

# MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

**DATE:** September 11, 2003

**SUBJECT:** Chemistry Determinations- Prussian Blues

**CC:** NDA 21-626, \_\_\_\_\_ IND 51,700,  
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This memorandum supercedes the memorandum of David A. Place, Ph.D dated July 11, 2003.

## Introduction:

Prussian Blue is a blue industrial and artist pigment first synthesized by Diesbach in 1704. The common name 'Prussian Blue' has been used for several different but related ferric(III) hexacyanoferrate(II) compounds; specifically potassium ferric(III) hexacyanoferrate(II),  $KFe[Fe(CN)_6]$  or  $KFeFe(CN)_6$ <sup>i,5</sup> and ferric hexacyanoferrate,  $Fe^{III}_4[Fe^{II}(CN)_6]_3$ <sup>ii,iii</sup>. The compound most commonly associated with the common name 'Prussian Blue' is  $Fe^{III}_4[Fe^{II}(CN)_6]_3$  with chemical names iron (III) ferrocyanide, ferric ferrocyanide, iron (III) hexacyanoferrate (II), and ferric hexacyanoferrate. This Prussian Blue is also commonly called 'Insoluble Prussian Blue' while potassium ferric(III) hexacyanoferrate(II),  $KFe[Fe(CN)_6]$ , is commonly named 'Soluble Prussian Blue'. Prussian Blue and related compounds, including ammonium iron(III) hexacyanoferrate(II),  $NH_4Fe[Fe(CN)_6]$ , have a very high affinity for cesium and thallium and have been investigated and used as a treatment for radiocesium contamination<sup>iv</sup> and thallium poisoning<sup>v</sup>. On February 4, 2003, FDA published a Federal Register notice announcing that the Agency has determined that Prussian Blue can be found to be safe and effective for the treatment of internal contamination with radioactive thallium, nonradioactive thallium, and radioactive cesium (68 FR 5645) and the availability of Guidance on submitting a new drug application.

## The question:

There has been much discussion regarding whether the different ferric(III) hexacyanoferrate(II) compounds, specifically  $Fe^{III}_4[Fe^{II}(CN)_6]_3$ ,  $KFe[Fe(CN)_6]$ , and  $NH_4Fe[Fe(CN)_6]$ , are all different active moieties and, therefore, 'New Molecular Entities' or are all different salts of the same active moiety. The reason for the difficulty in making this determination is that the information on the structure and mechanism of action for all three forms of Prussian Blue is incomplete, and in some cases conflicting.

### Definitions:

Before addressing this question, it is helpful to keep in mind the following definitions for a 'molecular entity', an 'active moiety', and a salt. A new molecular entity (NME) is defined as an active moiety that has not been previously approved or legally marketed as the active moiety in the United States in any drug product, either as a single ingredient, as part of a combination product, or as part of a mixture of stereoisomers.

Active moiety is defined in the Code of Federal Regulations (21 CFR 314.108(a)) as the molecule or ion, excluding those appended portions of the molecule that cause the drug to be an ester, salt (including a salt with hydrogen or coordination bonds), or other noncovalent derivative (such as a complex, chelate, or clathrate) of the molecule, responsible for the physiological or pharmacological action of the drug substance.

There is no regulatory definition of salts. Chemically, a salt is a molecule that contains two or more oppositely charged ions that are bound together by electrostatic attraction. These electrostatic attractions that bind the oppositely charged ions are called "ionic bonds".

### Discussion:

For the purposes of this discussion, ferric(III) hexacyanoferrate(II) compounds are divided into two groups, insoluble ferric(III) hexacyanoferrate(II) with the empirical formula  $\text{Fe}^{\text{III}}_4[\text{Fe}^{\text{II}}(\text{CN})_6]_3$  and those with the generalized empirical formula  $\text{M}_A\text{Fe}^{\text{(III)}}[\text{Fe}^{\text{(II)}}(\text{CN})_6] \cdot x\text{H}_2\text{O}$ . All of the ferric(III) hexacyanoferrate(II) compounds are practically insoluble in water with a solubility constant of  $10^{-40}$  g/L. Potassium ferric(III) hexacyanoferrate(II),  $\text{KFe}[\text{Fe}(\text{CN})_6]$ , forms a colloidal suspension in water, despite its very low ionic dissociation, and for this reason is commonly called "Soluble Prussian Blue". Ferric hexacyanoferrate,  $\text{Fe}^{\text{III}}_4[\text{Fe}^{\text{II}}(\text{CN})_6]_3$ , however, does not readily form a colloidal suspension in water and therefore, is commonly called 'insoluble Prussian Blue'. As stated in the introduction section, although the common name 'Prussian Blue' has been used for both of these compounds, for consistency with the published Federal Register Notice and Guidance, the common name 'Prussian Blue' will refer only to ferric(III) hexacyanoferrate(II) with the empirical formula  $\text{Fe}^{\text{III}}_4[\text{Fe}^{\text{II}}(\text{CN})_6]_3$ .

It is accepted in the literature that the crystal structure of Prussian Blue,  $\text{Fe}^{\text{III}}_4[\text{Fe}^{\text{II}}(\text{CN})_6]_3$ , is a cubic lattice with the  $\text{Fe}^{\text{II}}$  and  $\text{Fe}^{\text{III}}$  atoms occupying the corners of the cube and the  $\text{C}\equiv\text{N}^-$  groups positioned on the sides (Figure 1<sup>vi</sup>). The  $\text{Fe}^{\text{II}}$  atoms are always bonded to the carbon atom in the cyano group and the  $\text{Fe}^{\text{III}}$  atoms are bonded to the nitrogen atom of the cyano group. Therefore, the crystal matrix is an infinite 3-dimensional solid based on repeat units of the type  $\text{Fe}^{\text{(III)}}-\text{N}\equiv\text{C}-\text{Fe}^{\text{(II)}}-\text{C}\equiv\text{N}-\text{Fe}^{\text{(III)}}$  with the ratio of Fe(III), Fe(II) atoms and cyano groups in the crystal structure of 4:3:18. In aqueous media, this cubic structure is maintained because of the strong thermodynamic stability of the 3-dimensional structural architecture, a fact that accounts for the insolubility of the ferric(III) hexacyanoferrate(II).

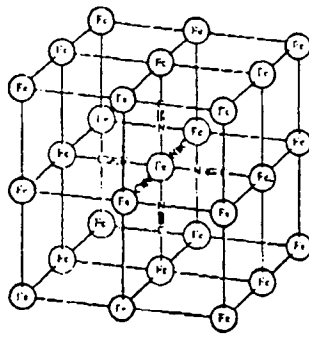


Figure 1: Portion of the basic crystal structure of ferric(III) hexacyanoferrate(II) compounds illustrating bridging by ambidentate cyanide ions. For clarity, any charge neutralizing cations, waters of crystallization, and most of the cyanide groups have been omitted

An important characteristic of the crystal structure of Prussian Blue is that not all of the atomic positions in the unit structure are completely occupied. Rather, the crystal lattice has “holes” in it with about one-quarter of the  $\text{Fe}^{\text{III}}(\text{CN})_6$  positions, particularly in the internal positions, either vacant or filled with water molecule. The distribution of these vacancies (or “holes”) within the crystal lattice range from random to more ordered depending upon the conditions in which the crystals were prepared<sup>vii</sup>.

Although the mechanism of cesium ( $\text{Cs}^+$ ) or thallium ( $\text{Tl}^+$ ) uptake by Prussian Blue is not fully understood, it is believed that these “holes” play an important role in Prussian Blue’s ability to strongly bind  $\text{Cs}^+$ . Literature reports indicate the mechanisms for cesium ( $\text{Cs}^+$ ) and thallium ( $\text{Tl}^+$ ) uptake are the combination of chemical ion-exchange and physical adsorption (physisorption). For Prussian Blue, the ion-exchange occurs with the hydrogen (hydronium ion) from the water molecules in the crystal. Evidence for this mechanism is the reduction in pH when  $\text{Cs}^+$  exchange is performed and hydronium ions are released as observed by Neilsen et al.<sup>viii</sup> In solutions containing  $\text{Fe}^{\text{III}}_4[\text{Fe}^{\text{II}}(\text{CN})_6]_3$ , a decrease of pH from 5.54 for  $\text{Fe}^{\text{III}}_4[\text{Fe}^{\text{II}}(\text{CN})_6]_3$  in water to pH 2.82 when  $\text{CsCl}$  was added. However, through mass balance calculations, this decrease in pH alone does not account for all the  $\text{Cs}^+$  uptake. It is believed that remainder of uptake of  $\text{Cs}^+$  is absorption into the crystal lattice with the  $\text{Cs}^+$  cation being mechanically or electrostatically trapped in the unoccupied cavities and “holes” within the crystal lattice. This is similar in concept to trapping balls in a box with a few holes in it. The larger the cation, the more difficult it is for the cation, once inside the interstices of the crystal, to diffuse out of the structure (a kinetic issue), i.e., it is “held more tightly” within the crystal lattice.

If the ferric(III) hexacyanoferrate(II) compound contains a monovalent alkali metal or ammonium cation, then the generalized empirical formula is of the general type  $\text{M}_A\text{Fe}^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6] \cdot x\text{H}_2\text{O}$  where  $\text{M}_A$  represents the charge neutralizing cation (e.g.,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{Cs}^+$ ) and  $x$  represents the number of water of crystallization (i.e., water molecules within the crystal lattice). Similar to Prussian Blue, the crystal structure of ferric(III) hexacyanoferrate(II) salts consists of a cubic lattice with the  $\text{Fe}^{\text{II}}$  and  $\text{Fe}^{\text{III}}$  atoms occupying the corners of the cube and the  $\text{C}\equiv\text{N}^-$  groups positioned on the sides. Unlike the transition metal  $\text{Fe}^{\text{III}}$  ions which are directly bonded to the nitrogen end of the cyanide ligand with a degree of covalency to the bonding, however, the alkali metal ions (e.g.,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cs}^+$ ,  $\text{Tl}^+$ ) or ammonium

(NH<sub>4</sub><sup>+</sup>) ions are held in place electrostatically by ionic bonds in the “holes” within the crystal framework (as “defects” in the perfect face-centered cubic unit cell) and within the cubic lattice (as charge neutralizing counterions). The presence of these cations contributes to the increased tendency of these compounds to form colloidal suspensions. The ratio of Fe(III), Fe(II) atoms and cyanide groups in the crystal structure for ferric(III) hexacyanoferrate(II) salts is 1:1:6.

Literature reports also indicate that the mechanism of cesium (Cs<sup>+</sup>) and thallium (Tl<sup>+</sup>) uptake by ferric(III) hexacyanoferrate(II) salts is a combination of ion-exchange and physical adsorption. However, unlike “pure” ferric(III) hexacyanoferrate(II), the ion-exchange for ferric(III) hexacyanoferrate(II) salts is primarily cation exchange with the monovalent cations. For these monovalent cations, the relative binding affinity is largely determined by the size of the cations. According to Nielsen *et al.*<sup>8</sup>, the binding affinity (i.e., stability) of the cations with the ferric hexacyanoferrate(II) anion increases in the order of Na<sup>+</sup> < K<sup>+</sup>, NH<sub>4</sub><sup>+</sup> < Cs<sup>+</sup> < Tl<sup>+</sup>. The ionic nature of these cations was observed by Nielsen *et al.* In that paper, it was reported that in artificial gastric juice, at a pH of 1.2, and artificial duodenal juice, at a pH of 6.8, where there are relatively large amounts of sodium ions, the sodium ion exchanges with the potassium cation. However, because the affinity for Cs<sup>+</sup> is so high, the competing sodium ions do not appreciably interfere with Cs<sup>+</sup> exchange. In contrast to Prussian Blue, there was no significant decrease in pH associated with the uptake of Cs<sup>+</sup> by ferric(III) hexacyanoferrate(II) salts suggesting that ion-exchange with the hydrogen (hydronium ion) from the waters of crystallization do not contribute appreciably to the mechanism of uptake for Cs<sup>+</sup>.

#### **Regulatory Chemical Classification Determination**

Determination of the chemical classification for new drugs, in particular the differentiation between a new molecular entity and a new salt, requires the determination of the active moiety. In the case of drugs containing metal ions, the active moiety may be a coordination complex or chelate, rather than a metal ion itself, when the metal-ligand complex is sufficiently stable *in vivo* to be responsible for its physiologic-pharmacologic action. In such cases, the metal-ligand complex usually needs to be of a clearly defined stoichiometry and contain coordinate bonds with bond strengths comparable to covalent bonds.

For Prussian Blue, Fe<sup>III</sup><sub>4</sub>[Fe<sup>II</sup>(CN)<sub>6</sub>]<sub>3</sub>, all of the atoms are incorporated into the frame work of the cubic crystal structure in an infinite 3-dimensional matrix of Fe<sup>(III)</sup>-N≡C-Fe<sup>(II)</sup>-C≡N-Fe<sup>(III)</sup> repeat units with a Fe<sup>(III)</sup>:Fe<sup>(II)</sup>:CN ratio of 4:3:18. All of the atoms that make up the crystal structure of Prussian Blue are tightly bound and the whole structure, represented by the empirical formula of the molecular solid Fe<sup>III</sup><sub>4</sub>[Fe<sup>II</sup>(CN)<sub>6</sub>]<sub>3</sub>, remains intact in aqueous media, and participates in the uptake of Cs<sup>+</sup>. Therefore, the active moiety for ferric(III) hexacyanoferrate(II), or Prussian Blue, is the whole molecule (i.e., Fe<sup>III</sup><sub>4</sub>[Fe<sup>II</sup>(CN)<sub>6</sub>]<sub>3</sub>).

In contrast, in the ferric(III) hexacyanoferrate(II) compounds that contain alkali metal or ammonium cations, the monovalent cations are electrostatically held in place (i.e., ionic bonds) within the crystal structure and are available for cation exchange with the Cs<sup>+</sup> ion. For ion-exchange resins, the function of the resin is determined by the chemical composition of the resin, excluding the ion to be exchanged, and the structure of the resin determines its performance. The “active moiety” could be thought of as a traditional ion-exchange resin excluding the ion being exchanged. Because of the relative insolubility of ferric(III) hexacyanoferrate(II)

compounds, the 3-dimensional cubic colloidal suspension based on  $[\text{Fe(II)-C}\equiv\text{N-Fe(III)}]^{-1}$  units acts as an ion-exchange resin. Therefore, the  $(\text{Fe}^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6])^{-1}$  anion that makes up the framework of the crystal structure is the "active moiety". Further, although the crystal structures for both Prussian Blue and ferric(III) hexacyanoferrate(II) salts are very similar, the ratio of atoms that make up the crystal framework of ferric(III) hexacyanoferrate(II) salts is different with a  $\text{Fe}^{\text{III}}:\text{Fe}^{\text{II}}:\text{CN}$  ratio of 1:1:6. Finally, because of the ionic nature of the monovalent alkali metal and other similar cations, and the fact that the crystal structures of the ferric(III) hexacyanoferrate(II) salts do not change with different monovalent alkali metal cations, all ferric(III) hexacyanoferrate(II) compounds containing different monovalent alkali metal cations should be considered salts of the same active moiety.

In summary, one can say that the Fe(III) is an integral part of a molecular solid of Prussian Blue,  $(\text{Fe}^{\text{III}}_4[\text{Fe}^{\text{II}}(\text{CN})_6]_3)$  where the Fe(III) ions are held by covalent bonding (chemical interactions) that are not merely electrostatic, whereas the alkali metal and ammonium ions in the ferric(III) hexacyanoferrate(II) compounds are physisorbed due to weak interactions with an anionic framework.

### Conclusion

After review of the current information available in the literature and careful consideration, based on the current understanding of the structure and mechanism of sorption of the ferric(III) hexacyanoferrate(II) compounds, we have concluded that Prussian Blue with the empirical formula  $\text{Fe}^{\text{III}}_4[\text{Fe}^{\text{II}}(\text{CN})_6]_3 \cdot x\text{H}_2\text{O}$ , also known as "Insoluble Prussian Blue," should be considered a different active moiety than ferric(III) hexacyanoferrate(II) salts that contain monovalent cations. Thus, Potassium ferric(III) hexacyanoferrate(II) and Ammonium ferric(III) hexacyanoferrate(II) should not be considered as different salts of Prussian Blue. Table 1 summarizes the difference between Prussian Blue and ferric(III) hexacyanoferrate(II) salts.

Table 1: summary of differences between "pure" ferric hexacyanoferrate and salts of ferric hexacyanoferrate

Compound(s)	Empirical formula	Mechanism of Cs uptake	Active Moiety
Ferric(III) hexacyanoferrate(I) (a.k.a., Prussian Blue or Insoluble Prussian Blue)	$\text{Fe}^{\text{III}}_4[\text{Fe}^{\text{II}}(\text{CN})_6]_3 \cdot x\text{H}_2\text{O}$	(1) Ion-exchange with the hydronium ion of the waters of crystallization. (2) Adsorption/mechanical trapping of the $\text{Cs}^+$ cation within the crystal structure.	The crystal structure incorporating the whole molecule, including the waters of crystallization, with a $\text{Fe}^{\text{III}}:\text{Fe}^{\text{II}}:\text{CN}$ ratio of 4:3:18.
Potassium ferric(III) hexacyanoferrate(I) & Ammonium ferric(III) hexacyanoferrate(I)	$M_A\text{Fe}^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6] \cdot x\text{H}_2\text{O}$	(1) Cation-exchange with the ionically bound monovalent cation. (2) Adsorption/mechanical trapping of the $\text{Cs}^+$ cation within the crystal structure.	The crystal structure defined with the $\text{Fe}^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6]^{-1}$ anion with a $\text{Fe}^{\text{III}}:\text{Fe}^{\text{II}}:\text{CN}$ ratio of 1:1:6.

<sup>i</sup> A.F.Wells, *Structural Inorganic Chemistry*, Third Edition, Oxford, 1962, pg. 739

<sup>ii</sup> H.J.Buser, D.Schwarzenbach, W.Petter, and A.Ludi. The Crystal Structure of Prussian Blue:  $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot x\text{H}_2\text{O}$ . *Inorganic Chemistry*, **16**, 2704-2710 (1977)

<sup>iii</sup> Bernd Dresow, Peter Nielsen, Roland Fischer, Alexander A. Pfau, Hellmuth H. Heinrich, In vivo Binding of Radiocesium by two forms of Prussian Blue and by ammonium iron hexacyanoferrate (II), *Clinical Toxicology*, 1993, **31**(4), pg. 563-569

<sup>iv</sup> Jaroslaw Rachubik and Bogdan Kowalski. Ammonium Ferric Hexacyanoferrate (AFCF) as a countermeasure for reducing the Radiocaesium Transfer to Muscles and Inner Edible Organs of Broiler Chickens, *Bull. Vet. Inst. Pulawy*, **45**, 57-61 (2001)

<sup>v</sup> Pau P.W., Management of thallium poisoning, 2000, *Hong Kong Med. J.*, **6**(3), pg. 316-318

<sup>vi</sup> James E. Huheey, *Inorganic Chemistry, Principles of structure and reactivity*, Harper & Row Publishers, New York, 1972, pg. 413-415

<sup>vii</sup> H.J. Buser, D. Schwarzenbach, W. Petter, and A. Ludi, The Crystal Structure of Prussian Blue:  $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot x\text{H}_2\text{O}$ , *Inorganic Chemistry*, 1977, **16**(11), pg. 2704

<sup>viii</sup> Peter Nielsen, Bernd Dresow, and Hellmuth C. Heinrich. In vitro Study of  $^{137}\text{Cs}$  Sorption by Hexacyanoferrates(II), *Z.Naturforsch*, 1987, **42b**, 1451-1460

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