic Pollutants Ten Years After the Stockholm Convention - Environmental and Analytical Update

Edited by Dr. Tomasz Puzyn

ISBN 978-953-307-917-2
Hard cover, 472 pages
Publisher InTech
Published online 24, February, 2012
Published in print edition February, 2012

Ten years after coming into force of the Stockholm Convention on Persistent Organic Pollutants (POPs), a wide range of organic chemicals (industrial formulations, plant protection products, pharmaceuticals and personal care products, etc.) still poses the highest priority environmental hazard. The broadening of knowledge of organic pollutants (OPs) environmental fate and effects, as well as the decontamination techniques, is accompanied by an increase in significance of certain pollution sources (e.g. sewage sludge and dredged sediments application, textile industry), associated with a potential generation of new dangers for humans and natural ecosystems. The present book addresses these aspects, especially in the light of Organic Pollutants risk assessment as well as the practical application of novel analytical methods and techniques for removing OPs from the environment. Providing analytical and environmental update, this contribution can be particularly valuable for engineers and environmental scientists.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Petr Kačer, Jiří Švrček, Kamila Syslová, Jiří Václavík, Dušan Pavlík, Jaroslav Červený and Marek Kuzma (2012). Vapor Phase Hydrogen Peroxide – Method for Decontamination of Surfaces and Working Areas from Organic Pollutants, Organic Pollutants Ten Years After the Stockholm Convention - Environmental and Analytical Update, Dr. Tomasz Puzyn (Ed.), ISBN: 978-953-307-917-2, InTech, Available from: http://www.intechopen.com/books/organic-pollutants-ten-years-after-the-stockholm-convention-environmental-and-analytical-update/vapor-phase-hydrogen-peroxide-method-for-decontamination-of-surfaces-and-working-areas-from-organic-

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