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New Investigations on Acute Toxicities of Vanadium Oxides

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Summary. The "in-vivo"-toxicity of the Vanadium-oxides V_2O_5 and V_2O_3 (administered orally, dermally and by inhalation) has been reinvestigated with particular emphasis on the safety and handleability of vanadium-oxides in the vanadium processing industry. Chemical-thermodynamic properties of vanadium-oxides make it likely that some earlier results on vanadium-toxicities have introduced artefacts as a consequence of the administration-techniques used. Special precautions have therefore been taken to avoid any chemical changes or artificial interactions during sample-preparation to ensure that the results significantly reflect the toxicities of the vanadium-compounds as exposure to them might occur. The LD_{50}(14d)-values indicate, that V_2O_5 should be classified as "hamful" (V_2O_5 techn. grade fused oral LD_{50}(14d): 716 mg/kg b.w. (rats m.) resp. 658 mg/kg b.w. (rats f.); inhal. LC_{50} 16.2 mg/l (rats m.) resp. 4.0 mg/l (rats f.) for a 4-hour exposure), while V_2O_3 should be classified as "relatively non toxic" (V_2O_3 tech. grade powder oral: LD_{50}(14d): 5639 mg/kg b.w. (rats f.) resp. 8713 mg/kg b.w. (rats m.)) according to the EEC-commission directive of July 29, 1983 (83/467/EEC). Based on interaction-studies and considering new results reported in literature, a 3-level-model of the mechanism of vanadium-toxicity via oxygen-radicals is suggested.

Keywords. Vanadium-in-vivo-toxicity; Oxygen-radicals; Toxicomechanism.

Neue Untersuchungen zur akuten Toxizität von Vanadiumoxiden

Zusammenfassung. Die "in-vivo"-Toxizität der Vanadium-Oxide V_2O_5 und V_2O_3 bei oraler, dermaler und inhalativer Applikation wurde neu untersucht. Aufgrund einer Analyse der chemisch-thermodynamischen Eigenschaften dieser V-Oxide wird nahegelegt, daß die Resultate einiger früherer Toxizitätsuntersuchungen durch chemische Veränderungen der zu untersuchenden Stoffe bei der Probenvorbereitung verfälscht wurden. Nach den in-vivo-LD_{50}(14d)-Werten ist V_2O_5 als "mindergiftig" (V_2O_5 techn. "fused" oral LD_{50}(14d): 716 mg/kg b.w. (Ratten m.) bzw. 658 mg/kg b.w. (Ratten w.); inhalotiv LC_{50} 16.2 mg/l (Ratten m.) bzw. 4.0 mg/l (Ratten w.) for a 4-hour exposure) bzw. V_2O_3 (techn. pulv. peroral LD_{50}(14d): 5639 mg/kg KG (Ratten w.) bzw. 8713 mg/kg KG (Ratten m.) als relativ nicht toxisch-nicht klassifiziert gemäß EEC-Commission-Directive vom 29. Juli 1983 (83/467/EEC) einzustufen. Basierend auf Studien der Interaktionswirkung bestimmter Substanzen und unter Eibeziehung der Resultate jüngst mitgeteilter Befunde zur Vanadium-Toxizität an Zellkulturen, wird ein Modell zum Vanadium-Toxizitäts-Wirkungsmechanismus vorgeschlagen, das 3 Hauptmechanismen – abhängig von der Konfrontations-Intensität (Konzentration und Expositionsdauer) – nahelegt.

Introduction

Vanadium is an element widely contained in natural sources such as South African, Australian, New Zealand, Ural's and Chinese magnetite iron ores. According to Cotton–Wilkinson [1], its average global distribution in the earth's surface is 0.02%.

Vanadium can also be enriched by some organisms, such as ascidiae and holothuriae, where it plays a similar role to that of iron, as an oxygen carrier similar to that of iron in the haemoglobin complex [2]. Consequently, many mineral oils, especially of Venezuelan origin, show relatively high vanadium contents (approximately 130 ppm). Such levels can be further concentrated in heavy oil fractions during oil refining. Thus, vanadium, and especially its oxides, occur much more widely in nature than frequently appreciated. As a result, vanadium oxides in fuel-oil-fly-ash and carbon-black from heavy oil firing electricity generating stations have been observed [3–5] to cause irritations, especially conjunctivitis, and lung toxic phenomena, especially bronchitis, in service personnel at installations where vanadium containing fuels have been burnt. After the first observations of vanadium compounds induced toxicity, Symansky [6] indicated that this feature of vanadium use has been exaggerated to a significant degree (Massmann[7]). Indeed, the toxicity of vanadium has been and still is frequently compared with that of chromates [8].

However, solubilities, chemistry, and thermodynamics of vanadium are significantly different from those of chromium and make it unreasonable to deduce similar behaviour. In contrast to chromates, dissolved vanadates polymerise easily to suspensions of insoluble vanadium-oxide or -oxide-hydrate particles, when the *pH* approaches neutrality.

1. Chemistry of Vanadium Oxides

Cotton and Wilkinson [1] describe the possible oxides of vanadium as being a V(II)-oxide VO, a V(III)-oxide V_2O_3, a V(IV)-oxide "VO_2" (also named "V_2O_4") and the V(V)-oxide V_2O_5, all of which have very low solubility in water.

For the solubility of V_2O_5 in water at ambient temperature there are given in literature several data – partially showing some divergence – but in any way indicating very low solubility. The rather recent analysis given in Cotton–Wilkinson or D'Ans Lax make likely that solubility does not exceed 5–7 mg/l [1, 10], while that of V_2O_3 is even significantly lower. These solubility-data given in the literature mentioned do not agree with the values of 0.8% at 20 °C as usually reported earlier [7]; the difference may possibly be explained by "solubilisation"-reactions as indicated below. "Solubilisation" can be achieved by treatment with strong acids which react with vanadium oxides to form vanadyl ions e.g. V(II): $V(H_2O)_6^{++}$; V(III): $V(H_2O)_6^{+++}$, VO^+, $V(OH)^{++}$; V(IV): VO^{++}; V(V): VO^{+++}; VO_2^+.

Alternatively, "solubilisation" can also be achieved by reaction with a strong alkali to give meta- or polyvanadates V(V) or hypovanadates V(IV). The ease with which this alkaline attack happens increases with increasing levels of oxidation.

This chemical behaviour explains why alkalis or acids may significantly increase the "solubility" of vanadium oxides. Such techniques e.g., solution of vanadium-oxide in aqueous Na_2CO_3, have been used in early toxicological experiments, in order to render the vanadium-oxide more easily administrable [7]. However, this effect is nothing other than a breaking up of the vanadium-oxide lattice by strong chemical reagents, such as OH^- ions, or hydrated protons, and conversion of the

vanadium-oxide into mobile ions, as described above. Such a method should not, therefore, be called "solubilisation", as it does not involve the creation of a simple solution, by normal solvation, in which the character of the solute remains unchanged. Real "solubilisation" only occurs when the ions, atoms or atomic groups remain broadly as they are preformed in the solid state and as they are linked together by chemical bonds in the crystal lattice of the solid substance. In such a case, the lattice energy is overcome by stabilising these ions, or molecules, in discrete form by solvation, i.e. in water: hydration (forming of hydrate shells), not by creating new atomic arrangements or new ions.

In the above-mentioned experiments [7] by "solubilisation" of vanadium by alkalis via a chemical reaction where new substances, namely vanadates, which were different from the original substrate, were formed resp. mobilised, Massmann found peroral acute toxicities for V_2O_5 at rats of "1.04 mg/100 g" which would classify this substance as highly toxic (as being below 25 mg/kg b.w.). In contrast to this, an example of real solubilisation were that of dissolving Barium-chloride ($BaCl_2$) by water to give Ba^{++} and Cl^- ions, which are already present in the $BaCl_2$-lattice and which ions are stabilised in discrete form in the aqueous solution by solvation i.e. in this case by hydration.

Toxicity tests which have been carried out on vanadium oxides, "solubilised" by treatment with acids or alkalis cannot, therefore, reflect the toxicity of unmodified vanadium oxides. They represent the results from chemically different vanadates, or vanadyl compounds, which have formed from vanadium oxides by chemical reaction with acids or alkalis. (In the example given above, this would be similar to the situation, where toxicity tests on $BaCl_2$-solutions indicating high toxicity of barium would not reflect the toxicity of $BaSO_4$, from which the $BaCl_2$ might have been synthesised by suitable chemical reaction. However, $BaSO_4$, because of its low toxicity resulting from its low solubility, is safely used in medicine as a medium for improving contrast in x-ray diagnosis.) Therefore, we suggest that there is a strong requirement to properly distinguish between "solubilisation" by chemical reaction and real solubilisation. The first of these processes involves conversion into a more highly soluble, but chemically different, compound, while the second involves simple solvation.

We would also like to suggest the adoption of a term of the "*toxicological relevancy*" of a substance. This would include not only the toxic effects which the substance in question might cause directly and only by itself, but also all the toxicities and toxic effects which might occur due to interactions with an organism and by any other substances (*toxic intermediates*) which would be chemically and/or biochemically created from the substance in question, but only under and by physiological or cellular conditions.

Thus, a complete description of the toxicological properties of any substance could be obtained.

Consideration of both, the possible chemical reactions and the thermodynamics of these reactions involving vanadium oxides indicates that mobilisation of vanadium, from either V_2O_5 or V_2O_3, under "in vivo" conditions, is likely to be blocked, in a way similar to that of the release of toxic Ba^{++} ions from $BaSO_4$, or its suspensions. The chemical equilibria, together with the relevant thermodynamic data, for acid or alkali attack of V_2O_5, in aqueous media, at ambient temperature, are described by Cotton and Wilkinson [1]:

Commencing with the equilibrium for strong acidic attack, following a chemical reaction like

$$10VO_2^+ + 8H_2O \rightleftharpoons [H_2V_{10}O_{28}]^{4-} + 14H^+, \qquad \lg K = -6.75,$$

a simple calculation (see Appendix A) using the thermodynamic equilibrium constant given in literature ($\lg K = -6.75$), shows, that this is only possible in an aqueous medium at $pH = 1$ (0.1 molar solutions), up to $pH = 2$ (1.7 for 0.01 molar solutions; see Appendix A).

At *pH* approx. 2:

$$[H_2V_{10}O_{28}]^{4-} \rightleftharpoons [HV_{10}O_{28}]^{5-} + H^+, \qquad \lg K = -3.6.$$

In the *pH*-range of approx. 3–5:

$$[HV_{10}O_{28}]^{5-} \rightleftharpoons [V_{10}O_{28}]^{6-} + H^+, \qquad \lg K = -5.8.$$

At "neutrality", i.e. $pH = 6$–8, no such equilibria are calculable, meaning that at such *pH*s no mobilisation of vanadium ions from vanadium oxides is possible, by either (such slight) acid or alkali attack. Consequently, only V_2O_5 (or V_2O_3), with its very low solubility in water (see also Sect. 2), exists. For all practical purposes, under these conditions, its participation in chemical and/or bio-chemical cellular reactions is strongly reduced and, thereore, it behaves mainly like an inert medium over a wide range of conditions.

When considering chemical equilibria, it is important to recognise that for each forward reaction there is a corresponding reverse reaction. Thus, such effects also occur if vanadyl-, or vanadate solutions are neutralised into this range of *pH*. This leads, spontaneously, to polymerisation to more highly condensed polyvanadate-ions and to precipitation of widely inert vanadium oxide. This highlights a significant difference in behaviour between vanadyl- and vanadate solutions compared with chromate solutions.

Following similar logic, it can be shown that alkali attack of V_2O_5, in aqueous solutions, at room temperature, gives:

In the *pH*-range of approx. 9.5–11.5

$$3[VO_3(OH)]^{2-} + 3H^+ \rightleftharpoons [V_3O_9]^{3-} + 3H_2O, \qquad \lg K = 30.7$$

and

$$2[VO_3(OH)]^{2-} + H^+ \rightleftharpoons [V_2O_6(OH)]^{3-} + H_2O, \quad \lg K = 10.6,$$

this can be alternatively described as a base dissociation as follows:

$$3[VO_3(OH)]^{2-} \rightleftharpoons [V_3O_9]^{3-} + 3OH^-$$

and

$$2[VO_3(OH)]^{2-} \rightleftharpoons [V_2O_6(OH)]^{3-} + OH^-.$$

In the *pH*-range of approx. 11.5–12.5,

$$[VO_3(OH)]^{2-} + H^+ \rightleftharpoons [VO_2(OH)_2]^-, \qquad \lg K = 7.7,$$

alternatively described as a base dissociation

$$[VO_3(OH)]^{2-} + H_2O \rightleftharpoons [VO_2(OH)_2]^- + OH^-.$$

In the *pH*-range of approx. 12.5–13.5,

$$VO_4^{3-} + H^+ \rightleftharpoons [VO_3(OH)]^{2-}, \qquad \lg K = 12.6,$$

alternatively described as a base dissociation

$$VO_4^{3-} + H_2O \rightleftharpoons [VO_3(OH)]^{2-} + OH^-.$$

However, substances which are able to create a higher electrical charge density, at the local molecular level, and which have a large number of flexible functional groups, with electrical charges and polarisability, able to initiate multi-dentate nucleophilic or electrophilic attack on vanadium, or on oxygen atoms, in the vanadium oxide lattice [12], might be strong enough to shift such solubilisation equilibria to dissolved and stabilised ions. This is demonstrated in the following sections on physics and in the results dealing with the influence of polycarboxylic polyelectrolytes on the toxicity of vanadium oxides. However, the required high electrical charge density cannot be realised by proteins, or other normal biochemical substances and can only be observed in synthetic polyelectrolytes [13].

On the other hand – as it is calculable thermodynamically and in good accordance with experimental results [14] – although proteins are incapable of realising sufficiently high densities of nucleophilic or electrophilic fully charged functional groups, (as is possible with synthetic polyelectrolytes), they do contain functional groups and units, with electrically strong positive or negative ends of dipole moments. Consequently this polarity enables adsorption of the protein molecules on to the surface of vanadates. Thus, soluble and insoluble proteins, along with the extracellular matrix, must act in the opposite sense to that of such synthetic polyelectrolytes, which have very high densities of charged functional groups. As a result, protein molecules must be capable of contributing to the detoxification of vanadium compounds.

This may be another reason why, depending on whether the tests were carried out "in vivo" or "in vitro", differing degrees of toxicity were observed.

Additionally, as noted in the introduction, the chemical behaviour of vanadate-ions and their equilibria, in an aqueous environment, are characterised by an extreme and spontaneous tendency to form ("on their way towards oxides") highly complex, aggregated, and highly charged poly-ions, for example

$$[V_2O_6(OH)]^{3-}, \quad [V_2O_9]^{3-} \quad \text{and} \quad [H_2V_{10}O_{28}]^{4-}\text{-ions [1].}$$

These poly-ions, as a result of their size and high electrical charge, creating a large hydration-sphere, have a very high virtual diameter, which must be considered when they attempt to penetrate a cellular membrane.

Alberts et al. [15], discussing the permeability of membranes in biological systems, describe the ability of (dissolved) particles to penetrate cellular membranes as strongly decreasing with electrical charge and with size of the particle. They ranked the results of ability to permeate at synthetic lipide-double-layers as models for cellular membranes (reciprocal to virtual diameters measured Dalton-units) for uncharged molecules as follows: water (MW = 18) > oxygen (O_2; MW = 32) > carbondioxide (CO_2; MW = 44) > urea (MW = 60) > glycerine (MW = 92).

They also estimated the coefficients of permeation (in cm/sec) at such membranes as being approximately $2 \cdot 10^{-2}$ for water, compared with $5 \cdot 10^{-5}$ for urea and

glycerine, 10^{-7} for trypthophane and glucose; and for ions: 10^{-10} for chloride and 10^{-12} for sodium ions.

Thus ions tend to have much lower permeability through cellular membranes than other particles. When considering the relatively large, highly charged, polynanadate ions, it would appear to be safe to say that it must be practically impossible for such a particle to penetrate through functioning cellular membranes at reasonable high penetration-rates, respectively to higher concentrations.

This highlights another difference between vanadate and chromate ions, due to the comparatively low tendency for chromate ions to polymerise. Nevertheless – apart from such vanadium compounds, in toxic-active-form i.e. where its reactive centres remain unblocked (i.e. not deactivated – see below) this does not preclude the operation of other mechanisms which would enable vanadium compounds to penetrate cells:

As stated above, proteins do have a significant affinity to be adsorbed on to vanadium-oxide-particles or on to vanadium-compounds by their functional groups and their polarity (dipole moments) and/or by their electron-donator-ability (especially at the amide-N).

Thus they may act as "wrap-around-agents" by the protein-molecule attaching itself to the vanadium, by its functional-groups, and thus allowing the tail of the protein-chain to wrap itself around the vanadium-bearing molecule. It is obvious that such wrapped-in vanadium-compounds, especially if they are not electrically charged and thus do not have a large-diameter-hydration-shell, could be transported into cellular systems even through intact cellular membranes. Such mechanisms might also be a type of "phagisation" etc.

Such mechanisms – being able to act as detoxification mechanisms – may explain the observed rapid and significant increases, or decreases, of vanadium-concentration in blood and its correlated concentration in urine, depending on the level of exposure (according to Syga [16] correlation-coefficients between blood- and urine-concentrations were found to be $r = ((0.54), 0.71, 0.85, 0.87, 0.87,$ and $0.99)$. However as an additional side-effect it should be borne in mind that as a result of the protein attaching itself by its functional groups to the vanadium-atom the one-electron-exchange-ability of that V-atom will be significantly reduced. Consequently a reduction in toxic activity of this atom will be achieved.

Syga [16] describes the "normal" vanadium-concentration for persons with no significant exposure to vanadium, as being in human blood 2.25 µg V/l and in urine $0.8(< 0.5 - 1.9(2.5))$ µg V/l, respectively. For blood, this compares with vanadium-concentrations for persons who have been exposed to vanadium of between 2.5 and 52.4 (average 2.9) µg V/l; for urine, the comparison is shown in Table 1.

This indicates that, in the upper limit, the correlation between exposure-level and the level of vanadium in the urine is not as simple as it is at the lower levels (even after making allowance for the number of sources from which the above data were obtained), indicating that with higher concentrations of vanadium compounds confronting the organism, also other mechanisms of the vanadium-activity must increasingly become of importance.

The lower figure as given in Table 1 led to the suggestion by Koehler [24] and Schaller et al. [25] that 5–8 µg V/l should be accepted as the upper limit in urine for people who had no significant exposure to vanadium. Further, Syga [16] stated that there were no clinical symptoms of vanadium-toxification observed up to levels of

Table 1. Exposure to V_2O_5 and corresponding levels in urine

	Level of exposure (mg V_2O_5-equivalents per m^3)	Level in urine (μg V/l)
Boiler maintenance personnel in generating stations	n.g.	6200–12200 (?) [17]
V-mining-personnel	approx. 58	300 max [18]
	$\leqslant 0.15$	200–500 [19]
Boiler-Cleaning-personnel (masks used?)	approx. 17	70–400 [20]
Labourers in vanadium-processing-plants (masks used?)	0.1–0.3	31.2–46.7 [21]
	n.g.	2.9–200 [16]
	0.2 ± 0.06	130 max [22]
	0.48–2.54	21–259 [23]

n.g. = not given

100 µg V/l in urine. From the above table it would appear that the majority of people exposed to vanadium in the working-environment have a maximum level in the urine of around 200–400 µg V/l [19], corresponding to an exposure to approx. 0.1 mg V_2O_5-equivalents per m^3 air at the working place roughly. It could be of serious interest to try to work out a correlation of mg/m^3 V_2O_5-concentrations and vanadium-concentrations in urine under standardised conditions of exposure and to define by this a scientific-based maximum working-place-concentration (MAK-value) safely below the level where the normal biological detoxification-mechanism (="phase-I-mechanism") is exhausted and consequently no clinical symptoms of vanadium-toxification at long-term (periodic) exposure are observed.*

The available data till now already do appear to be in good agreement with a model, in which the above mentioned detoxification-power of the organism is limited by the availability of such "wrap-around"-proteins and the efficiency of the mechanism of vanadium- excretion via urine. In the situation where this organism's natural detoxification-function is overloaded, two new mechanisms would appear to become of importance:

On the one hand, the effect of "unmasked" vanadium compounds, which although precluded from penetrating intact cellular membranes thus limiting their toxicity may still achieve toxicity via the action of any toxic intermediate (as, e.g., H_2O_2 – see Sect. 5; ="Phase-II-mechanism"). On the other hand, the effect of membrane-eroding reactions which could become more and more significant, leading finally to a break-down of some membranes and opening the way for strong toxic effects (="Phase-III-mechanism"). Such a model seems also to be supported by studies on cinetics of vanadium elimination [38].

Summarising

1. For chemical and thermodynamic reasons (solubility, *pH*-ranges for equilibria to form ions, tendency to polymerise) the risk of obtaining highly toxic effects with vanadates is significantly lower than it is with chromates.

* In preparation

2. Where soluble vanadates are concerned, e.g. ammonium meta-vanadate, within the normal range of *pH* found in intra- or extra-cellular biological environments, there is a tendency for *Chemical Detoxification* to take place, by spontaneous polymerisation, followed by precipitation, to suspensions of undissolved vanadium oxides and by proteins being able to adsorb on to the surface of vanadium-oxide particles, leading to their complex-chemical deactivation and finally also to excretion (*Biochemical Detoxification*).

3. Even if solubilisation is actually achieved, by chemical stabilisation of a soluble vanadate, or by a promoter such as a synthetic, high density, polyelectrolyte, the toxic effects of vanadium ions will be significantly less than those of chromate-ions, due to their low permeability through cellular membranes, resulting from their large diameter (poly-ions) and high electric charge density (charge and hydration-sphere).

4. This does not exclude the possibility of other (i.e. non-vanadate), soluble forms of vanadium from exhibiting toxicity: If the barriers preventing vanadate permeation were reduced and the ability of the 3d-defect-atom vanadium to act as a "one electron exchanger" still existed, lowly charged or non-ionic vanadium containing molecules, of small diameter, would be expected to exhibit similar toxicity to that of chromates. However, any such vanadium compounds able to overcome significantly the barrier presented by an intact cellular-membrane and being in toxic-active-form (i.e. not having blocked their one-electron-exchanging ability as f.e. by any complex with an adsorbed protein i.e. retaining its one-electron-exchange-ability), cannot be usual compounds as normally technically used. Even VCl_3 will show only moderate toxicity similar to vanadates as it is hydrolysed soon to vanadates or V_2O_3 at cellular *pH*s. Such significantly membrane penetrating, active vanadium-compounds must, therefore be considered to be "rather exotic" – like metallo-organic vanadium compounds. Obviously, the ability of the cellular membrane, to act as a barrier, will persist as long as it remains unperforated or unaffected by an erosive attack by, for example, extremely high concentrations of "vanadium acid" (i.e. by the acidity of the vanadium-oxide itself, if present in such high concentrations, that even its weak acidity may "overjump" the buffer-capacity of the extra-cellular environment) and/or by other erosive substances. Such effects, as a result of cellular membrane degradation, may explain why strong toxicity has been observed in personnel at power-stations, where they have been exposed to very high concentrations of vanadium-containing fly-ash, associated with strongly erosive acidic or alkaline contamination and that, after such exposure, vanadium has been found to appear extreme rapidly in very high concentrations in the blood and urine of such personnel (see Table 1) [38, 39].

2. Physics of Vanadium Oxides

V_2O_5 crystallises in ortho rhombic needles (space group D_{2h}^{13} Pmmm $a = 3.563$ Å, $b = 11.51$ Å, $c = 4.369$ Å) with two V_2O_5 molecules in each unit cell [9, 10].

Using x-ray diffraction techniques Bachmann [9, 27] and Byström [9, 28] have estabilshed the structure of the unit cell (Fig. 1) proving that there are two V_2O_5 molecules per unit-cell (see Appendix B).

The atomic-positions in the unit-cell mean that around each vanadium-atom there are four oxygen-atoms arranged in a trigonal pyramid of unequal sides. One

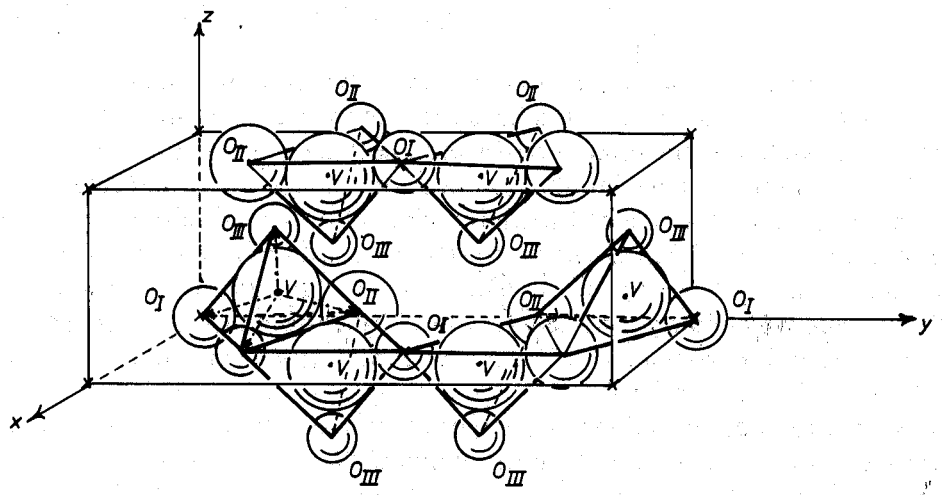

Fig. 1a. Unit-cell of V_4O_{10} ("V_2O_5") with base coordinated V-, O-type-I-, O-type-II- and O-type-III-atom-positions

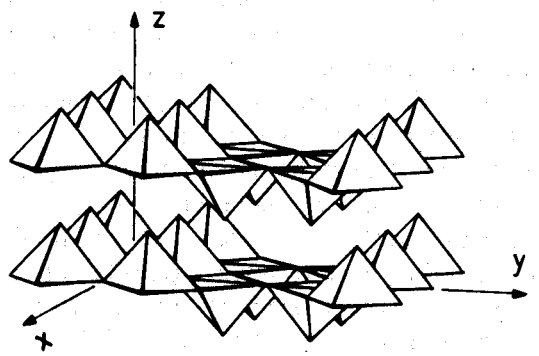

Fig. 1b. Unit-cells with upper and lower trigonal-bipyramids

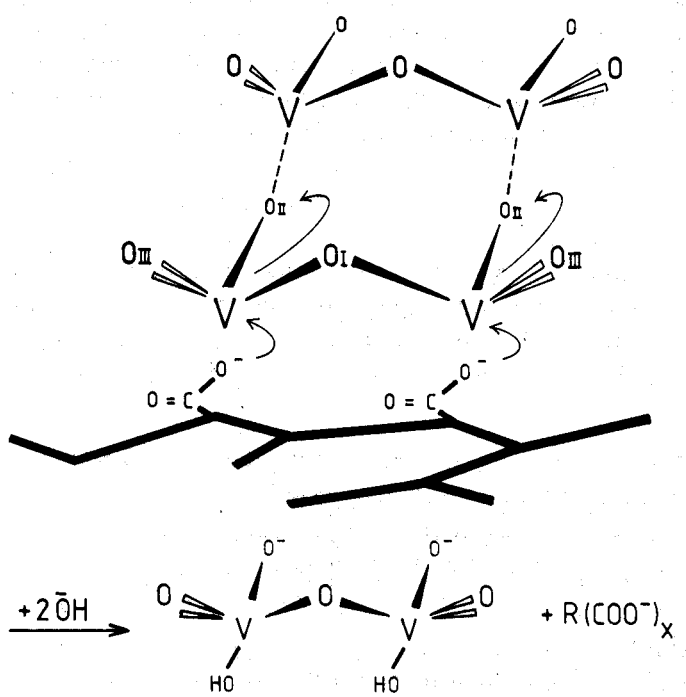

Fig. 1c. Trigonal-V-OI-OII-OIII-pyramid and V_2O_5-double-molecule with possible attack by a "geometrically suitable" polyelectrolyte

corner of this pyramid, that where the type-III-oxygen-atom is situated, is nearer to the vanadium atom ($d = 1.59$ Å). This indicates that there must be a covalent V–O bond, with a high double-bond-character. The distances from the vanadium atom to the type-I and type-$II_{2,3}$ oxygen-atoms ($d = 1.9$ Å) are in good accordance with a normal covalent bonding system, as they fit the sum of the well established covalent radii for vanadium ${}_cr_v = 1.3$ Å plus that of oxygen ${}_cr_o = 0.6$ Å, derived from measurements on other compounds. The only exception within the trigonal pyramid with respect to character of bonding is indicated by the distance from the vanadium-atom to the type-II-oxygen-atom ($d = 2.02$ Å), which indicates ionic-bonding with $r_{o^{2-}} = 1.4$ Å and $r_{v^{5(4)+}} = 0.5$ to 0.6 Å.

Because of the geometric position of the type-III-oxygen-atoms, two of them are located at the nodes of each pair of trigonal pyramids. This results in a network of trigonal pyramids, where each pair of pyramids shares a common edge (Fig. 1a, 1b and 1c*).

As a result of the highly covalent nature of the chemical bonds within the vanadium-oxygen-lattice, it is obvious that water is a very poor solvent for V_2O_5. Furthermore, such a highly covalent compound is amenable to substitution reactions, such as nucleophilic attack, which occur in organic chemistry. Such attack leads to the breakaway of $H_2V_2O_7^{--}$ ions and is aided by both the presence of water and the single vanadium oxygen ionic bond, noted above (Fig. 1c) and below.

The structure of V_2O_5 shown in Fig. 1a and 1c exhibits the possibility of a nucleophilic attack occurring at the vanadium atoms exposed on the faces of the pyramids (positioned there like an "ball of ice-cream" on its cone). Such an attack could occur in the presence of a polyfunctional i.e., polycarboxylic molecule, where the distances between the attacking groups, or atoms, fit the V–V distance in the vanadium oxide lattice, i.e. is geometrically suitable.

In such cases, a break away of $H_2V_2O_7^{--}$ ions is easily understood. It could explain why such special reagents result in a mobilisation effect in macroscopic *pH* environments, with water as a solvent, where equilibrium data would indicate that normal solubilisation would be impossible. Such interactions would, in turn, be expected to lead to an increase in the toxicity of vanadium oxides (see Sect. 5).

3. Technical Vanadium Oxides

With respect to the possible practical contact with vanadium compounds and the toxic effects which might arise, it is important to understand the sources from which technically applied vanadium compounds come. Vanadium compounds from different sources, being generated by different processes, may show significantly different effects as a consequence of:

(a) different crystal shape and size, resulting in a different degree of uptake-ability,
(b) different elubility, resulting from differences in active surface,
(c) contamination with other chemically reactive substances, generated during the processes in which the vanadium compound is formed.

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* With respect to Gmelin the above mentioned denomination has been changed into: *x*, a (H. Bachman [9, 27] → *y*, *b* (this paper); *y*, *b* → *z*, *c*; *z*, *c* → *x*, *a*; OI → OIII; OII → OII; OIII → OI

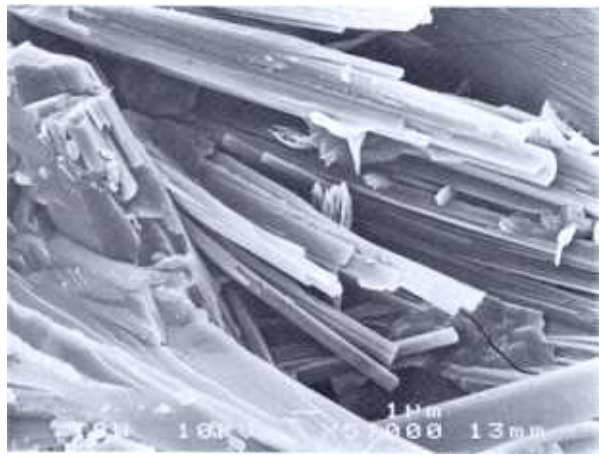

Fig. 2. Precipitated V_2O_5 (× 5000, BET-surface = 3.3 m^2/g)

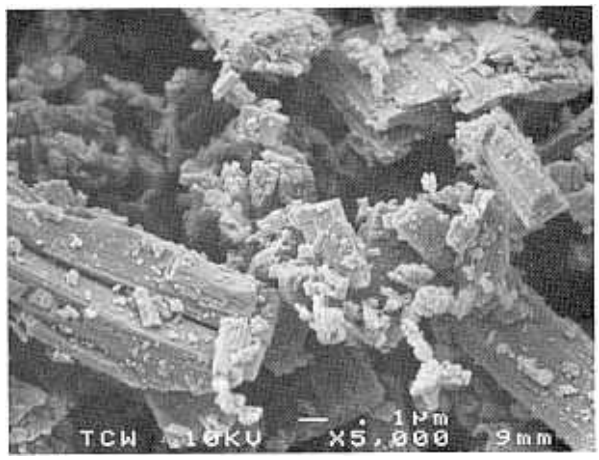

Fig. 3a. Fused V_2O_5: "flakes" × 5000, BET-surface = 2.1 m^2/g)

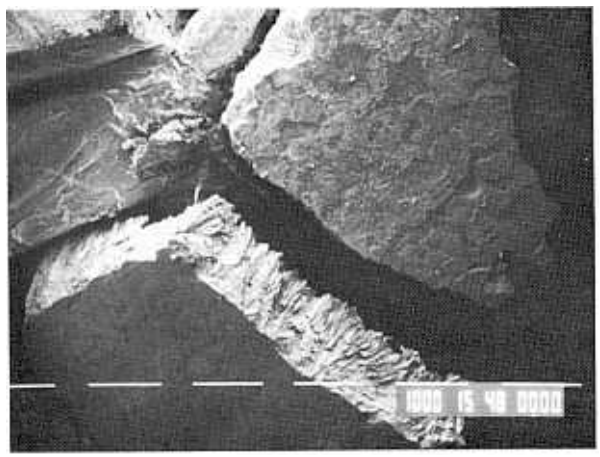

Fig. 3b. Fused V_2O_5: "Flakes" (× 15)

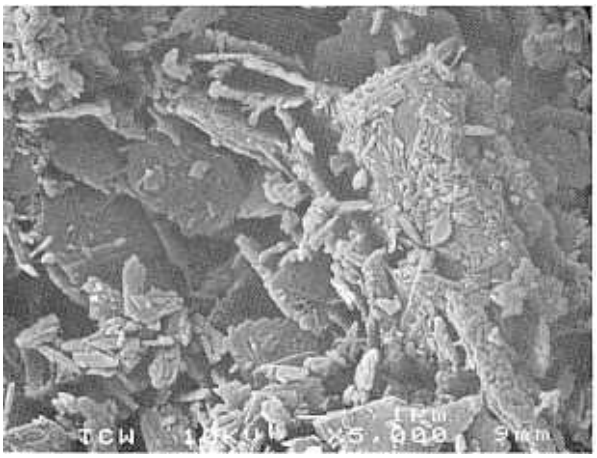

Fig. 4a. V_2O_5 produced by thermal decomposition of APV (× 5000, BET-surface = 3.11 m^2/g)

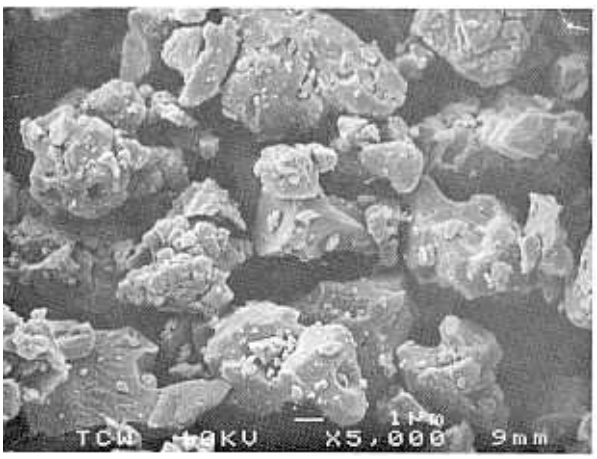

Fig. 4b. V_2O_3 produced by thermal decomposition of APV under reducing conditions (× 5000, BET-surface = 0.52 m^2/g)

Scanning electron microscope examination and identification of vanadium oxide, formed by precipitation from vanadium salts, indicates well shaped, well formed, large crystals of grain size 30–50 μm (Fig. 2).

Even larger crystals and agglomerates are created if the vanadium-oxides is converted by fusing V_2O_5 into the form in which it is normally handled, i.e., flakes (Fig. 3a, b).

Similar, blocky, material results from the production of V_2O_5 or V_2O_3 by thermal decomposition of ammonium polyvanadate (APV) (Fig. 4a, b).

All the scanning-electron macrographs, shown in Figs. 2–4, depict rather large, well-developed and compact blocks of crystals and crystal agglomerates. There are no amorphous or pseudo-amorphous particles and they have the same crystal habit planes. Such particles, because of their size and blocky nature, are not easily inhaled, or incorporated in any other way, provided that they are not dissolved and administered orally, dermally or via an aerosol. In contrast, particles such as fly-ash or carbon black, produced as a result of the combustion-processes in generating stations, have a rather high surface area and small particle size (Fig. 5a, b). Dry airborne dusts containing such particles could be more easily incorporated, especially by inhalation and could exhibit greater toxicity by being easier to eluate according

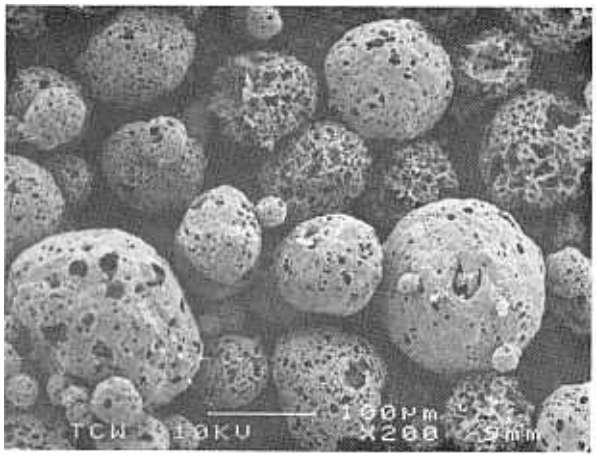

Fig. 5a. Fly-ash particles containing 10.93% V_2O_5 (× 200 BET surface = 55.8 m^2/g)

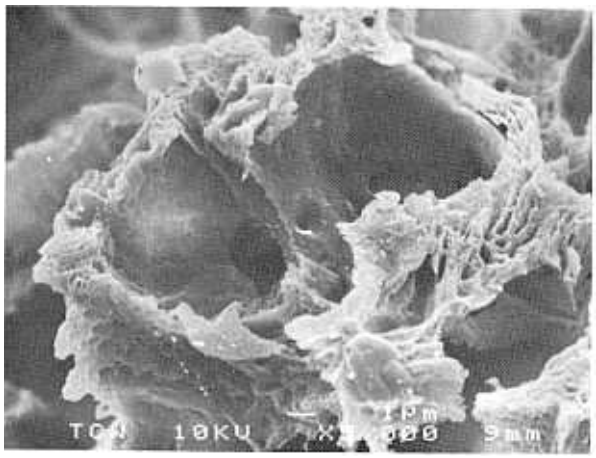

Fig. 5b. Fly-ash particles containing 10.93% V_2O_5 (× 5000, BET surface = 55.8 m^2/g)

to Fick's diffusion laws. Wet particles would of course have a significantly reduced tendency to remain airborne.

Such size and diffusion effects may explain the results of the early toxicological studies of the vanadium-induced bronchitis etc., after inhalation of fly ash and burner dusts at boilers fired with vanadium containing fuels. These toxic effects may be ehanced by the presence of chemically active acid, or alkaline impurities generated during the process of burning fuel. For example, aqueous suspensions, containing 1% by weight, prepared using samples of fly ash from different sources, exhibited values of *pH* which were in range of about 3, or about 9, indicating different types of burnt fuel.

4. Results of Toxicological Studies

All the substances tested by *dermal administration*, i.e. analytical grade, technical precipitated powder and fused V_2O_5, technical powder V_2O_3, AMV and PMV, exhibited very low values of toxicity. Consequently, no LD_{50} values could be determined, even at dosages as high as 2500 mg/kg body weight (2000 mg/kg body weight for AMV and PMV). *Inhalatively administered* V_2O_5 exhibited medium

toxicity, i.e., "harmful", according to EEC Directive 83/467/EEC. LC_{50} data (14d), fell into the range 4.04 mg/l air, for female rats, to 16.19 mg/l air, for male rats for a 4-hour exposure.

AMV and PMV should also be classified as "harmful": For AMV LC_{50} (14d) data fell into the range 2.4 mg/l air, for females and 2.61 mg/l air, for males. For PMV, the range was 1.85 mg/l air, for males, and 4.16 mg/l air, for females for a 4-hour exposure.

The toxicity of V_2O_3 administered by inhalation, appeared to be significantly lower than that of V_2O_5, AMV or PMV. Limit tests, performed at the maximum dust concentration possible in the inhalation chamber (6.65 mg/l), exhibited no toxic symptoms, nor lethality within 14 days.

In the industrial situation the most pertinent results are those from toxicity tests involving oral or inhalative administration. The above "in vivo" results indicate that, as far as these two parameters are concerned, V_2O_5 and the "soluble" compounds AMV and PMV should be classified as "harmful" according to EEC Directive 83/467/EEC. V_2O_3 has a significantly lower toxicity and should be classified as "relatively non toxic" if swallowed. However, the toxicity of V_2O_3 can be significantly increased in the presence of a synthetic polycarboxylate of high carboxyclic density.

Detailed Toxicological Data

The no-effect level, LD_{50} or LC_{50} values for the 7 compounds are given in Tables 2–4.

Following *oral* administration reduced motility, ataxia, muscular hypotonia, dyspnoea, reduced body weight gain and death were observed as toxic signs (see

Table 2. Route of administration: oral

Test substance	Sex	No-effect level in mg/kg b.w.	LD_{50} (14 d) in mg/kg b.w.
V_2O_5 analytical grade, powder	male	215	470
	female	215	467
V_2O_5 technical grade, fused	male	464	716
	female	316	658
V_2O_5 technical grade, powder	male	215	314
	female	147	221
V_2O_3 technical grade, powder	male	4640	8713
	female	4640	5639
V_2O_3/POC	male	918[a]	3289[a]
	female	918[a]	2846[a]
KVO_3	male	100	318
	female	100	314
NH_4VO_3	male	100	218
	female	46.4	141

[a] Values refer to V_2O_3-content

Table 3. Route of administration: dermal

Test substance	Sex	No-effect level in mg/kg b.w.	LD_{50} (14 d) in mg/kg b.w.
V_2O_5 analytical grade, powder	male		
	female	>2500	>2500
V_2O_5 technical grade, fused	male		
	female	>2500	>2500
V_2O_5 technical grade, powder	male		
	female	>2500	>2500
V_2O_3 technical grade, powder	male		
	female	>2500	>2500
KVO_3	male		
	female	>2500	>2500
NH_4VO_3	male		
	female	>2500	>2500

Table 4. Route of administration: inhalative

Test substance	Sex	MMAD* 50 (μm)	No-effect level in mg/l	LC_{50}(14d) in mg/l
V_2O_5 analytical grade,	male		0.90	11.09
powder	female	>2.01–2.44	2.42	4.3
V_2O_5 technical grade,	male		0.97	16.2
fused	female	>1.31–4.04	0.97	4.0
V_2O_5 technical grade,	male		1.62	4.4
powder	female	>2.72–4.15	1.11	2.2
V_2O_3 technical grade,	male			
powder	female	>15.14–19.48	>6.65	>6.65
KVO_3	male		0.52	1.85
	female	>3.36–17.28	0.90	4.16
NH_4VO_3	male		0.72	2.61
	female	>7.22–13.26	1.21	2.43

* Mass medium aerodynamic diameter

Table 2). No signs of toxicity were observed following *dermal* application (see Table 3). Animals died up to 8 days after the oral or *inhalative* exposure (see Table 4). Macrosopic inspection revealed haemorrhagic intestinal mucosa and a dark liver following oral exposure in the animals that died prematurely. Histopathological examination of the lungs of the animals that received inhalative exposure revealed haemorrhage, vascular congestion and oedema in the lungs and bronchopneumonia.

Classification

Following oral administration, the LD_{50}-values for the V_2O_5 test-substances lie between 200 and 2000 mg/kg. Hence according to the EC-guidelines on the classification packaging and labelling of dangerous substances, these compounds have to be labelled as harmful, if swallowed, whereas V_2O_3 requires no labelling, since the compound can be classified as relatively non-toxic if swallowed. The toxicity of V_2O_3 can be significantly increased by administration in combination with a synthetic polycarboxylate with high carboxyl density.

Potassium metavanadate has also to be classified as harmful if swallowed whereas ammonium metavanadate is toxic if swallowed according to the EC-guidelines.

None of the compounds requires labelling for contact with skin as no toxicity was observed following administration of 2500 mg/kg b.w. Based on the results following *inhalative exposure* all vanadium compounds tested should be labelled as harmful by inhalation. The toxicity of V_2O_3 by inhalation exposure appeared to be significantly lower than the other tested vanadium compounds. One reason may be the generally lower toxicity of this compound, as established by oral toxicity.

Interpretation

It may, at first glance, be surprising that AMV, PMV and V_2O_5 have similar, medium toxicities. However, comparison of the *pH*-ranges for stable, soluble vanadium-ions renders understanding of these results possible: In a neutral, especially a neutral buffered environment, similar to that existing in cellular media (intracellular $pH_i = 7.1–7.2$, the normal biological environment in living cells) – as long as not any mobilisers stabilise the soluble form of the vanadium-compound – thermodynamics immediately force such compounds to condense to highly aggregated polyions leading finally to precipitation and the formation of suspensions of vanadium oxides or oxide-hydrates. The toxic behaviour of these suspensions is then very similar to that of the "normal" corresponding oxide (see chemistry of vanadium oxides, above).

As already stated, undissolved aggregates of vanadium oxide crystals will seldom participate in cellular reactions. Additionally, they cannot be easily transported sufficiently close to cells to enable an heterogenous reaction to take place. This is in accordance with the relatively non-toxic, "in vivo", behaviour exhibited by V_2O_3 and in good relationship with the significantly lower toxicity found for fused (flaked) V_2O_5 compared with powdered V_2O_5. Real mobilisation to soluble forms, at least as the first step, is necessary to generate higher toxicity. This can only be achieved by conversion of the vanadium into compounds, complexes or environments which are stable enough, to survive long enough, to realise soluble vanadium, even in neutral and/or cellular media.

However, even this condition of mobilisation is insufficient, on its own, to generate anything other than moderate toxicity. It is also necessary that either the soluble vanadium should be able to penetrate the cell or, as a second order effect, the vanadium compound must be transported to the close proximity of the cell and then generate molecules of another substance (toxicological intermediate), which is uncharged and small enough to permit it to penetrate the cell and, hence, result in toxicity.

To meet the first of these additional conditions, the soluble vanadium should be stable, in a soluble or solubilisable form, and have a cellular membrane penetration diameter sufficiently small to allow significant penetration to take place. To meet the second additional condition, the vanadium could be solubilised and then precipi-

tated as a vanadium-oxide-suspension, the effectiveness of this route depending on the toxicity of the toxicological intermediate, its longevity under intra- and extracellular conditions, and its ability to penetrate cells. Popper, in a recent paper [8], presented results from "in vitro" experiments on V-79 cells from Chinese hamster lungs, pneumocytes type II of Fisher – 344 rat lungs and alveolarmacrophages. He found a high level of toxicity for all the vanadium compounds tested, i.e., 0.05 μM for V_2O_5; 0.01 μM for V_2O_3, which was found to be more toxic than V_2O_5, which he claimed to be significant, despite the known lower solubility of V_2O_5 and despite the fact that Popper himself states in his paper that toxicities of vanadium-compounds were in dependance of their solubilities.

Popper also proposed what is probably the main bio-chemical mechanism, to explain the toxicity of vanadium compounds: His results excluded the possibility of simple heavy-metal-toxicity, such as by disturbing or blocking the normal detoxifying enzyme systems. *EDTA* did not exhibit any antitoxic effects while, in contrast, dithiotreitol (*DTT*) and a water soluble derivative of tokopherole (*TROLOX*), (both known to be classified chemically by their function as so-called radical-"regulators" or "catchers"), acted as strong, toxicity-reducing agents. This makes it very likely that the main mechanism of vanadium toxicity is based on an increase in the formation of, or an increase in concentration of intracellular radicals – especially oxygen radicals ($^{\cdot}OH$). Further, the facts that only *DTT* (which is able ot penetrate cellular membranes and is metabolised in the cell to glutathion) is able to detoxify, while glutathion (in methylated or reductive treated form) being only able to act outside the cell in these experiments, was shown to be ineffective. This proves that oxygen radicals, which might have been generated outside of the cells (e.g., by heterogeneous reaction on the surface of the vanadium oxide particles in suspension), do not significantly contribute to toxicity. The critical substances which create the oxygen-radicals must have been transported into the cell, at an earlier stage. This confirms our observations and deduction that as long as vanadium compounds are unable to penetrate cells, they are not highly toxic.

Nevertheless there are also known additional other, very likely effects of Vanadium-toxicity. Symansky [6] describes the irritating effect of V on the mucous membranes of the respiratory tract and on the conjunctiva. This were, almost certainly, a result of the erosive effect of "vanadium-acid" in high concentrations or V_2O_5-accompanying aggressive contaminations. Mountain et al. [32, 33] report an effect on cysteine- and cystine-metabolism. This may explain the reduction of the cystine-content in finger-nails from approx. 9.9 mg/100 mg for "normal" (i.e. unexposed) persons to approx. 8.9 mg/100 mg for labourers who have suffered a vanadium exposition (Syga [16]). Mascitelli et al. [34, 35] found an influence to coenzyme A – possibly as a consequence of the a. m. effect on cysteine and with this on the formation of its decarboxylation-product, cysteamine. (Cysteamine is known to be a precursor in the biosynthesis of coencyme A, which it forms together with pantothene-acid via pantethein as the "tail" and adenosine-3'mono-5'diphosphate.) Curran [36] observed that vanadium caused a decrease in cholesterol-synthesis, probably as a consequence of an influence on the fatty-acids-coencyme A-metabolism. Missenard et al. [37] demonstrate an impairment of hem-synthesis due to an influence on the protoporphyrin-metabolism, etc. All effects – from a chemical point of view – being understandable as the consequence of the chemical affinity (or reactivity) of $^{\cdot}OH$-radicals against –SH-groups (or –S–S-groups as their precursors); exactly as it was biochemically well proven by Popper when he demonstrated the (re)activity of *DTT* against these $^{\cdot}OH$-radicals.

Examination of the details of the mechanism suggested by Popper, indicates that the possibility of either vanadium oxide, or vanadate ions, causing high toxicity by generating oxygen radicals outside the cell is practically excluded.

This point of view is supported by the fact that there is not found any indication of a detoxifying effect from the enzymes catalase, or superoxiddismutase: Both of these enzymes would destroy ·OH-radicals, but are unable to penetrate cells. If oxygen radicals were formed in appreciable concentrations outside the cell, they would also be immediately detoxified and, until deactivated, would destroy cellular membranes and thus allow those enzymes to enter the cell.

This suggests the following alternatives:

(a) After (if) penetrating the cell, dissolved vanadium compounds generate oxygen radicals in the cell; or
(b) a precursor of the oxygen radicals is formed outside the cell; such precursor being small, chemically stable and being uncharged. It would be able, to rapidly penetrate the cellular membrane, be able to survive outside the cell until its penetration (to escape from the ·OH-radical-destroying enzymes catalase, or superoxiddismutase) and be chemically designed to break down into toxicity-creating ·OH-radicals. It should also refrain from acting as a main-destroyer of the cellular membrane.

Alternative (a) is unlikely in the case of vanadium oxide or vanadates. Vanadate ions would, depending of *pH*, immediately polymerise and then precipitate as vanadium oxide. Additionally, they are large, have a high virtual diameter, are highly charged and, consequently, show poor cell permeability. In the case of alternative (b), H_2O_2 molecules could act as such precursors (see Fig. 6). Such molecules could be generated outside the cell by vanadates, or vanadium-oxide-hydrates located there, for example by being transported there in the form of a solubilised vanadium-compound and precipitated there. As a consequence of the ability of vanadium-oxides to adsorb proteins or to be adsorbed at proteins, indicating that they also would have a tendency to be adsorbed at the outer side of the cellular-membranes, they could tend to be located sufficiently close to the outer surface of the cellular membrane. Thus they could be near enough to enable an H_2O_2-molecule, created by them, to survive the short distance from the point of its generation to the inner of the cell, penetrating any cellular membrane in its path. An attack by catalase or superoxidismutase would be primarily directed at ·OH-radicals, formed for example, by the decomposition of H_2O_2-molecules, so that a H_2O_2-molecule itself could escape them. Therefore, whole (i.e. not decomposed) H_2O_2 molecules could possibly survive until they had penetrated the cell. Furthermore, the physico-chemical properties and mobility of vanadates, as compared to vanadium oxides, support the interpretation that, in comparing these two types, vanadates, are the more likely source of such H_2O_2 molecules and, consequently, of ·OH-radicals: As already noted, as a result of their electron configuration (V(O): $3d^3 4s^2$; V(V): $3d^0 4s^0 \leftrightarrow$ V(IV): $3d^1 4s^0$), facilitating V(V) $\leftrightarrow$ V(IV) transitions, vanadates are well known as "one electron catalysts". (This is similar to transitions in the Fe^{+++}/Fe^{++}

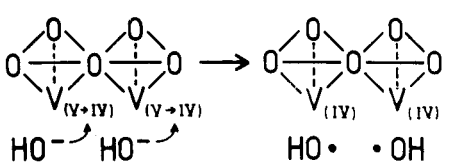

Fig. 6. V_2O_5–H_2O_2 reaction scheme

system, where the $Fe-H_2O_2$-system (for example at Ruff's aldose-degradation) is known as "classic" ·OH-radicals-donor-reagent-system. It is also similar to the OsO_4-reagent for cis-hydroxylation of alkenes to glycols. But in contrast to V, in these cases complexing agents (such as *EDTA*) would (for reasons associated with the ligand-field- and molecular-orbital-energetics [14]) easily fill up the d shell of Fe^{++} and strongly reduce its "one-electron-transfer"-capability by preferentially stabilising the Fe^{++} state.

Similar "one-electron-transfer" could be introduced by dissolved vanadium, or vanadyl cations, capable of undergoing $V(IV) \leftrightarrow V(V)$ transitions. On the other hand, in solid vanadium oxide particles, as a result of crystal physics, the electron-band-structure does not promote such catalytic activity. Its behaviour is similar to that of Fe-ions masked by complexing agents. This further reflects the real significant difference in "in vivo"-toxicity observed for vanadium oxides (being lower) and vanadates.

All of the above physico-chemical facts help illustrate why Popper, in his work using "in vitro"-techniques, found soluble vanadium compounds, such as VCl_3 to be "highly toxic", while the present "in vivo" test results show vanadium oxides to be merely "harmful". Popper's work is also contradictory in stating that the toxicity of vanadium compounds depends on their solubility: While he finds the highly soluble VCl_3 as being of high toxicity [8], but also the practically insoluble oxides V_2O_5, V_2O_4 and V_2O_3 as well. This is further compounded by the fact that he observed V_2O_3 to be significantly more toxic than V_2O_5, despite the known lower solubility of V_2O_3.

The discrepancies in toxicities found for V_2O_3, by Popper's "in vitro"-tests and by our in-vivo-experiments might be explained if there was a mobilising agent present in Popper's work. (It should be noted that "in vivo", such an effect does not appear to happen, yielding results which are more easily explained and in accordance with the substances' solubilities). In particular, V_2O_3 really exhibited "in vivo" definitely, significant lower toxicity than V_2O_5.

The possible mechanism of such an influence of a mobilising agent is known, and described above under Synthetic polyeletrolytes of Sect. 1 and nucleophilic attack of a V–O trigonal bipyramid of Sect. 2.

In Popper's work, such a synthetic polyelectrolyte was present as a result of the cell culture membrane used. These membranes, manufactured by Costar, Mass., USA and given the trade name Transwell, are synthetic polycarbonate membranes, which have been negatively ionised by carboxylate groups. They are, therefore, synthetic, polycarboxylic polyelectrolytes. The fact that such mobilisation can result in a significant increase in toxicity, even "in vivo", has been demonstrated in the present work. In the test series, in which equivalent mixtures of V_2O_3 and a synthetic polycarboxylate were administered orally, (even without adjustment of the selected polyelectrolyte to the geometry of the V_2O_3 molecule), the toxicity was doubled.

In addition to the effect which the presence of the synthetic polycarboxylic polyelectrolyte may have had on Popper's results, there is one further factor to be considered: If it is assumed that it really is H_2O_2 which is the toxic intermediate, it is the stabilising effect in decomposing environments, by the presence of complexes of such polycarboxylic polyelectrolytes with magnesium ions [14]. Such Mg^{++} ions may be present in the cell-culture-cocktail. This could have been an additional feature which assisted in permitting the H_2O_2 molecules to survive long enough, to the enable them to build up a sufficiently large concentration and to create inside the cell highly toxic effects after having penetrated through the cell membranes. As a side-effect: by being stabilised against early decomposition into ·OH-radicals, they also would exhibit a lower degree of aggression against the proteins and lipids making up the cellular membrane. However, once inside the cell, in the absence of any stabilising Mg^{++}-polyelectrolyte complex, such H_2O_2 molecules would behave as normal: They would decompose rapidly into highly aggressive ·OH-radicals, in high concentrations and with resulting corresponding toxicity.

Experimental Part

Materials

The vanadium oxides and chemicals investigated in the present work included: V_2O_5 analytical grade powder, V_2O_5 technical grade powder, V_2O_5 technical grade fused, V_2O_3 technical grade powder, KVO_3 potassium metavanadate, and NH_4VO_3 ammonium metavanadate. V_2O_5 analytical grade powder and the vanadates were provided by Gesellschaft für Elektrometallurgie GmbH., Nürnberg, Germany. The technical grade oxides were provided by Treibacher Chemische Werke AG., Treibach, Austria.

In addition, a 40% solution of a polycarboxylate (POC), with high carboxylic density and V_2O_3, at a ratio of 1:1 (carboxylic groups: V atoms), was prepared. The POC was delivered by Österreichische Chemische Werke Ges.m.b.H., Weissenstein, Austria, and had a molecular weight of 6000 (Na form) and an equivalent weight of 100.4. It contained 90 mol% carboxylate groups in Na form and 10 mol% OH groups in primary alcoholic form ($-CH_2OH$).

Animals

The animals used were Sprague–Dawley rats (Lippische Versuchstierzucht Hagemann GmbH, Extertal, Germany) weighing between 250 g and 290 g, slightly heavier rats were used for the inhalation studies. Rats were provided with standard rat diet and water and libitum. The diet was withdrawn approx. 16 hours before application. At the onset of the main experiment animals were randomly divided into group of 5.

Toxicological Methods

Oral Toxicity. The test substance was suspended in 0.8% aqueous hydroxypropyl-methyl-cellulose gel and administered by gavage. The volume of application was 20 ml/kg b.w.

Dermal Toxicity. The hair on the site of application was clipped with hair-clippers, without causing injury, approximately 24 hours before application. The site was situated on the animal's back between the fore and hind extremities and had an area of approx. 5 × 6 cm (= 1/10 body surface). The test substance was applied to 8 layers of gauze and then to the application site. The patch was covered with a plastic sheet and secured with adhesive plaster. Exposure time was 24 hours. At the end of the exposure period, residual substance was removed with tepid tap water. The test substances was suspended in 0.8% aqueous hydroxypropyl-methyl-cellulose gel and administered at an application volume of 20 ml/kg b.w.

Inhalation Toxicity. The study was carried out using a dynamic inhalation apparatus with a nose only exposure of the animals according to Kimmerle and Trepper. The apparatus consists of a cylindrical exposure chamber (volume 40 l) which holds a maximum of 20 animals in pyrex tubes at the edge of the chamber in a radial position.

The dust was generated with a dust generator and dosing apparatus. The generator was fed with compressed air from a compressor. At the bottom of the exposure chamber the air was sucked off at a similar rate as created by the dust generator, in order to produce a homogeneous distribution in the exposure chamber. At least 12 air changes per hour were carried out. Air-flow meters were used to control the constant supply of compressed air and vacuum. Flow rates were checked at least once/hour and corrected if necessary. Exposure time was 4 hours.

Analysis of the Particle Size Distribution. An analysis of the particle size distribution was carried out twice during the exposure period using a cascade impactor according to May [29].

The dust from the exposure chamber was sucked through the cascade impactor for 1.5 to 5 min at a constant flow rate of 5 l/min. The slides covered with adhesive tape were removed from the impactor

and were weighted on an analytical balance (precision 10 μg). The mass median aerodynamic diameter (MMAD) was estimated by means of nonlinear regression analysis (Litchfield and Wilcoxon [30]).

Analysis of the Dust Concentration. The dust concentration in the inhalation chamber was measured with an air sample filter (Sartorius Minisart SM 17598). Dust samples were taken during the first half and during the second half of the exposure. For that purpose, Minisart SM 17598 filters were placed close to the animals' nose and sucked through with a constant flow of air of 300 l/h for 1 to 3 min. The filters were weighed before and after sampling. Concentration of the test substance in the air was calculated as mg/l.

Clinical Evaluation. Following administration, observations were made and recorded systematically with individual records being maintained for each animal. Observations were performed immediately, 5, 15, 30 and 60 min, as well as 3 h, 6 h and 24 h after administration. Then twice daily for a period of 14 days. The time of death was recorded as precisely as possible. Individual body weights were recorded before administration of the substance, thereafter in weekly intervals up to the end of the study, and at death. At the end of the experiments all surviving animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. Autopsy and macroscopic inspection of rats which died prematurely were carried out as soon as possible after exitus. The lungs of the animals used for inhalative exposure were evaluated histopathologically.

Statistical Evaluation. The LD_{50} was calculated according to Finney [31].

Acknowledgements

We would like to thank Prof. *H. Popper* (Anatom. bakteriolog. Abteilung der Karl-Franzens-Universität, A-8036 Graz) for the scientific discussion we have had.

For many critical remarks and contributions as to where results described in literature needed interpretations from the point of view of our findings and mechanistic results, we have to thank Dr. *Roller* from GfE (D-90431 Nürnberg).

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Appendix A

Example of Calculation for Strong Acidic Attack ($\lg K = -6.75$)

The equality of the sum of the Gibb's free energies ($G \equiv H - TS$; german: Freie Entalpien) of reactants and reaction-products (concentrations in moles/liter are indicated in { }-brackets) reads:

$$10\,{}_0G_{vo_2^+} + 10\,RT\ln\{[VO_2]^+\} + 8G_{H_2O} = {}_0G_{[H_2V_{10}O_{28}]^{4-}} + RT\ln\{[H_2V_{10}O_{28}]^{4-}\} + 14\,{}_0G_{H^+} + 14\,RT\ln\{H^+\};$$

after reformulation this gives

$$\ln\frac{\{[H_2V_{10}O_{28}]^{4-}\}\cdot\{H^+\}^{14}}{\{[VO_2]^+\}^{10}} = \frac{10\,{}_0G_{[VO_2]^+} + 8G_{H_2O} - {}_0G_{[H_2V_{10}O_{28}]^{4-}} - 14\,{}_0G_{H^+}}{RT},$$

or after delogarithmation (and assuming, that the concentration of the solvent ($= H_2O$) can be assumed to be approximately constant → i.e. the term $8G_{H_2O}$ may be included into the equilibrium-constant K; – as in such cases it is usually assumed if such K-values are given in physico-chemical-literature [11]) resulting in the well-known law-of-mass-action

$$\frac{\{[H_2V_{10}O_{28}]^{4-}\}\cdot\{H^+\}^{14}}{\{[VO_2]^+\}^{10}} = \frac{\Delta_0G}{RT} \quad \text{with} \quad \ln K = -\frac{\Delta_0G}{RT}$$

(where $\Delta_0G = {}_0G_{[H_2V_{10}O_{28}]^{4-}} + 14\,{}_0G_{H^+} - 10\,{}_0G_{[VO_2]^+} - 8G_{H_2O}$).

Taking into account that, during the "forward"-reaction, for each $[H_2V_{10}O_{28}]^{4-}$-ion generated by hydrolysis of a $[VO_2]^+$-ion, 14 H^+-ions also are cogenerated, so that it can be assumed that the concentration of H^+-ions in the solution is approximately 14-times the concentration of the $[H_2V_{14}O_{28}]^{4-}$-ions (the start-concentration of H^+ in water, which is approx. 10^{-7} mol/l may be neglected as it were only affecting the 7th number behind the comma), it follows

$$\{[H_2V_{10}O_{28}]^{4-}\} = \frac{1}{14} \cdot \{H^+\},$$

so that the law-of-mass-action now gives

$$\frac{1}{14}\{H^+\}^{15} = K \cdot \{[VO_2]^+\}^{10}.$$

Thus, using the value of the equilibrium constant obtained from literature, as shown above, the concentration of H^+-ions which are available in such a reaction at equilibrium, can be calculated as

$$\{H^+\}^{15} = 14 \cdot 10^{-6.75} \cdot \{[VO_2]^+\}^{10},$$

which can be expressed in logarithmic form,

$$\lg\{H^+\} = \frac{1}{15} \cdot (\lg 14 - 6.75 + 10 \cdot \lg\{[VO_2]^+\})$$

and

$$pH \approx -\lg\{H^+\} = -\frac{1}{15} \cdot (1.15 - 6.75) - 0.67 \cdot \lg\{[VO_2]^+\},$$

i.e.,

$$pH \approx 0.37 - 0.67 \cdot \lg\{[VO_2]^+\}.$$

Assuming that there is a reasonable (i.e. significant) concentration of the vanadyl-cation (VO_2^+) present (e.g. $\{VO_2{}^+\} = 0.1$ to 0.01 mol/liter minimum) this is only possible in an aqueous medium at $pH = 1$ (0.1 molar solutions), up to $pH = 2$ (1.7 for 0.01 molar solutions). If lower vanadium-concentrations are chosen, this obviously results in a higher level of pH, where the vanadyl-concentrations in the equilibrium becomes insignificant meaning that, at such higher levels of pH, other equilibria must control the system as described below (e.g. at pH approx. 2), with the equilibrium:

$$[H_2V_{10}O_{28}]^{4-} \leftrightharpoons [HV_{10}O_{28}]^{5-} + H^+, \qquad \lg K = -3.6,$$

etc.

Appendix B

Unit-Cell-Structure of V_2O_5

Using x-ray diffraction techniques Bachmann [9, 27] and Byström [9, 28] have established the structure of the unit cell (Fig. 1).

Within the lattice, the vanadium atoms are situated in two positions in the (y, z) plane ($(0/y/z)$ and $(0/-y/z)$, with $y = 0.1487$, $z = 0.1086$), resulting in 2 times 1/2 → one vanadium atom, on each of the rear and facial planes of the unit cell, which is shared by the adjacent cell, giving two vanadium atoms in total. There are further two positions, within the unit cell, ($(0.5/0.5 + y/-z)$ and $(0.5/0.5 - y - z)$), counting as 2 × 1 vanadium atoms, giving a total of four vanadium atoms per unit cell: two in planes and two in space.

The type I-oxygen-atoms are situated at each corner of the unit cell and in the centre of both: the top and bottom x, y plane. The (0/0/0) position gives 8 × 1/8 oxygen-atoms and the (0.5/0.5/0) position gives 2 × 1/2 oxygen-atoms, a total of two type I-oxygen-atoms. The type II-oxygen-atoms are situated

slightly above and below the bottom plane at $(0/y/z)$, $(0/-y/z)$, $(0.5/0.5+y/-z)$ and $(0.5/0.5-y/-z)$ with $y = 0.32$ Å and $z = 0.003$ Å, a total of four type-II-oxygen-atoms. The type -III-oxygen-atoms are situated at $(0/y/z)$ and $(0/-y/z)$, with $y = 0.146$ and $z = 0.4713$. Thus two of the type-III-oxygen-atoms are situated in the rear (i.e. the y,z-) and two in the facial (y,z-) planes, giving 4 times $1/2 = 2$ type-III-oxygen-atoms. A further two type-III-oxygen-atoms are located at $(0.5/0.5+y/-z)$ and at $(0.5/0.5-y/-z)$, giving totally four type-III-oxygen-atoms. Thus, the sum of the oxygen-atoms in the unit cell is $2+4+4=10$, proving that there are two V_2O_5 molecules per unit-cell.

Appendix C

Summary of Experimental Data Received After Oral Administration

V_2O_5:	LD_{50} (14d) from (minimum) 221.1 mg/kg b.w. (for V_2O_5 techn. powder; female rats), to (maximum) 715.7 mg/kg b.w. (for V_2O_5 techn.fused; male rats).
V_2O_3:	LD_{50} (14d) 5639.0 mg/kg b.w. (female rats), resp. 8713.1 mg/kg b.w. (male rats).
V_2O_3/POC:	LD_{50} (14d) 2846 mg/kg b.w. (female rats), resp. 3289 mg/kg b.w. (male rats).
POC:	LD_{50} (14d) approx. 38740 mg/kg b.w.
AMV(NH_4VO_3):	LD_{50} (14d) 141.4 mg/kg b.w. (female rats, resp. 218.1 mg/kg b.w. (male rats).
PMV(KVO_3):	LD_{50} (14d) 313.8 mg/kg b.w. (female rats), resp. 317.9 mg/kg b.w. (male rats).

In the industrial situation most pertinent results are those from toxicity tests involving oral or inhalative administration. The above "in vivo" results indicate that, as far as these two parameters are concerned, V_2O_5 and the "soluble" compounds AMV and PMV should be classified as "harmful" according to EEC Directive 83/467/EEC. V_2O_3 has a significantly lower toxicity and should be classified as "relatively non toxic" if swallowed. However, the toxicity of V_2O_3 can be significantly increased in the presence of a synthetic polycarboxylate of high carboxyclic density.

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