Chapter 1
Physical-Chemical Properties of Ozone – Natural Production of Ozone: The Toxicology of Ozone

As I already mentioned, ozone (from the Greek means to give off a smell) is a natural but unstable molecule. The pure gas has a soft sky-blue colour with a pungent, acrid smell. The molecule is composed of three oxygen atoms (O_3) and, the molecular weight, in comparison to the oxygen diatomic molecule (32.00) is of 48.00. Ozone has a cyclical structure with a distance among oxygen atoms of 1.26 Å and exists in several mesomeric states in dynamic equilibrium. For the physician it is useful to know that **the solubility (ml) in 100 ml water (at 0°C) of either ozone or oxygen is either 49.0 ml or 4.89 (ten fold lower), respectively**. Consequently the great solubility of ozone in water allows its immediate reaction with any soluble compounds and biomolecules present in biological fluids. Either traces of oxygen polymers (O_4) or ozone polymers (O_6 and O_9) can be generated but the idea that superactive ozone polymers/clusters may have a therapeutic role remains speculative (Cacace et al., 2001; Murai et al., 2003).

It is believed that during ozone generation, a trace of singlet oxygen ($^1O_2$) may be formed but again its practical significance remains negligible.

Among oxidant agents, ozone is the third strongest, after fluorine and persulphate, a fact that explains its high reactivity.

Ozone is formed from pure oxygen via an endothermic process allowed by very high voltage gradients set up between the electrodes of the Siemens' tube:

$$3O_2 \leftrightarrow 2O_3 - 68{,}400 \text{ cal.}$$

The reader can note that this reaction is reversible, practically meaning that ozone decomposes spontaneously and therefore it is hardly storable. Moreover the life of the ozone molecule depends on the temperature, so that at 20°C the ozone concentration is halved within 40 min., at 30°C within 25 min., while at –50°C is halved only after 3 months.

What is known about the natural production of ozone?

In the stratosphere, at about 22 km from the earth's surface, there is an ozone layer that may reach a maximal concentration of 10 ppmv (parts per million volume), equivalent to 10 micrograms/ml (mcg/ml). The maintenance of the ozone layer is very important because it absorbs most of the ultraviolet (UV) radiation (<290 nm) emitted by the sun. UV rays include band A (316–400 nm) responsible

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for suntan and bands B and C (from 100 up to 315 nm), which are far more mutagenic and responsible for enhancing skin ageing and carcinogenesis that has been shown by a progressive increase of carcinomas and melanomas in recent times.

Nature has been provident because, thanks to cyanobacteria, as soon as that oxygen started to increase in the terrestrial atmosphere about 2.3 billion years ago, UV solar emission catalyzed the production of ozone (Chapman mechanism), which then could control the UV irradiation and protect biological systems on earth:

$$O_2 + UV\ (<242\ nm) \rightarrow O + O$$

$$2O_2 + 2O \rightarrow 2O_3$$

The protective ozone layer in the stratosphere was fairly constant as it was the result of a dynamic equilibrium between the ozone-forming reaction and the natural dissociation of ozone. This equilibrium has been partly subverted during the last century owing to a progressive increase of pollutants, namely nitrogen oxides (NOx) and chlorine derived from chlorofluorocarbons (CFCs) used as refrigerant fluids and incautiously dispersed in the environment. One single chlorine atom, through a catalytic chain reaction mechanism discovered by Molina and Rowland (1974), can destroy thousands of ozone molecules before being transported back into the troposphere.

The excessive destruction of ozone has caused the thinning of the protective ozone layer and the famous "Antarctic ozone hole"; only thanks to an international effort to substitute CFC, the ozone layer will be probably restored to normal by 2050 (Schrope, 2000)!

Once again chaotic human activities (industrial processes, vehicular traffic, etc.) have led to a dangerous environmental pollution of the air present in the troposphere, which extends 8–17 km from the earth's surface. Exaggerated anthropogenic emissions of nitrogen monoxide (NO) and dioxide (NO_2), of carbon monoxide (CO), of methane (CH_4), sulphuric acid and other acid compounds have favoured an almost intolerable increase of ozone concentration up to 0.3 mcg/l or more. In large metropolis, **ozone, mixed with the other compounds, composes the photochemical smog: it has become the main toxicant for the lungs, eyes, nose and, to a lesser extent, the skin because particularly the respiratory mucosa does not contain enough neutralizing substances for this murderous acid mixture**. Indeed the respiratory tract lining fluids (RTLFs), that amounts to only 20–40 ml dispersed as an aqueous film layer over the alveolar space of about 70 m^2 is easily overwhelmed by this acidic mixture of strong oxidants. Particularly children, asthmatic and other broncho-pulmonary patients are at risk and the ozone "toxicomania" is well justified (Devlin et al., 1991; Aris et al., 1993; Broeckaert et al., 1999; Jerrett et al., 2009). **Certainly ozone toxicity at the street level has contributed to support the dogma that ozone is always toxic and the layman can well wonder why ozone can be used as a therapeutic agent**. Toxicologists and Health Authorities are correctly concerned about this problem, which is clearly not only due to ozone and that should not lead to the sweeping conclusion that ozone "is always toxic". The

findings that human activated leukocytes can probably produce ozone (Babior et al., 2003; Nieva and Wentworth, 2004) may be important in pathological situations.

We will come back to this point with another three gaseous molecules, namely CO, NO and H_2S (Moncada, 1992; Verma et al., 1993; Pannen et al., 1998; Nakao et al., 2009a, b), which, like ozone, surprisingly can also behave at physiological doses as essential effectors and become toxic at high concentrations. In other words, **the concept valid for any molecule is that it is the right dose that differentiates between a therapeutic and a toxic agent**.

Thus, for the safety of patients and personnel, not a trace of ozone should be present and a suitable exchange of air can be insured by an aspirator supplied with an ozone destructor. Moreover a monitor analyzer with warning lights and a loud alarm must be turned on all the time to immediately alert in case of a little contamination. I must say that our odour perception threshold for ozone is about 0.01 ppmv (0.01 mcg/ml), 10 times lower than the maximum work site concentration (WSC) of 0.1 ppmv over a breathing period of 1 h. The World Health Organization (WHO) permits to work for 8 h when the ozone concentration is 0.06 ppmv that is well perceived as a fairly strong ozone smell. Needless to say we should never trust our nose because our olfactory receptors become quickly tolerant and, in any case, the air in the clinic must be ozone-free.

It is unfortunately confusing that ozone concentrations are reported as either ppmv or as mcg/ml in USA or Europe, respectively. The conversion is as follows:

$$1 \text{ ppmv} = 1.0 \text{ mcg/ml or } 1.0 \text{ mg/l or } 1.0 \text{ g/m}^3$$

After prolonged breathing of air contaminated with ozone, the seriousness of symptoms and pathological changes are in relation to the ozone concentration and the exposure time (Table 1.1)

The toxicological effects are worse if the subject has breathed ozonated air contaminated with NO_2, acidic compounds, CO, etc because the RTLFs of the mucosa

Table 1.1 Toxic effects of gaseous ozone in humans

O_3 concentrations in air (ppmv)	Toxic effects
0.1	Lachrymation and irritation of upper respiratory airways
1.0–2.0	Rhinitis, cough, headache, occasionally nausea and retching
	Predisposed subjects may develop asthma
2.0–5.0 (10–20 min)	Progressively increasing dyspnoea, bronchial spasm, retrosternal pain
5.0 (60 min)	Acute pulmonary oedema and occasionally respiratory paralysis
10.0	Death within 4 h
50.0	Death within minutes

have a very weak buffering and antioxidant capacity. It must be emphasized that **the toxicity of ozone for the respiratory tract cannot be extrapolated to blood owing to quite different anatomical, biochemical and metabolic conditions (Bocci, 2006b)**. An intoxicated patient must lie down possibly breathing humidified oxygen. A slow intravenous (IV) administration of ascorbic acid and reduced glutathione (GSH) in 5% glucose solution may limit the damage. Ascorbic acid, vitamin E and N-acetylcysteine (NAC) can also be administered by oral route but this type of treatment is more rational as a preventive than curative therapy. Indeed, the higher is the anti-oxidant capacity of biological fluids; the lower is the possible oxidative damage.

1.1 Conclusions

Ozone is a natural, highly reactive, gaseous molecule produced by an electric discharge or/and UV radiation, alone or with NOx. Remarkably, even activated leukocytes seem to generate ozone in vivo. It can be protective or offensive depending upon its concentration and location. Ozone should never be inhaled because the pulmonary lining fluid has, in comparison to blood, a negligible protective capacity. Today, the use of ozone for industrial applications and water disinfection has received a wide consensus while its use in medicine remains controversial because medical scientists and clinicians remain sceptical and do not want to learn and understand the usefulness of ozone.