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From:	IT delegation
To:	Working Party on Technical Harmonisation (Dangerous Substances - Fertilisers)
Subject:	Italian comments on the CZ Delegation comments on the Proposal for a Regulation of the European Parliament and of the Council laying down rules on the making available on the market of CE marked fertilizing products (doc. 11718/17) regarding the Chromium content

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DIPARTIMENTO DELLE POLITICHE EUROPEE ED INTERNAZIONALI E DELLO SVILUPPO RURALE
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Oggetto : Italian comments on the CZ Delegation comments on the Proposal for a Regulation of the European Parliament and of the Council laying down rules on the making available on the market of CE marked fertilizing products (doc. 11718/17) regarding the Chromium content

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Hereafter you can find the Italian Comments about the document provided by the CZ Delegation, on the Chromium content in fertilizing products, because we think that some clarification are needed.

Our comments, remarks and integration are highlighted within the text proposed by the CZ Delegation.

Some bibliography has been added, too.

MILESTONES

The Commission's Proposal for a Regulation on the placing on the market of CE marked fertilising products published on 17th of March 2016 proposes limit values for hexavalent chromium (Cr(VI)) for all the product function categories (PFCs). Such limit derives from a very long discussion started in 2012 in the ad-hoc Working Group 3 (Contaminants, Hygiene and other Risks) and then continued in the Fertilizer Working Group of the DG ENTR (now DG GROW).

The proposal of Commission to limit Cr(VI) is supported by a very wide scientific literature, the official positions of European Authorities such as EFSA, European institutions such as the European Commission (DG-GROW, DG-JRC, DG-SANTE, DG-ENVI) and European and international legislations on fertilizers.

The proposal of a limit for total Chromium would not guarantee that Cr(VI)-contaminated fertilizers will be placed on the European market. While it is fundamental to avoid that Cr(VI) is contained in the fertilizing products, the presence of Cr(III) in such products does not present any risk by a health point of view.

As widely known and accepted, Cr(III) may be ingested safely (it is normally contained in the dietary supplements) and there is no evidence that the transformation from Cr(III) to Cr(VI)

happens significantly under natural environmental conditions as well as in natural and cultivated soils.
On the contrary a limit for total Chromium would impact fertilising products containing by-products from both the leather and steel industries very negatively.
On this matter several European authorities and institutions expressed their position:

- From a health point of view, in 2010 **EFSA** (*EFSA Journal 2010;8(12):1882*) delivered a scientific opinion affirming that **Cr(III) is not carcinogenic**.
- In 2014 **DG JRC** after a meeting with the Italian association Assofertilizzanti and Prof. Claudio Ciavatta of the University of Bologna regarding organic fertilizers based on tanning industry by-products (hides and skins), reported that "*it should likewise be taken into consideration that the chemical characterization of the hydrolysed hides and skins-based fertilizers from the tanning industry revealed no detectable concentrations of Cr(VI) (method EN ISO 17075). The hydrolysed hides and skins-based fertilizers from the tanning industry do however contain Cr(III), which is the non-toxic form of Cr and is very immobile in soils as it is precipitates as Cr hydroxide and is strongly adsorbed to soil organic colloids. Moreover, soil organic matter, Fe(II) and other reductants are able to decrease the possibility of the oxidation of Cr(III) to Cr(VI). These are the reasons why **avoid the presence of Cr(VI) in fertilizers, both organic and inorganic, represents the best way to market fertilizers not contaminated***".
- In 2016 **DG GROW D.2 in cooperation with DG ENV, DG SANTE, DG JRC and EFSA** published a note with the subject "*Limiting the presence of total chromium in fertilising products*" where concluded that "*there is no evidence that Cr(III) would have negative effects at current exposure levels, we would consider it **unjustified to establish limit values for total Cr** (thus including Cr(III))*" and that "*since **there is no evidence that transformation from Cr(III) to Cr(VI) can happen under natural environmental conditions in any significant scale**, that hypothesis cannot serve as justification for limit values on total Cr*".

Our comments are highlighted in the position of the Czech Republic.

Chromium, a short review
Chromium(III) in low concentration is essential trace element for living organisms. ~~At higher level~~Chromium(VI) in the environment it is highly toxic and belongs to so called toxic metals. In soilCr is present in two forms with different oxidation states: Cr(III) ~~Cr3+~~and Cr(VI) ~~Cr6+~~. Cr(III) ~~Cr3+~~ has lowertoxicity and it is immobile in alkali or slightly acidic soils. Mobility is decreased

by adsorptionand binding on the organic matter, Fe and Mn hydroxides[1]. Solubility of Cr(III) ~~Cr3+~~ increases in very acid soils, where it is presentin the form of soluble complexes (the soluble inorganic form of Cr(III) is only present when soil pH is less than 4[2]). In alkali soils it forms slightly soluble hydroxides involved in adsorption processes[1]. Inpresence of O_2, Cr(III) ~~Cr3+~~may be oxidized through biotic or abiotic oxidation processes to Cr(VI). Such reaction is possible only when particular conditions occur. Oxygen or MnO_2 are possible oxidizing agents of Cr(III) ~~Cr3+~~ in soil, even if Mn oxides represent the only naturally occurring oxidant of Cr(III).However oxidation could occur with the simultaneous presence of other factors, such as: low organic matter content, alkaline pH and high redox potential values[2,3]: all these conditions limit greatly the possibility of oxidation.Moreover oxidation of Cr(III) to Cr(VI) is a phenomenon occurring very rarely even in the presence of Mn oxides and favorable pH conditions, due to the poor availability of mobile Cr(III): exchangeable trivalent Cr(III) is practically inexistent in soil where the pH overcomes 5[4,5]. Cr(VI) ~~Cr6+~~ is highly toxic andmobile in soil. As strong oxidation agent it is reduced in anoxic conditions to Cr(III) ~~Cr3+~~. Reductionis promoted by organic matter (Banks et al., 2006). In a natural environment Cr(VI) can be reduced to Cr(III) in the presence of organic and inorganic electron donors (reductants) such as organic matter, Fe(II) and sulfides[1,6]. Organic reductants of Cr(VI), such as organisms and residual organic materials, in presence of soluble organic chelating agents may form soluble chelated complexes with Cr(III)[2]: when Cr(III) is bound to organic complexes in soil, it becomes immobile or insoluble[4]. Inorganic oxidants, such as Fe(II) species, reduce Cr(VI) concentration and simultaneously decrease the possibility of a transformation back to Cr(VI) because liquid Cr(III) is included in Fe, $Cr(OH)_3$ solids characterized by low solubility.

- ➔ **In conclusion, only under very oxidative conditions, Cr(III) could be transformed into Cr(VI). Under normal soil conditions, the transformation of Cr(III) into Cr(VI) appears to be highly unlikely.**

There are numerous reports of Cr(VI) ~~Cr6+~~elimination in soil with added organic matter (Chiu et al., 2009; Park et al., 2004; Banks et al., 2006). Organic matter may be directly oxidized by Cr(VI) ~~Cr6+~~, as Cr(VI) ~~Cr6+~~ is strong oxidation agent, orCr(VI) ~~Cr6+~~ may be reduced by microbial activity. The sources of Cr in soil are both natural and anthropogenic. The most important natural

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[6] B.R. James, "The Challenge of Remediating Chromium Contaminated Soil," Environmental Science and Technology/ News, Vol. 30, No. 6, 1996, pp. 248-251

source is weathering of rock. The most significant anthropogenic source is industry. Chromium(VI) is less frequently reported as contaminant of fertilizers. On the contrary Cr(III) contained in fertilizers based on hydrolyzed hides and skins does not represent a problem by an environmental point of view because it is linked to the organic components and it is not present as soluble/insoluble salt.

- ➔ **A very wide scientific literature carried out for over 40 years has demonstrated that once in soil Cr(III) contained in fertilizers based on hydrolyzed hides and skins:**
  - **is not oxidized to Cr(VI)[7,8,9,10,11,12,13]**
  - **is not leached in the groundwater[7,8,13]**
  - **is absorbed by plant roots only in small concentrations (and lower than the test)[14] and it was not observed contamination of the edible parts of several crops (maize, wheat, bean, radicchio, …)[7,11]**
  - **does not increase soluble Cr concentration in soil[7,9,10]**
  - **does not inhibit soil microbial activity[15,16,17].**

More often, Cd, Zn or Cu are found as contaminants of mineral or organic fertilizers. However, Cr(VI) also contaminates organic wastes, especially biosolids (Silveira, et al., 2003; Sloan et al., 1997). Tannery sludge
is another source of Cr (Gondek, 2008).

~~Both Cr3+ and Cr6+ are toxic to plants.~~ Toxicity of Cr in plants depends on its oxidation state, being Cr(VI) much more toxic than Cr(III). Cr(III) produces reactive oxygen species and may be toxic at high concentrations but the low solubility of Cr(III) in soil generally leads small

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[17] S. Mocali, "Studio della Diversità Microbica in Suoli Concimati con Cuoio Idrolizzato," Ph.D. Thesis, Università degli Studi di Firenze, Florence, 2003

concentrations of Cr in plants. The toxic properties of Cr(VI) are originated from its oxidizing action and formation of free radicals during its reduction to Cr(III) within the plant cells[18,19].

- ➔ **However the contents of Cr in plants growing in normal conditions range between 0.02 and 2 mg/kg and rarely exceed 5 mg/kg[20,21]. Since the level of phytotoxicity is around 10 mg/kg, it means that Cr is never contained at toxic levels in plants[20].**

Cr toxicity is based on oxidative damage of cellular structures (Panda and Choudhury, 2005). Chromium interfere with plant growth, nutrient uptake and photosynthesis, induces enhanced generation of reactive oxygen species, causes lipid peroxidation and alters the antioxidant activities. Plants tolerate Cr toxicity via various defense mechanisms such as complexation by organic ligands, compartmentation into the vacuole, and scavenging ROS via antioxidative enzymes. Thanks to such mechanisms of defence, translocation of Cr from roots to shoots is extremely limited. Roots accumulate Cr in a quantity 10-100 folds more than shoots independently on Cr state[22,23]. High accumulation in roots may be due to Cr immobilization in the vacuoles of the root cells[19]. It was demonstrated that Cr(III) uptake may drastically decrease the stability of Ca-polygalacturonates which play a key role in binding cell walls of the rhizodermis and cortical cells. The collapse of these substances stops the passive translocation of Cr(III) through the apoplast[24].

~~Consumption of Cr contaminated food can cause human health risks by inducing severe clinical conditions (Shahid et al., 2017).~~The food chain is well protected from the possible excesses of Cr in plants by a system known as "soil-plant barrier": the term was introduced by Chaney to describe "the waste-soil-plant-animal relationship of toxic elements". A soil-plant barrier prevents contamination of the food chain from trace elements limiting their levels in edible plant tissues. Protection is carried out by one or more of the following processes:

- if the element is not soluble in soil, uptake does not occur
- if the element is absorbed by roots but it is immobilized in fibrous roots, translocation is stopped
- phytotoxicity occurs when the element is present in edible plant tissues at concentrations significantly lower than those harmful to humans or animals.

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➔ All of these processes apply perfectly to Cr: indeed **Cr(VI) is readily immobilized in soil by adsorption, reduction and precipitation processes; only a fraction of the total Cr concentration is available for plant uptake. When this fraction is taken up by plants, quantities superior to 90% of absorbed Cr are retained in roots where they are reduced to Cr(III) species in a short time. Finally phytotoxic levels of Cr in the plant are less than 10 mg/kg[20].**

Chromium(III) nutritional supplements are widely consumed for their purportedantidiabetic activities.X-ray fluorescence microscopy(XFM) and X-ray absorption near edgestructure (XANES) studies have now shown that nontoxic doses of [Cr3O(OCOEt)6(OH2)3]+(A), a prospective antidiabetic drug that undergoes similar H2O2 induced oxidation reactionsin the blood as other Cr supplements, was also oxidized to carcinogenic CrVI and CrV inliving cells. Single adipocytes treated with A had approximately 1 mm large Cr hot spots containing CrIII, C rV, and CrVI (primarily CrVI thiolates) species. These results stronglysupport the hypothesis that the antidiabetic activity of CrIII and the carcinogenicity of CrVI compounds arise from similar mechanisms involving highly reactive CrVI and CrV intermediates, and highlight concerns over the safety of CrIII nutritional supplements

Effect of Cr content in soil on plant growth is reported in the study of Han et al.,(2004). Growth of Brassica juncea was inhibited at soil ~~Cr3+~~Cr(III) content higher than 500 mg kg^{-1} and ~~Cr6+~~Cr(VI) content higher than 100 mg kg^{-1}.The study was made in contaminated soils (and not natural agricultural soils) with Cr salts (and not Cr(III)-containing fertilizers) – Please see the note about the references.

➔ On the contrary **studies carried out using Cr(III)-containing fertilizers in agricultural soils report negligible risks of environmental pollution[7,8,10,13,25].**Indeed the organic constituents of the solid fertilizers play an important role in reducing the amount of Cr available to plants and no traces of Cr(VI) were found in the soils collected from various farms where these fertilizers had been used for decades to fertilize the fruit orchards[26]. Generally, Cr(III) added to soil through these fertilizers is gradually released due to mineralization of the organic matter: this process permits that Cr(III) is consequently fixed into soil and then unavailable to microbial and plant metabolism.

Content of Cr in plants and soil in real conditions of the Czech Republic reports Kacálková et al. (2014). They performed field experiment at Cr contaminated location near Hradec Králové. Content of Cr in soil did not exceed the limit according to regulation13/1994 and there was limited effect of increasing soil Cr on plant Cr content. Again, the study was made in contaminated soils (former waste incineration plant) and not in natural agricultural soils. Nevertheless, although soil

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was hugely contaminated by heavy metals, it was observed that Cr availability for plants was lower than the other heavy metals (6% Ni, 14% Cd, 1.3 % Pb, and less than 1% of Cr), it was largely retained at roots level (6.83 mg Cr·kg^{-1}, 5.04 mg Ni·kg^{-1}, and 7.76 mg Pb·kg^{-1}) and poorly translocated to the above ground biomass.

Chromium is also toxic for microorganisms (Cervantes et al., 2001). Inhibitory effect on soil microbial population observed Shi et al., (2002).The first study was carried out with Cr salts, in the second one the study was done in highly Cr-contaminated soil (a former tannery site) - Please see the note about the references.

- ➔ On the contrary **the main enzymatic activities of soils fertilized with Cr(III)-containing fertilizers did not show any statistically significant differences among the samples[15,26]. It was also demonstrated that adding these solid fertilizers to soil, a higher microbial biodiversity occurs and that the higher microbial activity is maintained in years[27].**

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Note: about the references on Cr issue, it is fundamental they report correct information. On the contrary often the references are based on incorrect contextualization.

Studies and data **must always be referred** to:

- soils (and not other media such as water where Cr behaviour is completely different in comparison to soil)
- soils with agricultural destination (and not polluted soils),
- the use of Cr(III)-containing fertilizers and not of Cr soluble salts (in the case of organic fertilizers, Cr(III) is linked (chelated) to organic matter and once in soil it is slowly released simultaneously to the slow degradation of organic matter. In this way Cr(III) is precipitated to Cr-hydroxide, adsorbed by soil colloids and it is not more available for any kind of transformation, including oxidation to Cr(VI)
- agronomical rates of the application of fertilizers (500-2000 kg/ha and not 10,000-20,000 kg/ha for a sludge).

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Ministero delle politiche agricole

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