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Toxicological Assessment

Expert report concerning the toxicological assessment of the STEREX atmospheric low-temperature plasma process against the background of the active disinfectant plasma products and other plasma by-products

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4/10/2021

1 Introduction:

The **STEREX plasma disinfection process** uses electrochemical effects to generate active disinfectant plasma products in the form of hydroxyl radicals, which are formed by oxygen and water vapor in the air.

In terms of **physics**, **"plasma"** is a **state of an electrically conductive gas**, which is conductive to the extent that a low flow of electrical current becomes possible. Two electrodes are positioned, which are positively and negatively polarized with a direct-current voltage. The relevant gas is located between the electrodes.
An ignition impulse causes individual gas molecules to decay into positively and negatively
charged atoms (ions, or ionized gas), meaning that the gas becomes conductive (the gas then acts as an ionic conductor, i.e. there is a second-level conductor present; commensurate with the name "ion", they move in the electrical field and, among other things, determine the flow of electric current through the gas).
If this has happened, an inverter is used to limit the flow of electric current through the gas to a low level in the µA range, and at the same time the voltage is reduced. The flow of electric current is reduced to the extent that conductivity is only just present, i.e. the state of the plasma is maintained between the electrode plates.

The flow of electric current through the conductive gas generates **chemical-physical effects** on the gas, or on the components of the mixture of gases (air).
The plasma used in the STEREX procedure is, as already mentioned above, an atmospheric low-temperature plasma with a potential difference of a maximum of 1.75 kV. As a result, one **chemical-physical effect** achieved is that a proportion of the oxygen in the air decays into oxygen radicals, which combine with other oxygen molecules in the air and water vapor to form so-called **hydroxyl radicals**.

The hydroxyl radicals are the active disinfectant product of the physical plasma, which kill off microorganisms and therefore determine the antimicrobial effectiveness.
This means that it is not necessary for the entire room air volume to go through the plasma generator within a certain period in order to kill off microbes. In actual fact, the plasma generator emits active products (hydroxyl radicals) into the room air, which are formed from oxygen and water molecules, disperse in the room air and destroy microorganisms found there.

A high antimicrobial effectiveness is observed in relation to hydroxyl radicals in various experimental and therapeutic applications.

2. General Overall Assessment of the STEREX Plasma Process:

In the event of high antimicrobial effectiveness, it is legitimate and necessary to ask whether and to what extent hydroxyl radicals may have a detrimental effect on human health.

The assessment of a detrimental effect on human health is carried out within the area of toxicology.

In this area, a differentiation is made between an **acute toxic effect** in the event of single exposure, a **subacute toxic effect** in the event of repeated or frequent exposure and a **chronic toxic effect** in the event of exposure to the agent over an extended period, usually at a low concentration.

The acute toxic effect may correlate to:

- Detrimental irritation/caustic effect on mucous membranes, skin, respiratory passages or the digestive tract (depending on the substance/agent and the route of exposure)
- Detrimental effect on organ systems

The chronic toxic effect often correlates to:

- Detrimental effect on cell division = mutagenic effect (altering the genetic make-up), often resulting in a carcinogenic effect (causing cancer)
- Detrimental effect on the germline = teratogenic effect (toxic to reproduction), a specific consequence of the mutagenic effect in the area of the gamete cells, or the somatic cells in the embryonic and fetal phase.

The subacute toxic effect displays detrimental effects of acute *and* chronic toxicity *in parallel*.

The **methods to test** for **acute, subacute and chronic toxic effects** primarily include, in addition to animal testing, cell culture tests (e.g. AMES test), which can be used to identify carcinogenic or mutagenic effects. Acute toxic effects are direct reactions of the individual to the harmful agent, which is why effects such as these need to be identified in animal tests.
In addition, data on misuse that may, for example, have resulted in higher concentrations and therefore in an acute toxic effect, can be assessed. This data is collected in the area of medical product safety and toxicological emergencies. As a result, valuable information is obtained about the effect on humans, because animal testing data can often not be entirely transferred to humans.

Scientific medical and chemical specialist literature available via www.pubmed.com is used as the basis for assessment. This portal also provides the evidence level of the studies and the impact factor of the article. This can be used to estimate the scientific relevance and the specialist value of the relevant article.

It must be deemed that the **core of the assessment is the hydroxyl radical,** which determines the antimicrobial effect as the plasma reaction product.

In relation to the hydroxyl radicals as an **agent that is possibly toxicologically relevant**, the following possibly **detrimental effects must be assessed**:

2.1. Products/By-products of the Plasma Reaction:

Direct products of the plasma reaction:

- hydroxyl radicals (active product)
- ozone (unwanted by-product)
- nitrogen oxides (unwanted by-product)

2.2. Product that is Directly Effective for the Intended Process (Disinfection):

The direct product of the plasma reaction is the **hydroxyl radical**, which can be described as an oxygen radical state with an antimicrobial effect.

2.3. Possible Secondary Products and Reaction Products

In this area, it is necessary to consider and assess possible secondary products of the reaction of hydroxyl radicals with components of the ambient air.

The three stated points will be considered and assessed in detail below:

Concerning
Point 2.1:
Products/By-products of the Plasma Reaction

Depending on the potential difference of the electrodes of a plasma reaction cell, various plasma reaction products from the mixture of gases in air are possible:

- Up to around 1.75 kV, hydroxyl radicals are mainly formed from oxygen and water vapor contained in the air. Note: These are the desired product of the plasma reaction and are required for the antimicrobial effectiveness.
- Between 2 and 5 kV, ozone O_3 is the main product of the plasma reaction, primarily formed as a result of the reaction of the oxygen.
- Above 5 kV, the triple bond of the nitrogen molecule N_2 is split, meaning that nitrogen radicals are available as a reactant and there is increased formation of nitrogen oxides (NO_x).

Ozone as a product has a high acute toxic effect in terms of an irritation of mucous membranes (eyes, nose, respiratory passages), as well as a possible chronic toxic effect in the event of constant exposure. The chronic toxic effect is identified as carcinogenic. Currently (2021) there are conjectures that a carcinogenic effect may occur, but this has not been fully confirmed.
Therefore the formation of ozone is undesirable and technical adjustments must be made to the process to prevent it, or reduce it to a level at which no acute or chronic toxic effects can occur.

In addition to the acute toxic effect (in particular irritation of the mucous membrane), **nitrogen oxides** display various chronic toxic effects. The chronic toxic effects must be described in terms of carcinogenic effects in the animal test and effects on the cardiovascular system. The effect on the cardiovascular system is a significant rise in the diastolic blood pressure values and an increase in coronary heart syndrome depending on the dose in the event of constant exposure.
Therefore nitrogen oxides, like ozone, are undesirable.

The plasma reaction of the STEREX process is controlled by a special inverter. This forces the plasma reaction at potential differences below 1.75 kV. In addition, the electrodes are specially customized. The amplitude of alternating frequencies, which are required e.g. during the ignition, is regulated so that even during the ignition process no higher potentials, and therefore other undesirable plasma reaction products, occur.

As a result of these special technical features of the STEREX process, the plasma reaction generates hydroxyl radicals as the reaction product and the formation of ozone and nitrogen oxides is prevented.

The **formation of nitrogen oxides** is not possible in the **STEREX process** from a technical procedural perspective, therefore it is not necessary to assess possible nitrogen oxides.

Ozone is identified in the STEREX process in traces no longer relevant in terms of health, which are, for example, less than 1% of the workplace limit value. The evidence is provided at the limit of determination for occupational health measurement procedures carried out on site (this means that as a rule the ozone formation is then lower than the limit of determination, which is usually 0.01 ppm).
Therefore the ozone formation in the STEREX process can be practically ignored. This means that further toxicological assessment is also not necessary in relation to ozone.

General toxicological information about nitrogen oxides and ozone is provided in specialist scientific literature.

Concerning
Point 2.2:
Product that is Directly Effective for the Intended Process
(Disinfec
tion):

The **hydroxyl radicals as the effective agent** (see considerations under point 1) will be presented further and assessed below.

Concerning
Point 3:
Possible Secondary Products and Reaction Products

In relation to oxidative disinfectants, we are definitely aware of the formation of disinfection by-products or products in parallel reaction with organic compounds in the indoor air.

The problem of disinfection by-products primarily exists in the case of halogens (chlorine, bromine, iodine) and halogen-releasing compounds (sodium hypochlorite, Chloramine-T, dichloroisocyanuric acid). Due to the high reactivity of halogens, various chemical reactions (radical substitution, electrophilic addition) occur, which generate reaction products that are organohalogen compounds. These chemical reactions destroy microorganisms (as a disinfectant effect), and in addition other organic compounds are chemically altered.
Chloramines, chloroform, carbon tetrachloride and other small-molecule compounds with organically bound halogens may be formed as the products of these extensive reactions.
Halogenized organic compounds, primarily compounds with chlorine substitutes, have a chronic toxic potential, particularly in relation to the kidneys.
Therefore, in systems that use halogens as a disinfectant, certain limit values need to be considered for (organically) bound chlorine compounds and trihalomethanes (chloroform and certain other organic chlorine compounds) (drinking water, bathing pool systems, certain technical disinfections).

Oxygen compounds in the form of singlet oxygen (oxygen radicals) cause a direct oxidation of organic compounds. Possible oxygen-organic compounds include alcohols and ketones. Even alcohols and ketones are immediately converted in the human metabolism (e.g. as the reactant in the citric acid cycle) and have no acute or chronic toxic potential.
Oxygen is also not a reactant of radical substitution or electrophilic addition. The reactions of radical substitution and
electrophilic addition take place with molecules that have *one* bond (such as the halogens, Cl_2 or Br_2).
However, there are *two* bonds in the oxygen molecule, which cannot be split for radical substitution or electrophilic addition.

In relation to ozone O_3, undesirable reaction products due to chemical radical reactions are possible. These often limit the use of ozone as a medium for technical room disinfection or room deodorization. In some cases, for example, unwanted odors cannot be completely removed because reaction products are produced which in turn have an odor. There have also been reports of the destruction of plasticizer substances in plastics if ozone is used too often for vehicle deodorization.
However, these are examples of targeted ozone application at a high and frequent concentration, often without specialist knowledge of the application of the relevant ozone synthesis devices.
The low ozone formation, if there is indeed any at all, in the STEREX process is at such a low level that it can in no way be compared with targeted
ozone
application.
An ozone application and the STEREX process are completely different processes. In the STEREX process, hydroxyl radicals occur and there is almost no ozone.

Oxygen radicals from hydroxyl radicals therefore cause no relevant organic radicals, such as organic peroxides, because hydroxyl radicals and ozone, or elemental oxygen as oxygen radical, are completely different compounds, the reactions of which are not the same.
It must therefore be assumed that organic peroxides, such as ethylene oxide, cannot be formed by hydroxyl radicals.

Ethylene oxide, as a gas that is toxic but has a strong biocidal effect, cannot be formed in normal atmospheric pressure conditions and due to the fact that the reaction takes place in the gas phase. The chemical synthesis of ethylene oxide for use for instrument sterilization (almost no longer used today) takes place under considerable excess pressure, in the presence of catalysts in the reaction mixture and with the reaction in the liquid phase. This has nothing to do with the plasma reaction, the hydroxyl radicals and the use of hydroxyl radicals under normal atmospheric conditions. The conditions of chemical synthesis for the controlled production of the active ingredient ethylene oxide

are therefore in no way comparable with the conditions of atmospheric low-temperature plasma.
Furthermore, short-chain organic carbon compounds need to be present in order for ethylene oxide to be able to be formed. In addition, C2 compounds such as ethanol or acetic acid or longer-chain C compounds are required, which usually do not occur in room air in relevant concentrations that make the formation of ethylene oxide possible.

Therefore laborious investigations of plasma reaction products in 2020 in relation to ethylene oxide were not able to provide evidence of or confirm the formation of ethylene oxide.

As a rule, water and carbon dioxide are created via the reaction of hydroxyl radicals with organic compounds. Short-chain alcohols or ketones may possibly be formed, but they are not relevant in relation to detrimental effects on health.

In addition, in verbal communication with the company Steurer Systems it was indicated that a **question had arisen in relation to a possible formation of formaldehyde by hydroxyl radicals**.
In relation to this it must be determined that hydroxyl radicals are oxidative disinfectants, which have an oxidative effect similar to hydrogen peroxide on an organic substance, whereby oxygen is reduced to water.
Formaldehyde is a reductive disinfectant, which is oxidized via reaction with organic substance, or which reacts directly with organic
substance (e.g. aldol addition or acetal/ketal formation).

Formaldehyde and hydroxyl radicals are completely different compounds. They are not created as reaction products of the STEREX process.

It is not possible for hydroxyl radicals to facilitate the formation of formaldehyde. In actual fact it is known that the effect of oxidants (in the acidic environment with catalysts) is to convert formaldehyde into formic acid, which can be metabolized by living systems as a source of carbon.
This means that e.g. formaldehyde in the basic structure can be broken down into less toxic and biologically convertible reaction products (e.g. formic acid) via treatment with peroxide, acid and a catalyst.
Therefore, in a similar way, hydroxyl radicals would be more likely to break down any formaldehyde load in the room air than to increase it.
The formation of formaldehyde due to the presence of hydroxyl radicals cannot be described in due consideration of known chemical reaction pathways.

The occurrence of reaction products relevant for health from hydroxyl radicals can therefore be considered to be unlikely according to our critical observation and assessment, because against the background

of the assessment of chemical reaction mechanisms (e.g. radical substitution, electrophilic addition and others) as recognized state of the art in science it is not possible to create possibly toxic plasma reaction products under the intended application conditions of the STEREX process.

3. Special Assessment of Hydroxyl Radicals as Active Components:

Following this in-depth consideration of the STEREX process in relation to the basic materials, products and possible reaction products, it is now necessary to **assess hydroxyl radicals in terms of detrimental effects on humans in the event of exposure.**

The general examination of the scientific database PubMed did not provide any entry of a specialist article on this subject that described the experimental testing of exposure of cell cultures or animals to hydroxyl radicals with exposure via the air/breathable air. From a scientific perspective, this must be seen as a gap that urgently needs to be filled in via experimental toxicological assessments.
To date, hydroxyl radicals have not been of significant scientific or
medical relevance. The question of application for room air disinfection or the general question of room air disinfection had not existed in recent decades before the Coronavirus pandemic in 2020.
Before 2020, therefore, it was only attributed a particular significance even in the clinical environment of room air quality if certain clean rooms
or minor surgery rooms or operating theaters needed to be assessed. These were referred to
as room class I and assessed both from a microbiological perspective and in terms of particle count. All other rooms, as room class II, were considered as practically equivalent to outdoor air.

Since the SARS-CoV-2 pandemic of 2020, preventing the transmission of airborne infection chains indoors is now also relevant. The normal room air in indoor areas that do not have aseptic use (room class II) is now becoming relevant for the transmission of airborne viruses, as a result of which sufficient consideration now needs to be given to the room air quality.

Questions now need to be asked about room air optimization and the reduction of germ accumulations indoors.

In addition to the dilution of the indoor air (ventilation as displacement; passively via window ventilation or actively via a ventilation system) or the filtration of indoor air, the treatment of indoor air with hydroxyl radicals is another important option for interrupting the infection paths of airborne microorganisms in a targeted manner.

This has been demonstrated via numerous investigations of the STEREX process in 2020. The process was initially tested experimentally under laboratory conditions in the accredited laboratory at Umwelthygiene Marburg and then under realistic application conditions in various areas (restaurants, schools, judicial authorities/security agencies, vehicles, etc.). The tests achieved sufficient microbe inactivation in all settings investigated, as a result of which it was possible to show that the process is microbiologically effective. The process therefore causes safe inactivation of airborne pathogens and therefore also the SARS-CoV-2 pathogen (an enveloped RNA virus).

Therefore the use of the process to contain transmission paths of the SARS-CoV-2 virus is possible and sensible from a microbiological perspective and must be implemented urgently. For this reason, the STEREX process provides a plasma disinfection process, because it offers a high benefit for public welfare by interrupting transmission paths of the SARS-CoV-2 virus.

As mentioned above, the general examination of the scientific databases did not identify any study that describes acute or chronic toxic effects of hydroxyl alcohols on humans.

In order to be able to carry out an assessment of possible detrimental effects in spite of everything, it is initially necessary to ask whether **hydroxyl radicals occur in other areas of relevant environmental compartments**.

It must be noted that **hydroxyl radicals occur if water is finely atomized with the ingress of sunlight.** This is observed after heavy rain when the weather subsequently brightens up in summer or at waterfalls.
However, it is not possible to deduce possible dose-effect relationships here, because the observations tend to be individual depictions or factual descriptions.
The fact is thus that hydroxyl radicals are primarily a natural substance that occurs in environmental compartments.

In addition, it is possible to check whether **currently used medical procedures make use of plasma reaction products**. Then the studies presented in this area can be used to assess whether hydroxyl radicals have displayed detrimental health effects in these accounts.

One such parallel application of **atmospheric low-temperature plasma** with **air as plasma gas** is **wound treatment.**

Here plasmas of this kind are used in a similar way as a medical product directly on people.

In geriatrics there are often problems involving chronic wounds in older, often immobile patients. These may be caused by infections of the wound with antibiotic-resistant microorganisms, which cannot be sufficiently eliminated using antibiotic treatment.

Wounds at pressure points of the body (decubitus, bedsores) are particularly at risk, among other things because in this area the blood flow, and therefore the supply of immune cells or antibiotic substances distributed in the blood, is not sufficient.
To date these wounds have often been treated surgically (debridement) or directly with strong oxidative disinfectants (iodine or hydrogen peroxide).
This procedure has been able to restrict the surface microbial colonization and improve the healing of the wound to a certain extent, however the
mechanical (debridement) or chemical (peroxide) impact resulted in parallel
damage to healthy tissue, meaning that healing was accompanied by heavy scarring, or healing was generally severely delayed.

Since the start of the 2000s, plasma generators have increasingly been used in the area of local wound treatment for chronic, infected wounds, in particular when antibiotic-resistant bacteria are present.
The treatments are time-consuming, daily treatment is usually needed, and the entire treatment time may take months.
However, it can be shown that the direct effect of the
plasma reaction products (mainly hydroxyl radicals) results in a suppression of pathogens relevant for infections and in promotion of wound healing.

Wounds treated with the use of plasma display considerably less scarring, and additional surgical treatment is often not required.

By the start of the SARS-CoV-2 pandemic, the process had become very important in certain areas of medicine (nursing, wound treatment, geriatrics).
To date no publication has described a detrimental effect of plasma reaction products on human tissue.

The important point is that the plasma treatment of wounds is carried out directly, i.e. in the near field of the plasma. The concentration of hydroxyl radicals in the area of the wound to be treated is a factor of around 100 to 1000 times higher than would be possible in the area of plasma disinfection of air.
In the plasma disinfection using the STEREX process, at 20°C, 1013 hPa and 50% rel. humidity, a maximum accumulation concentration of 650000 negative ion products from hydroxyl radicals per g air is identified.
This is a saturation concentration, which cannot increase further due to thermodynamic reasons relating to the plasma reaction.

In the near field of the plasma reaction during wound treatment, the concentration of hydroxyl radicals that is observed is therefore still 100 to 1000 times higher.
Even in these high concentrations, which can only occur in the oversaturation in the plasma state of the air, there is no relevant toxicological impact on the patient. Quite the contrary: It has been demonstrated that the wound healing sequences take place quicker and that there is less scarring on the wounds following the patient's recovery in comparison with conservative wound treatment (with antimicrobial chemical compounds) or even in comparison with surgical wound treatment (as here the granulation tissue is often recurrently destroyed in a non-selective manner).

In addition, when examining literature between 2000 and 2020 no indication can be found of a carcinogenic, mutagenic or teratogenic effect of hydroxyl radicals on humans.
The results of wound treatment also display no indications of the emergence of epithelial tumors or sarcomas.

The following must be considered when looking at the circumstances as a whole:

The SARS-CoV-2 pandemic is a global health emergency. All individuals must contribute to containing the pandemic.
At this point, the STEREX process is a room air disinfection process that is particularly relevant for the public good because there has been reliable confirmation of effectiveness in terms of interrupting infection chains in various applications.
On the other hand, there are hydroxyl radicals as active disinfectant substances. With regard to the hydroxyl radicals, there is no detailed, scientific assessment suitable for citing concerning the concentration-dependent exposure in cell culture testing and animal testing. It is therefore not possible currently in 2021 to state, for example, an LD_{50} value in the event of acute exposure and therefore an effect of high concentration on cells or animals, or to state a chronic toxic potential in the event of long-term exposure in humans or animals.

An important point is that in relevant medical areas and areas on the periphery of medicine (in some countries the treatment of chronic wounds is carried out by specially certified wound specialists, in other countries the treatment is carried out by nurses) we have seen plasma reaction products – namely hydroxyl radicals – being used in direct form (plasma source in the near field of the wound) with significant success since the 2000s.
When wounds are treated using plasma, the wound healing/granulation improves and (mainly bacterial, but also viral) pathogens are impeded.
A particular benefit is observed for patients with chronic wounds due to antibiotic-resistant bacteria.
It is not possible to find any citation since 2000 that describes a detrimental effect of plasma products on patients or personnel in attendance.

In addition, almost without exception, reports describe progression of wound healing with regular application of plasma products. There is also no evident possible carcinogenic potential in this area, because to date relevant tumors (in the area of skin/skin wounds this would mainly be squamous cell tumors or sarcomas) have not been described in association with the use of plasma in wound treatment since 2000.

When viewed as a whole, it must therefore be finally determined that the STEREX process generates hydroxyl radicals as active plasma reaction products.

Toxicological relevant by-products, such as ozone or nitrogen oxides, are reduced to below relevant concentration limits (ozone) or entirely prevented from forming (nitrogen oxides).

The hydroxyl radicals, as material correlates of the plasma reaction, do not produce any by-products that could be harmful to health and therefore need to be considered from a toxicological perspective.
No by-products should be expected that could be explained by generally known and relevant chemical reaction mechanisms.
Any such relevant substances are definitely observed when halogens are used as disinfection agents, but do not occur in the case of hydroxyl radicals. Halogens and hydroxyl radicals are different substance groups from both a chemical and material perspective.

Hydroxyl radicals are used via direct application of plasma generators, or via application of plasma generators in the immediate near field of the patient when used in wound treatment.
No acute or chronic toxic properties are described in this area.
The **acceleration of wound healing and the reduction of scarring** are medical indications of protective health effects rather than detrimental health effects.

When viewed as a whole, it must be assumed that under critical assessment of the information available and discussed here, there is no toxicological relevant, detrimental health effect of hydroxyl radicals (as plasma reaction products) in the event of the intended use of the STEREX process.

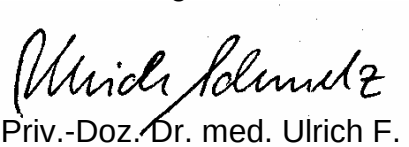
In general it has been determined that there has not yet been an explicit toxicological assessment of hydroxyl radicals in the air (e.g. by using the STEREX process). This matter was not relevant in prior decades, but the question has now arisen because the SARS-CoV-2 pandemic is forcing urgent optimization of indoor air because the relevant virus is transmitted in the air and optimizing the indoor air can break the infection chains here (this had already been demonstrated before).

In our view, the risk of a SARS-CoV-2 infection (case mortality rate, depending on age and state of health between 0.1 and 2%; chronic complications are possible, precise assessments are not yet possible with the usual scientific certainty because this is a novel disease) is considerably higher than a risk which may emerge from hydroxyl plasma reaction products.
The consideration of the use of hydroxyl radicals in humans, among other things for therapeutic care of wounds that are beyond pharmacological treatment, is an important aspect that shows the antimicrobial efficacy on one hand, but on the other hand shows that for around 20 years there has been no detrimental effect of hydroxyl radicals on patients and personnel (during the course of plasma treatment of wounds).
Therefore, in the overall view of considerations relating to the STEREX process, this is a safe process that can reduce and contain a considerably higher health risk, namely infection with the SARS-CoV-2 pathogen and therefore the further progression of the pandemic.

Against the background of the extraordinarily low risk of hydroxyl radicals and the high risk for the individual and public welfare during the SARS-CoV-2 pandemic, we cannot see practically any medical and toxicological risks that would oppose the intended use of the STEREX process in indoor areas.

Please feel free to contact the expert directly on 0049 175 915334 if you have any further questions.

With kind regards,



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