July 14, 2021 IRIS Public Science Meeting

Vanadium and Compounds (Inhalation Exposure) IRIS Assessment Plan (IAP)

Comments on Issues #3 pertain to consideration in interpreting nonneoplastic lesions in the upper and lower respiratory tract and alveolar/bronchiolar neoplasms in rodents

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Problem to be Assessed

The following key science issues were identified on the basis of the preliminary literature survey results (see Section 2.3.1) and review of past assessments on inhalation exposure to vanadium and compounds (see Section 2.1).

Issue #1 relates to issues surrounding chemical speciation of vanadium,

Issues #2 and #3 pertain to consideration in interpreting nonneoplastic lesions in the upper and lower respiratory tract and alveolar/bronchiolar neoplasms in rodents,

Issue #4 pertains to evaluating the MOA information relevant to potential carcinogenicity.

Issues identified in U.S. EPA. ORD Staff Handbook for Developing IRIS Assessments (Public Comment Draft, Nov 2020). U.S. EPA Office of Research and Development, Washington, DC, EPA/600/R-20/137, 2020

Problem to be Assessed

Issues #2 and #3 pertain to consideration of biologically observed effects. At the center of both issues is **what is the compound in the treatments** and how does it **interchange in the biological system.**

Issue #2 – non-carcinogenic

The 2-year NTP (2002) study reports **increasing incidences of nonneoplastic lesions** in the upper and lower respiratory tract of rats and mice (both sexes) **with increasing V_2O_5** exposure. All V_2O_5 exposure groups had leasions **highly elevated compared to controls**.

Issue #3 – Carcinogenic

The NTP (2002) study also reports that **tumor responses (alveolar/bronchiolar neoplasms) in male and female mice were highly elevated** at all concentrations of vanadium pentoxide exposure: 70–80% increased incidence at the lowest tested vanadium concentration; control incidence in male mice was high (44%), but background incidence in females was very low (2%).

In summary, aspects of the rodent leasons and tumor data noted above and the uncertainties will be considered in the assessment.

I will be particular focus on what species are present under the studies

Key points from Issue #1 relevant for #2 and #3

Science Issue from #1 and needed:

Specific to V_2O_5

- **What form** of V_2O_5 (or V_4O_{10}) is used for treatment – **from solid or solution**?
- Methods used for **inhalation studies**, **aerosolizing vanadium pentoxide** (or other vanadium compound) from solution, **rather than exposure to vanadium as a dust**.

General

- Consideration of **vanadium speciation under physiological conditions**
- Important parameters are **pH, concentration, and redox potential of conditions**
- **Critical because different species have different biological activities**
- **Conversion between vanadium oxidation state *(should include "species")* in the rodent**

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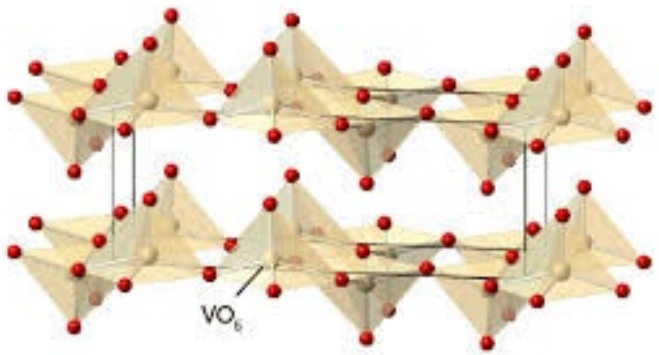
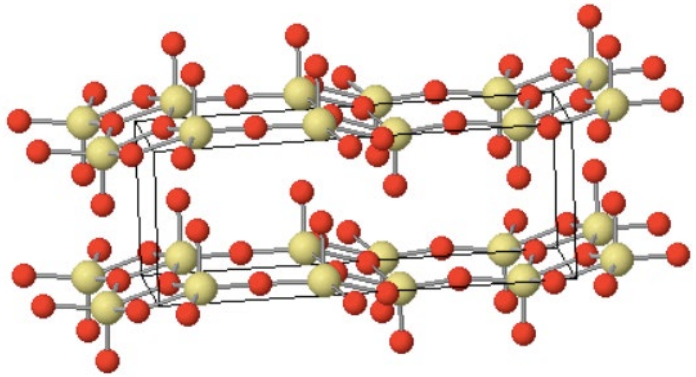
Structure of V_2O_5 and $V_{10}O_{28}^{6-}$ (1c)

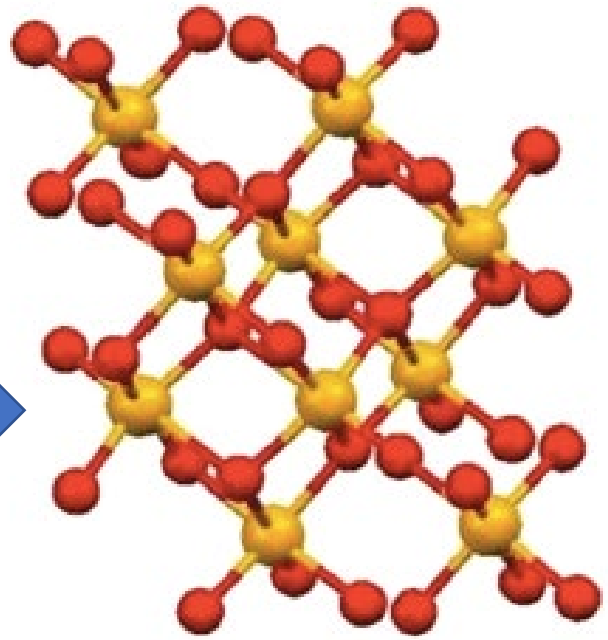


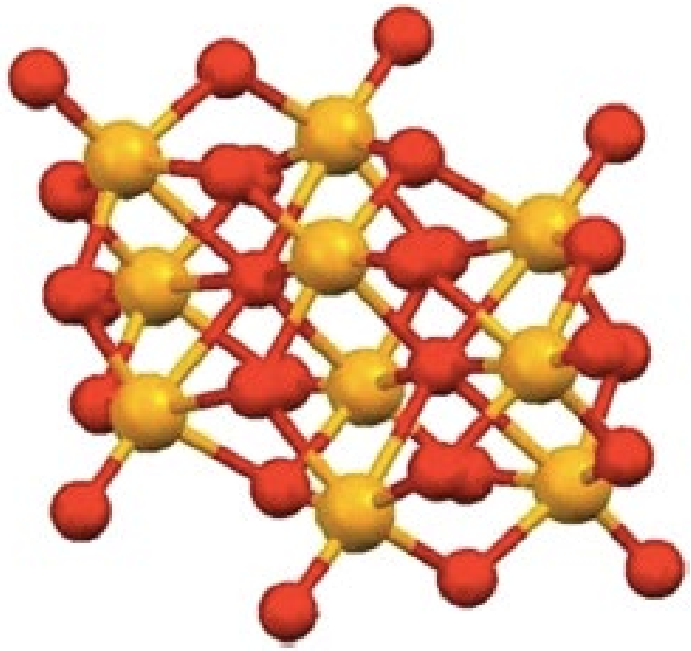


V_2O_5 - Solid state
Nick Greeves, Creative Comments Lincence

V_2O_5 have been modeled by $V_{10}O_{28}^{6-}$

V_2O_5





The partial structure for **V_2O_5 sheet** is shown (left). The structure for the **discrete anion (V_{10})** is shown (right). While V_2O_5 falls apart in solution, the discrete V_{10} anion retains its structure upon dissolution.

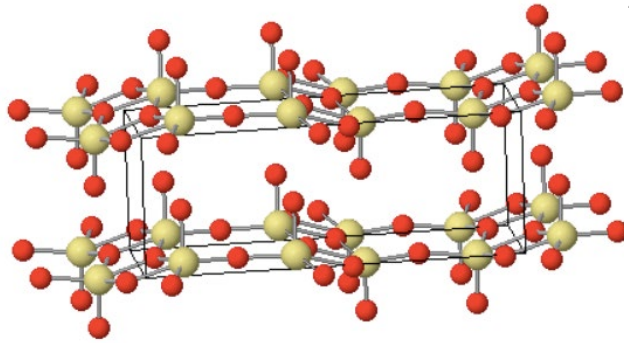
V_{10} used a s model for V_2O_5 Al-Qatati et al. *Dalton Trans.*, 2013, 42, 11912–11920

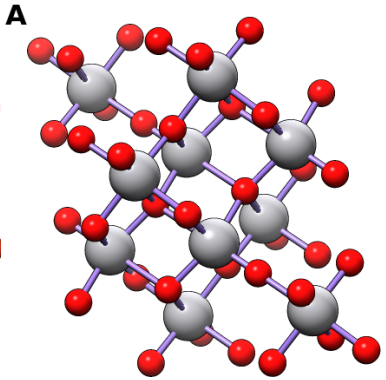
Comparing solid V_2O_5 with $V_{10}O_{28}^{6-}$ (Vanadium(V)) 1a)

V_2O_5

Pure V_2O_5 (orange & brown) – China 99.9%

V_2O_5 - catalyst



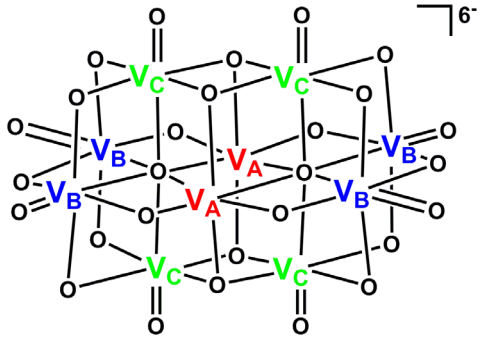


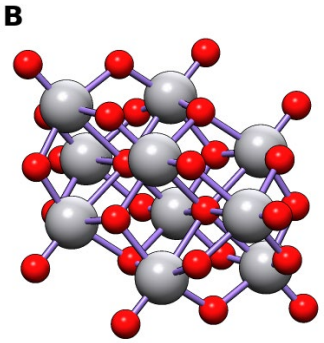
Sheets - polymers

$V_{10}O_{28}^{6-}$

$K_3Na_3V_{10}O_{28}$

$(NH_4)_6V_{10}O_{28}$

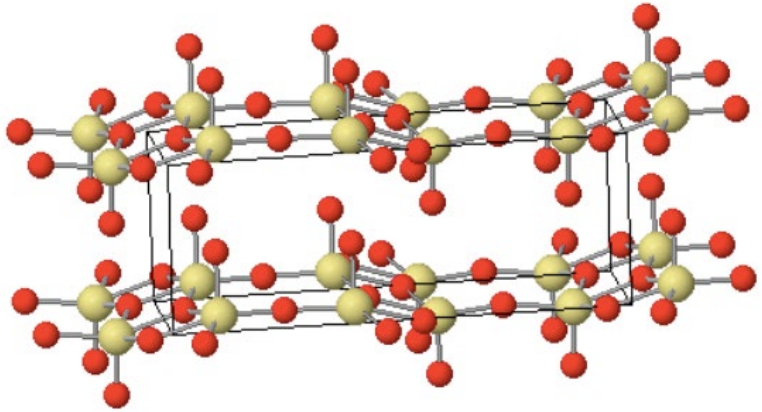




Discrete molecule

How does this translate to the biological experiments?(1c)

- Airborne V_2O_5 **are delivered in aerosols**
- What is speciation in aerosols?
- How are aerosol prepared? From solid or from solution?



V_2O_5 - Solid state
Nick Greeves, Creative Comments Lincence

Fragmenting sheets → What are the speciation in these aerosols?

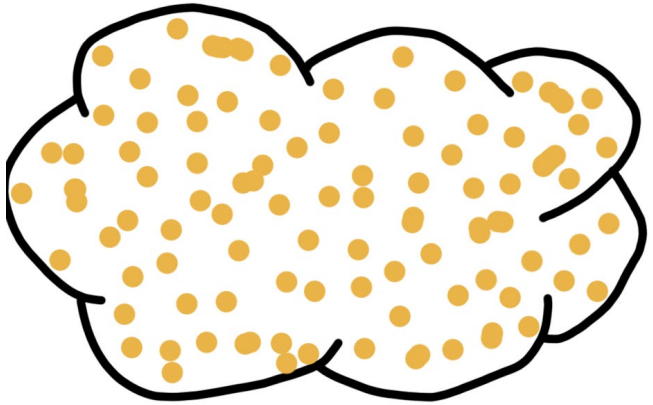
Dissolution → Various protonation states of V_{10}
So what information is available to do accessment?
Still imortant: pH, concentration, and redox potential

Speciation Studies in Confined Spaces

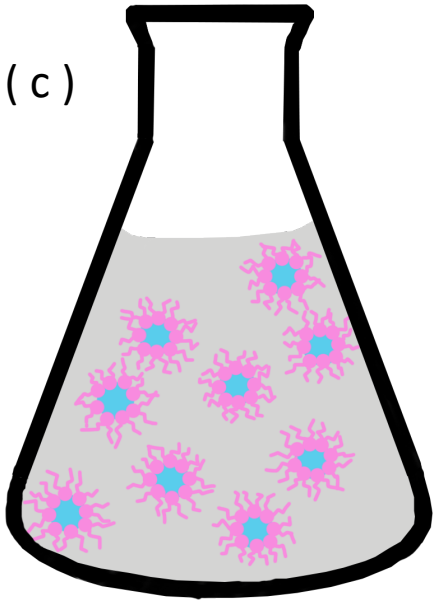
Aerosol definition: A colloidal suspension of particles dispersed in air or gas.

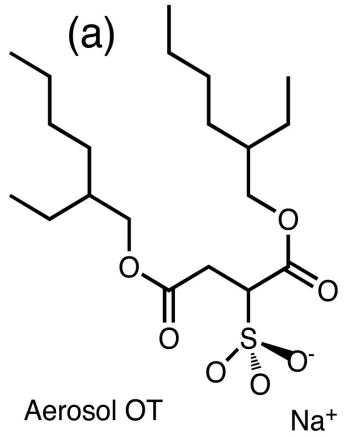
Related System: A colloidal suspension of particles dispersed in solution

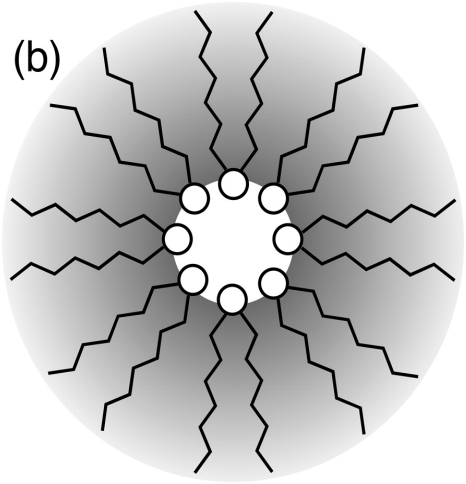
Ternary system consisting of aqueous water pool, Aerosol-OT, organic solvent



Aerosols



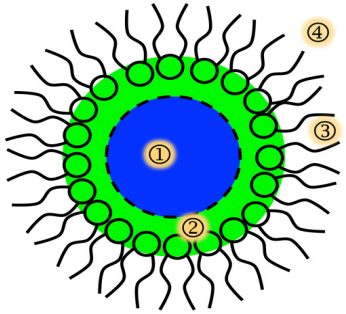




(a) Surfactant aerosol OT (AOT)
(b) Reverse micelle (RM), water pool, surrounded by AOT molecules (gray), organic solvent
(c) Solution of RMs

What is known about speciation in confined spaces? (1c)

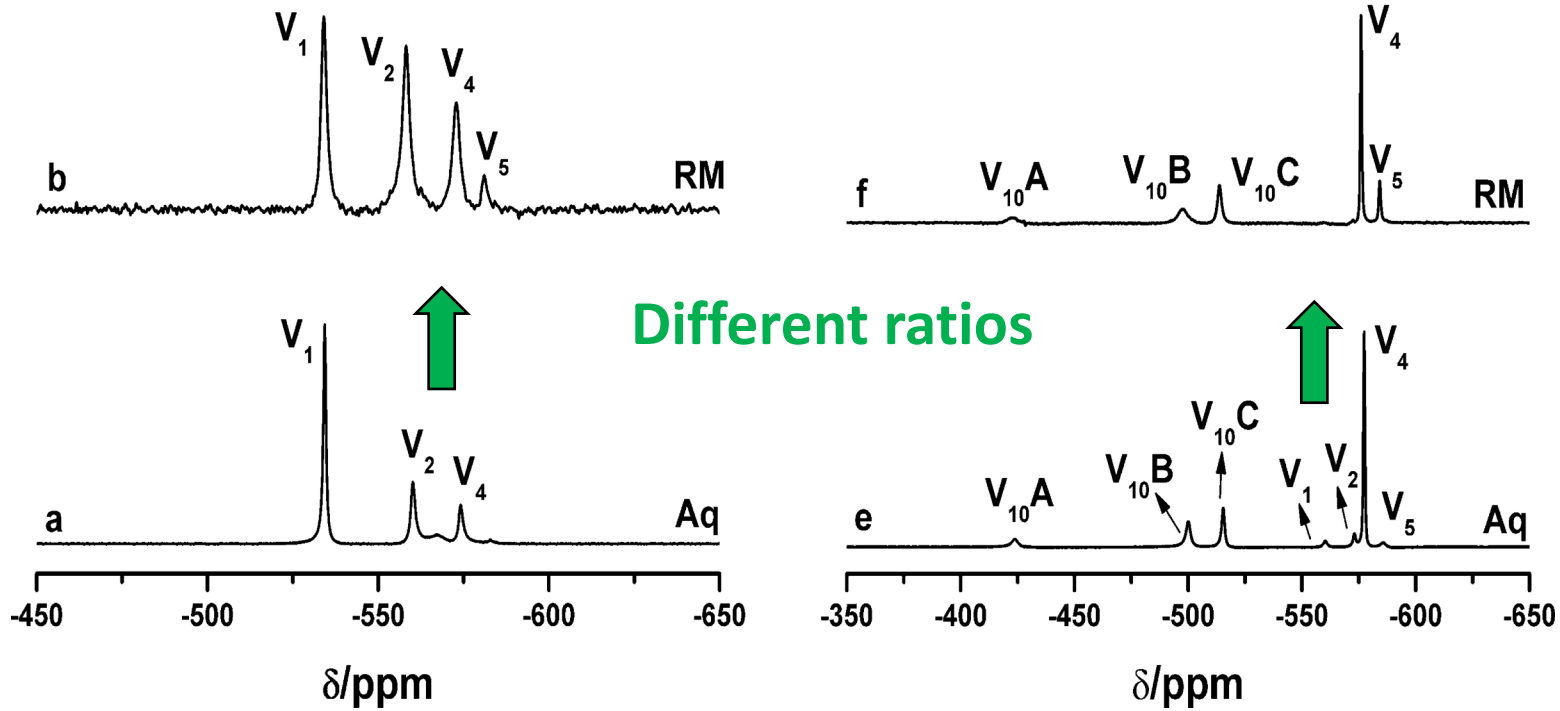
Aerosol OT
Reverse micelle
RM



- Aerosols prepared from Aerosol OT
- Speciation exists in confined space
- **Speciation changed from H_2O to confined space**

Data suggest speciation will change also in aerosols containing dissolved V_2O_5

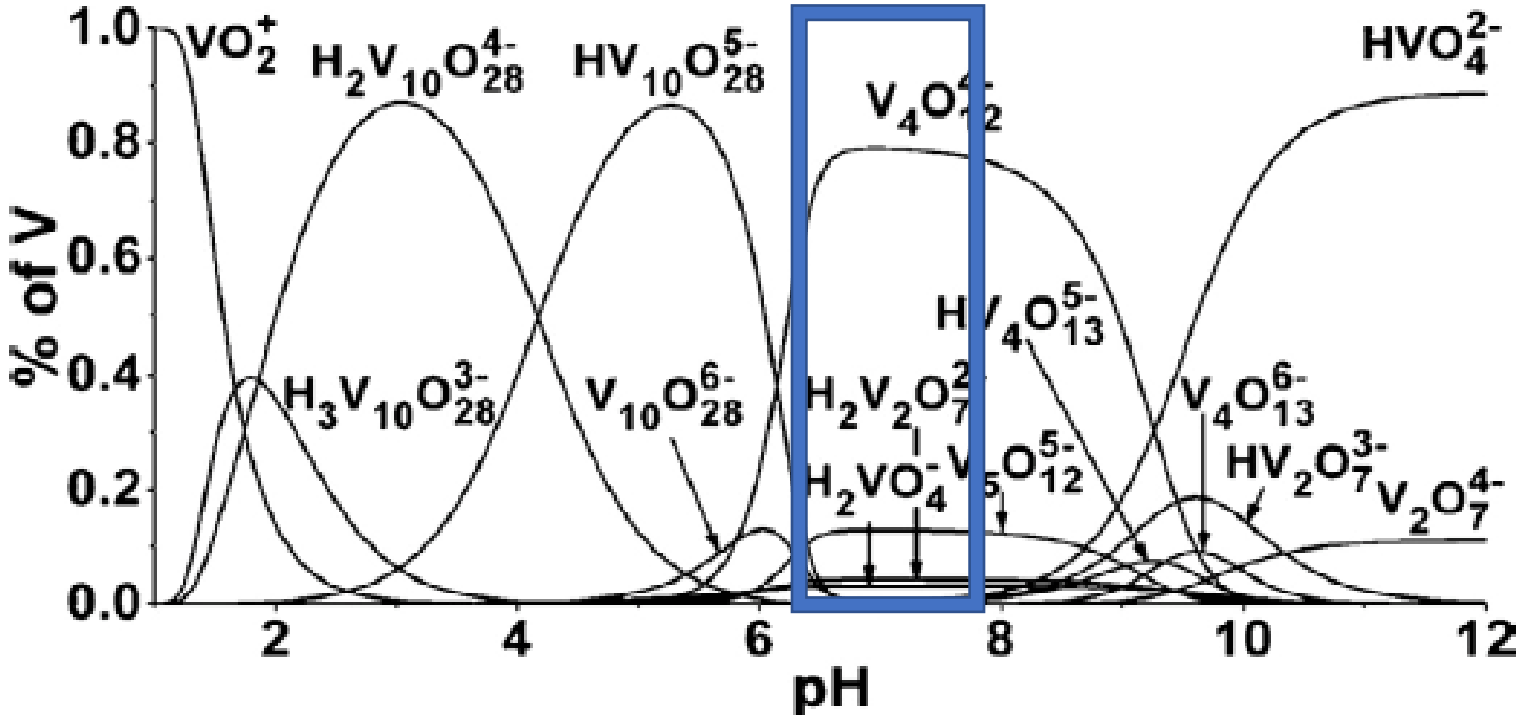
Aqueous solutions were: pH 9.8 and pH 6.3



^{51}V NMR spectra of vanadate in aqueous and reverse micelle samples collected at 78.9 MHz of aqueous vanadate solution (50 mM) or in 50 mM vanadate in w_0 = 12 AOT/isooctane RM suspension.

Levinger et al. JACS, 2011, 133, 7205-7213

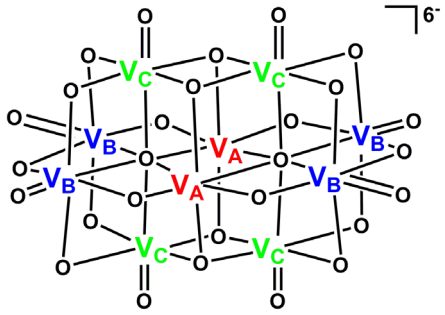
Speciation – Under Physiological Conditions



D.C. Crans, N.E. Levinger, Acc. Chem. Res. 45 (2012) 1637–1645.

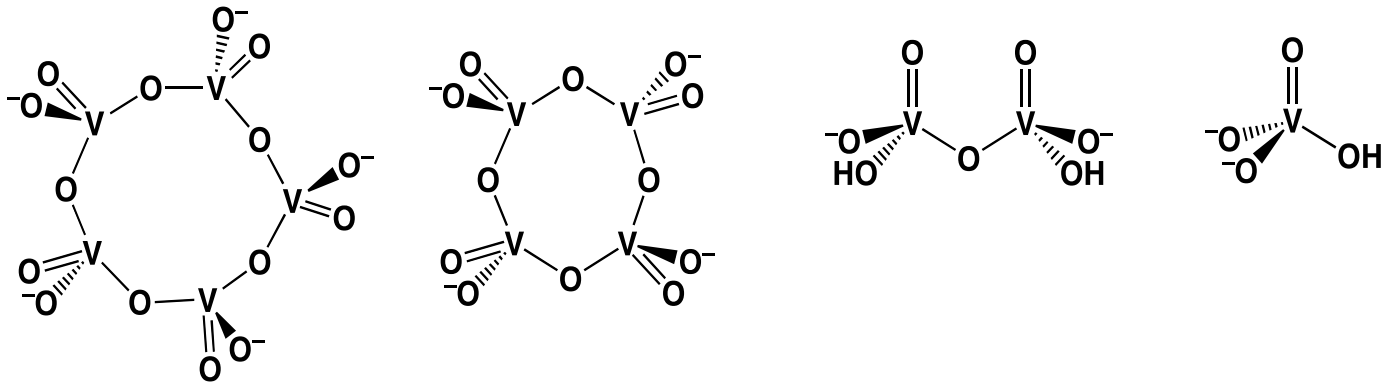
No matter where we start that is where we end up

$K_3Na_3V_{10}O_{28}$

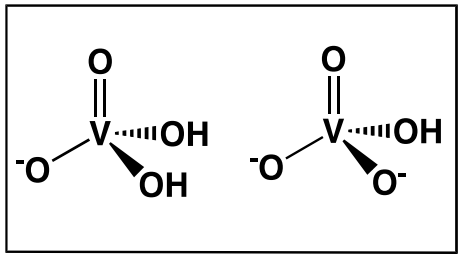


Decavanadate not stable at neutral (physiological) pH

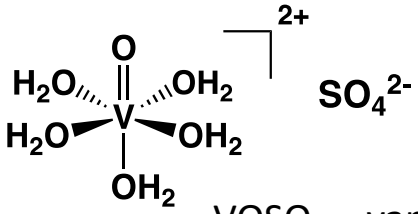
V_2O_5 hydrolyse to V_{10} that hydrolyse to vanadate



Oxidation state - V(V) & V(IV) speciation - solid and solution



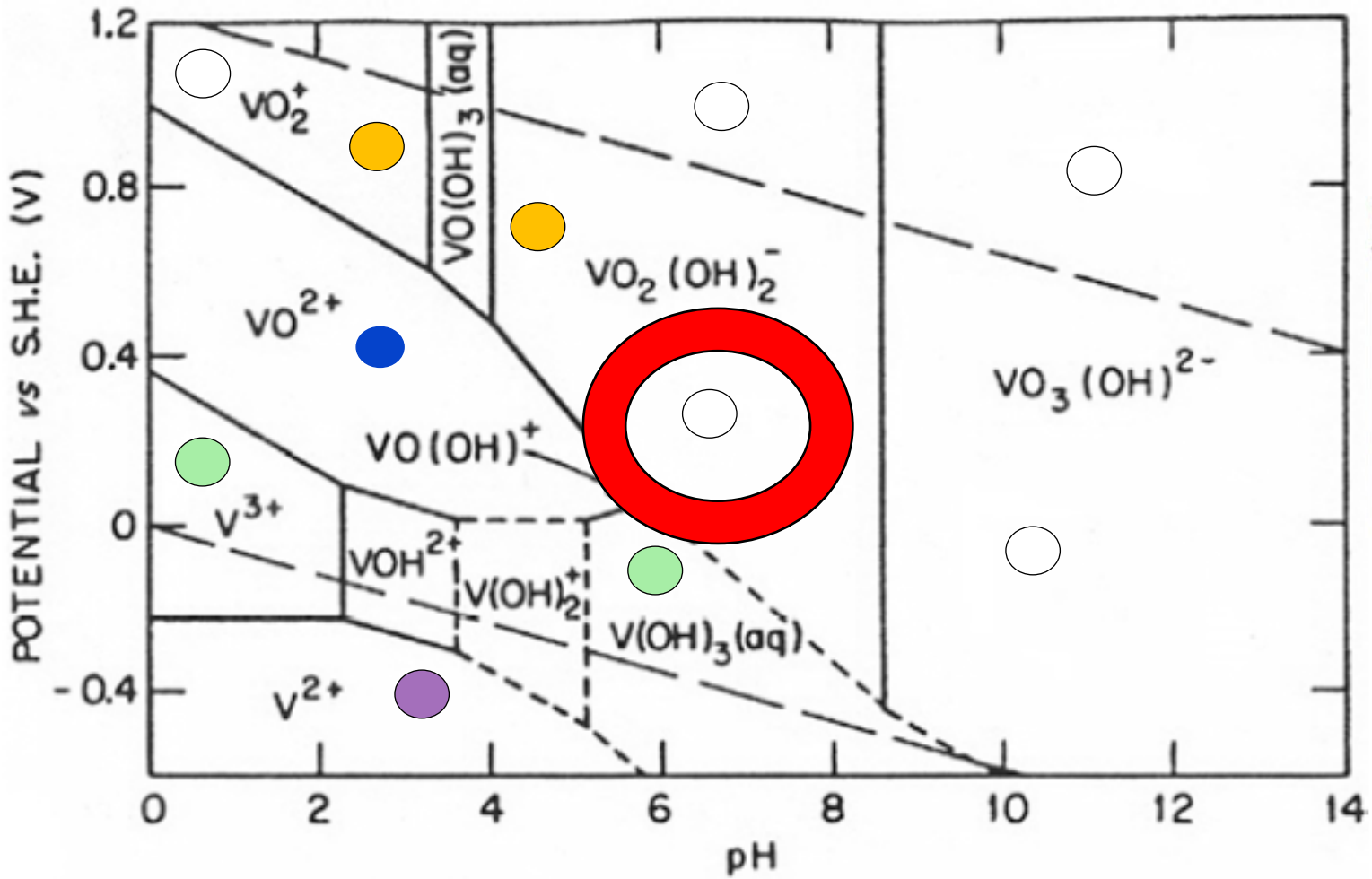
$NaVO_3$ – vanadium(V)



$VOSO_4$ – vanadium(IV)

Spectroscopic studies in solution
Iannuzzi, Rieger *Inorg. Chem.* ***1975,*** *14,* 2895
EXAFS and LAXS (Krakowiak et al *Inorg. Chem.* **2012**

Physiological range of speciation: See Circle



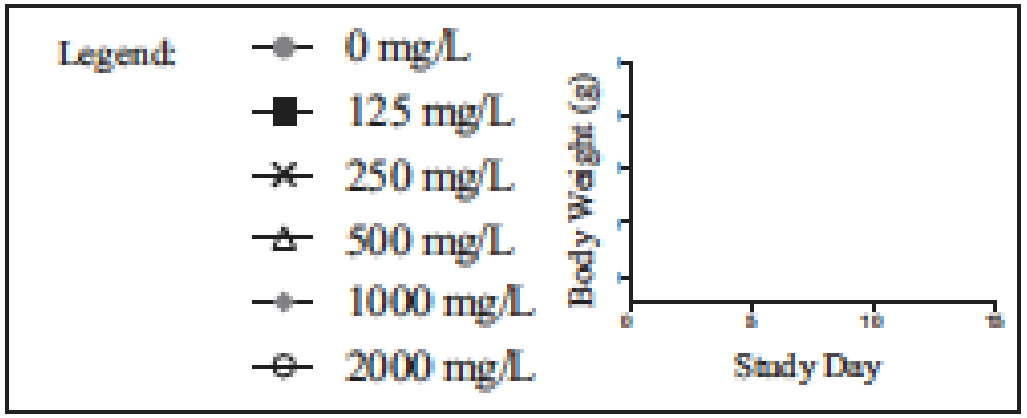
Pourbaix diagram

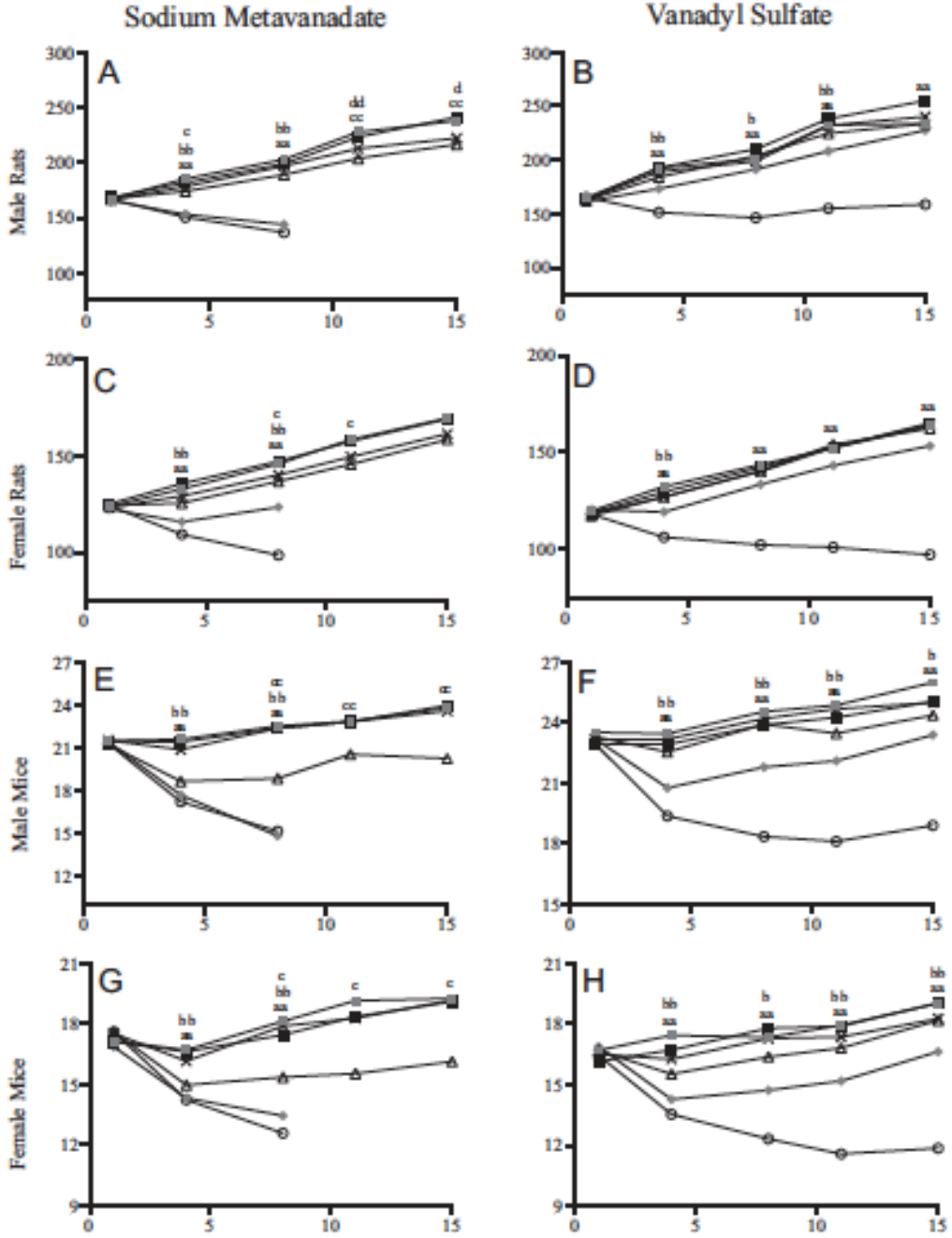
V(V) white and yellow

Effects of oxidation states on Harlan Sprague Dawley rats & B6C3F1/N mice

Body Weight Curves. The x-axes represent study day and the y-axes are body weight in grams. Body weights for Harlan Sprague Dawley (HSD) rats and B6C3F1/N mice animals exposed to sodium $NaVO_3$ are found in the left column and $VOSO_4$ in the right column

Males rats are the first row (A, B), female rats the second row (C, D), male mice the third row (E, F) and female mice the fourth row (G, H). All standard errors were less than 10%.

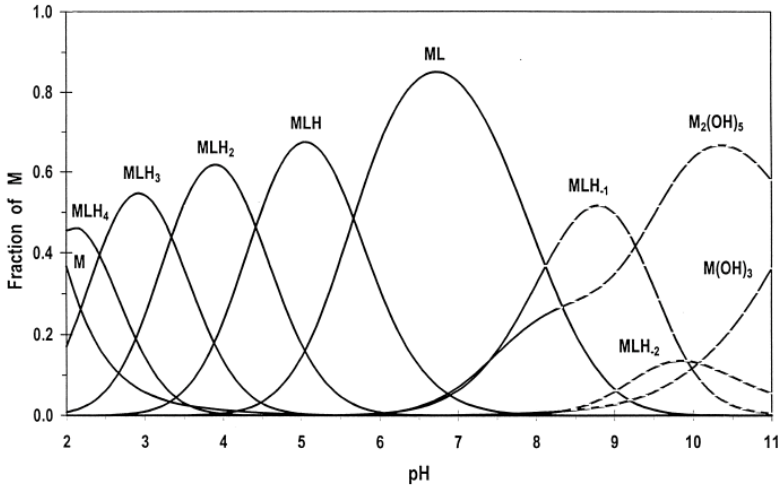




Roberts et a

In Biologial Systems: Vanadium and gluthathione

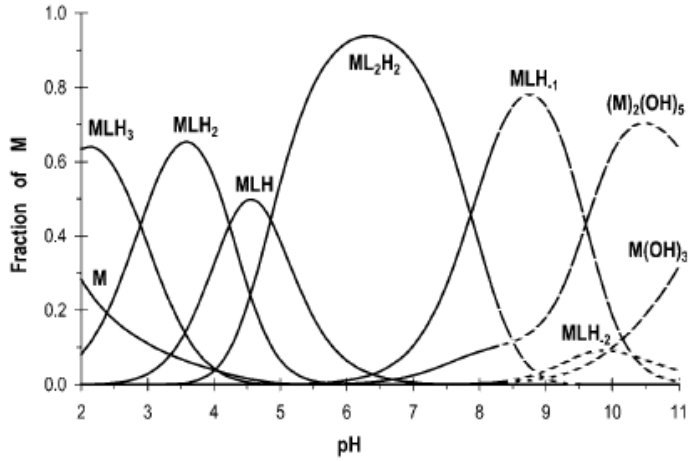
- Vanadium(V) and vanadium(IV) form both complexes with gluthathione (GSH)
- Vanadium(IV) form complexes with gluthathione (GSSG)
- Vanadium(V) can be reduced by GSH; metabolizing and can form both the V(IV)-GSH and V(IV)-GSSG complexes



The $V(IV)^{2+}$-GSSG system V(IV) 7 mM and 70 mM GSSG

Pessoa et al. *J. Inor. Biochem.* 2001, 84, 259-270

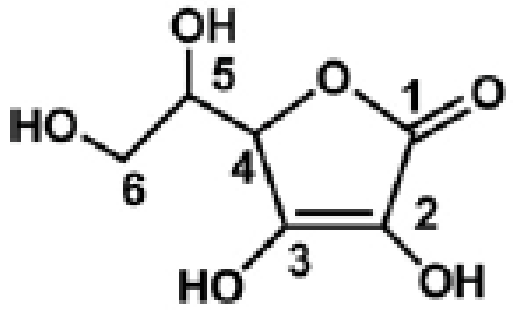
- **Relevance:** Vanadium in blood or plasma is likely to be in part in the form of a V(IV)-GSH and possible also a V(IV) complex



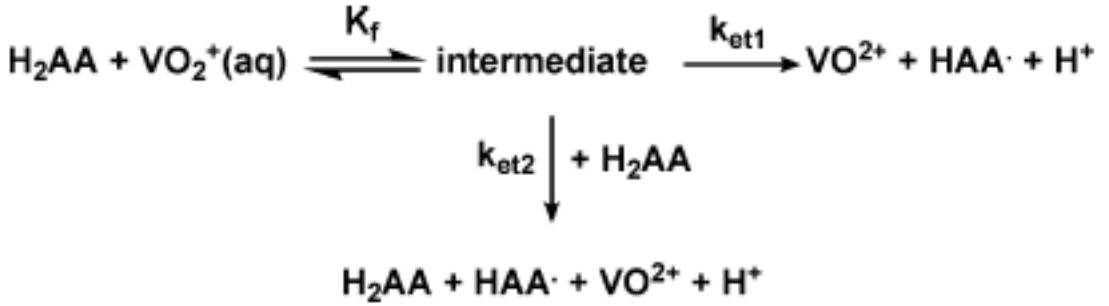
The $V(IV)O^{2+}$-GSH system with V(IV) 10 mM and 250 mM GSH

Pessoa et al. *J. Biol. Chem.* 2002, 7, 225-240

In Biologial Systems: Vanadium and Ascorbate



Ascorbic acid



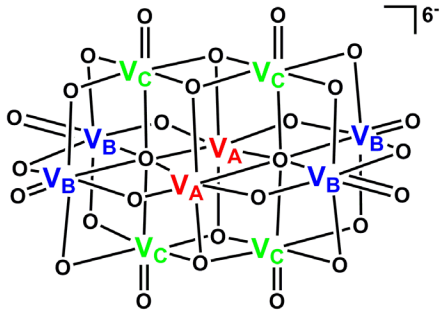
Scheme 1. Detailed mechanism for the ascorbic acid reduction by VO_2^+ presented previously [14].

- Vanadium for complexes with ascorbate
- Vanadium(V) is reduced by ascorbate
- The reaction contribute to convert any potential vanadium(V) compounds to vanadium(IV)

Reaction supportive of the possibility that vanadium(V) complexes converts to vanadium(IV) in blood or plasma

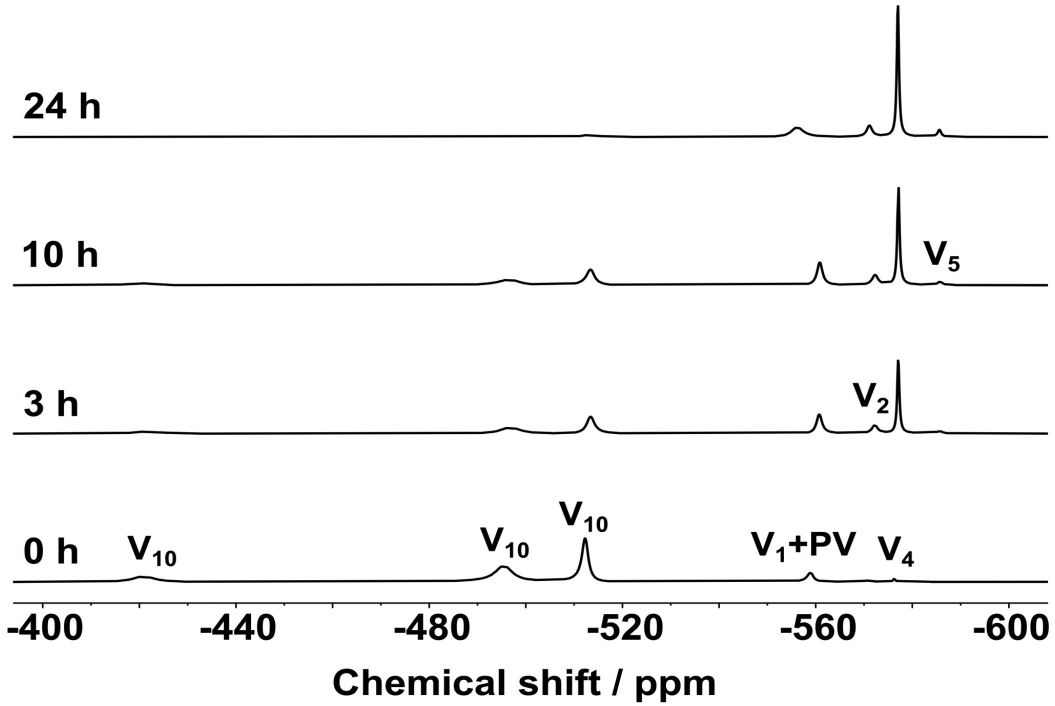
"Impairment of ascorbates' anti-oxidant properties in confined media: Inter and intramolecular reactions with air and with vanadate at acidic pH," Debbie C. Crans, Bharat Baruah, Ernestas Gaidamauskas, Brant G. Lemons and Michael D. Johnson, *J. Inorg. Biochem,* **2008**, *102*, 1334-1347 and references therein

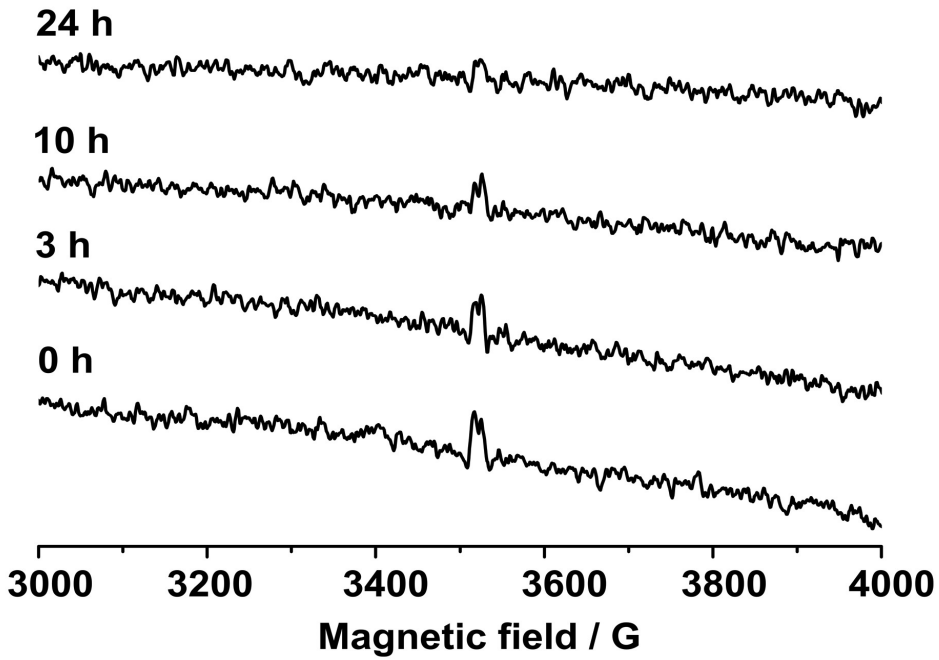
In Biologial Systems: V_{10} added to cells



Result: V_{10} convert into smaller species and also a little redox chemistry

Implication: that added V_2O_5 would also convert





Althumairy et al. *Metallomics*, 12, 2020, 1044–1061

Different Vanadium Species have variable Biological Effects

Even in solutions of converting vanadium species specificity is observed. This was first demonstrated in 1989/1990

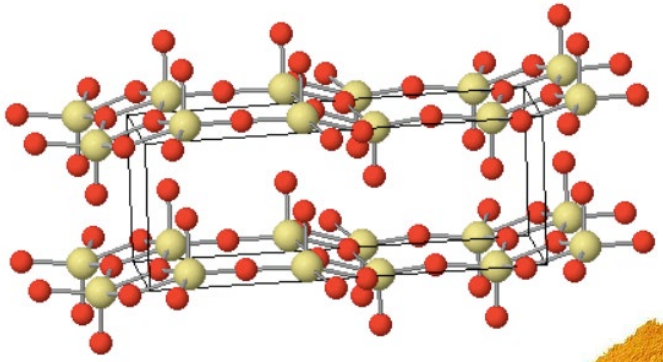
- Simple forms of vanadium species are potent inhibitors for phosphatases
- Some forms of vanadium species facilitate signal transduction
- Some oxovanadates (oxidovanadates) are inhibitors of enzyme activities

Effects on cells, rodents and humans

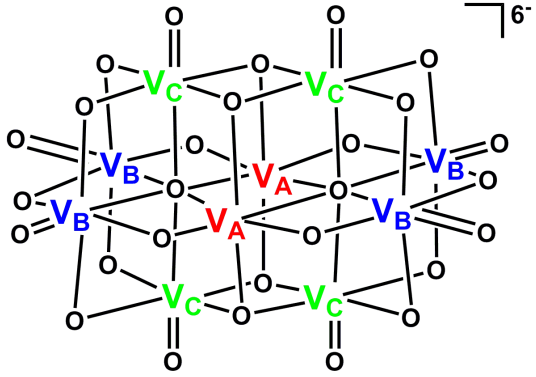
- Some forms of vanadium species are inhibiting cellular growth
- Some forms of vanadium are toxic
- Some forms of vanadium are alleviating cancer
- Some forms of vanadium are alleviating high blood glucose levels in diabetes

Crans, *J. Org. Chem.* **2015**, 80 (24), 11899-11915;. McLauchlan, et al., *Coord. Chem. Rev.*, **2015**, 301-302, 163-199; Crans, et al. *Met. Ions Life Sci,* **2018,** *18*, 251-279; Crans, et al., *Met. Ions Life Sci,* **2019**, *19*, 203-230; Crans, et al. Chapter 6 in Metal Toxicology Handbook, Taylor & Francis Group; Samart et al. *Coord. Chem. Rev.* **2020**, *416*, 213-286; Lima, et al., *Inorganics* **2021**, 9, 42.

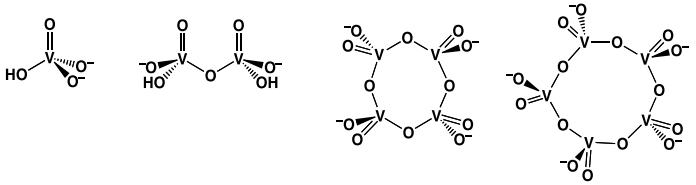
Summery: How these facts impact the interpretations in the inhalation experiments



V_2O_5 - Solid state



Vanadium(V) in solution



- **What form** of V_2O_5 (or V_4O_{10}) is used for treatment – **from solid or solution**? **Most studies are using of V_2O_5 dissolved**
- Methods used for **inhalation studies**, **aerosolizing vanadium pentoxide** (or other vanadium compound) from solution, **rather than exposure to vanadium as a dust are likely to yield different results: comparison studies are desirable**
- **Vanadium speciation under physiological conditions are sensitive to pH, concentration, and redox potential - yes**
- **Critical because different vanadium species have different biological activities - yes**
- **Conversion between vanadium oxidation state *(should include "species")* in the rodent - yes**