VERSITA VOL. 24, NO 1(55): 27–31
DOI 10.2478/OSZN-2013-0007

Edyta Chrzanowska*, Radosław Kalinowski*, Marek Brytan*

Determination of PNEC for selected decontaminants based on seed germination and vigor indexes of terrestrial plants

Wyznaczenie wartości PNEC dla wybranych odkażalników w oparciu o indeksy kiełkowania i wigoru roślin lądowych

** Mgr Edyta Chrzanowska, dr inż. Radosław Kalinowski, ppłk dr Marek Brytan – Department of Pharmacology and Toxicology. Military Institute of Hygiene and Epidemiology. Kozielska 4 St, 01-163 Warsaw, phone: 22-681-61-05, e-mail: e.chrzanowska86@gmail.com, rkalinowski@wihe.waw.pl*

Keywords: Chemical warfare agents (CWA), military decontaminants, germination index (GI), seed vigor index (SVI)
Słowa kluczowe: Bojowe środki trujące (BST), odkażalniki wojskowe, indeks kiełkowania, indeks wigoru nasion

Abstract
Due to the existing threat of use of CWA, many countries developed special chemical formulations dedicated to remove CWA – the so-called military decontaminants. The function of the decontaminant is to neutralize the toxic properties of the contaminant through chemical reactions: oxidation and nucleophilic substitution.
The decontaminants released to the environment may transform to toxic products which have a harmful impact on plants and other soil biota. To evaluate the impact of these chemicals on plants phytotoxicity, early growth tests with *Sinapis alba, Lepidium sativum* and *Sorghum saccharatum* were used. Parallely with standard toxkit endpoints (seed germination and root elongation, data not presented here), seed vigor indexes and germination indexes were calculated. GI is a more sensitive endpoint in phytotoxicity testing than SVI. The EC50-72h values based on GI are about 2–3 times lower than those based on SVI. Very low PNEC soil values indicate that wide usage of such compositions in case of CWA release will cause damage to vegetation in the environment.

Streszczenie
W związku z nieustannie istniejącym zagrożeniem użycia bojowych środków trujących, wiele państw Świata wprowadziło w wyposażenie armii specjalnie przygotowane formulacje chemiczne – tzw. odkażalniki wojskowe. Zadaniem odkażalników jest neutralizacja toksycznych właściwości bojowych środków trujących, poprzez reakcje utleniania oraz substytucję nukleofilową. Odkażalniki te, po przedostaniu się do środowiska naturalnego, mogą tworzyć produkty o wysokiej toksyczności w stosunku do składowych ekosystemu, w tym organizmów roślinnych. W celu oceny fitotoksyczności badanych odkażalników wojskowych, przeprowadzony został krótkoterminowy test Phytotoxkit z *Sinapis alba*, *Lepidium sativum* oraz *Sorghum saccharatum*. Oprócz standardowych punktów końcowych (kiełkowanie nasion i wydłużanie korzeni, dane nieprezentowane w artykule) w teście oznaczony został indeks kiełkowania oraz indeks wigoru nasion. Wartości uzyskane dla indeksu kiełkowania nasion były ponad 2–3 razy niższe niż analogiczne wartości uzyskane dla indeksu wigoru. Obliczone w pracy wartości PNEC wskazują na możliwość wystąpienia znacznego zagrożenia dla ekosystemów glebowych w przypadku masowego zastosowania badanych odkażalników wojskowych w celu dekontaminacji skażonego terenu.

1. INTRODUCTION

The Chemical Warfare Agents (CWA) are defined as any toxic substances used to kill, injure or incapacitate an enemy during military operations [Sanderson et al. 2007].
The CWA differ in structure and toxic properties. They include broad class of chemical compounds like: nerve agents (tabun, soman, sarin, VX), blistering agents (nitrogen- and sulfur mustard, lewisite), pulmonary agents (chlorine, chloripicrin, phosgene, diphosgenenitrogen oxides), blood agents (cyanides, arsines) and incapacitating agents (LSD-25, ketamine, fentanyl, carfentanil) [Salem et al. 2008, Shannon et al. 2006].
The CWA have been used in several military conflicts but their use reached a peak during the World War I, when the French were the first to use these as tear gases (ethyl bromoacetate, chloroacetone). It was followed by the use of o-dianisidine chlorosulphonate, chloroactetate, chlorine, hydrogen cyanide, diphenylchloroarsine ethyl- and methyldichloroarsine and sulfur mustard [Chauchan et al. 2008, Szinicz 2005]. The consequence of this military operation was the death of nearly 10 000 people and over million casualties. Before World War II, the CWA were still stockpiled by many countries but the CWA were not used in the field due to the fear of massive retaliation, except for the Germans, who used gas chambers for mass genocide of the Jews [Chauchan et al. 2008]. According to the current information, about 40 000 tons of chemical munitions (13 000 tons of chemical warfare agents) were dumped in the Baltic Sea in the south areas of the Little Belt, around Bornholm and South of Gotland [Sanderson et al. 2007, Szinicz 2005].
After the Second World War, the CWA were used in the Iraq-Iran war [Mansour et al. 2012, Zafarghandi et al. 2013] and as an act of terrorism in Japanese underground rail station [Salem et al. 2008]. Throughout the world about 70 different chemicals have been developed, produced and stockpiled as CWA or Chemical Weapon of Mass Destruction (CWMD) during the 20th and 21st centuries [Sanderson et al. 2007].
Due to the existing threat of the use of CWA many countries developed special chemical formulations dedicated to remove CWA – the so-called military decontaminants. The classical composition of decontaminants includes an active substance that causes contaminant inactivation, suitable sorbent designed to bind and separate the contaminants and the filler or carrier to facilitate deposition [Antkowiak et al. 2010]. The decontaminant neutralizes the toxic properties of the contaminant through chemical reactions: oxidation (by chlorine, peroxides or reactive gases) and nucleophilic

substitution by means of alkaline hydrolysis and oximes [Waysbort et al. 2009].

Many pieces of research contributed to development of numerous technologies to decontaminate CWA from human body, equipment and environment.

Surface-applied technologies allow us to apply a decontaminant directly on the surface contaminated with biological or chemical agents, the examples are: hypochlorite, aqueous hydrogen peroxide, aqueous chlorine dioxide and nanoemulsions. Hypochlorites were used as the very first decontaminants [Yang 1992, Waysbort et al. 2009]. Calcium hypochlorite, in particular, easily penetrates the biofilm and effectively destroys microorganisms in it. Based on these strong compounds decontaminants like STB (Super Tropical Bleach), HTH (High Test Hypochlorite) were created. Despite their high efficacy to inactivation of yperite (HD), soman (GD), sarin (GB) and VX, they are very corrosive to other materials like: soldier's equipment, skin or surfaces. The alternatives of such decontaminants are compositions based on NADCC (sodium dichloroisocyanurate) like: powder decontaminant – BX24 and chloramines [Boone 2007, Jang 2009, Love et al. 2011]. Chloramine B is an active compound of American military decontamination kits: M258A1 and M280 (chloramine B soaked towels, used to decontaminate the body and individual soldier equipment) and Polish IPP95 military kit [Love et al. 2011, Yang et al. 1992].

The chlorine-based decontaminants are very well recognized. Their decontaminant properties were known in the beginning of 20th century; for example halazone was used as a water disinfectant in 1917 due to the possibility of storage in the form of tablets, similarly chloramines, that were widespread decontaminants against CWA since 1916 [Al-safi 2011].

Active decontaminant's compounds after release to the environment may transform to another toxic products. Examples of such processes are transformations of another common decontaminant – triclosan – introduced to chemical industry in 1972. After release to the environment, triclosan may be converted into toxic dioxins (polychlorinated dibenzo-p-dioxin) and after contact with chlorinated water – even chloroform [Canosa et al. 2005, Kookana et al. 2011, Latch et al. 2003]. The decontaminants released to the environment may have a harmful impact on plants, both in a normal usage – performing contamination clean up, as well as during an accidental release.

The toxicants and their degradation products may contribute to the disturbance of many physiological processes in plant cells. Interactions of these stress factors with plant's cellular components may lead to oxidative stress and release of reactive oxidative species (ROS). The ROS may be produced as the result of the metabolism of these chemicals by cytochrome P450s. Rapid and non-specific reactions of ROS may lead to the damage of all classes of biomolecules including lipid peroxidation, inactivation of enzyme, destruction of proteins, nucleic acids and other functional molecules [Breen and murphy 1995, Fridovich 1985, Stadtman 1992]. In the chloroplasts, primary protein targets for ROS are thiol-containing enzymes participating in the CO_2-fixalion cycle: fiuctose-l,6-bisphosphatase, glyceraldehyde-3-phosphate dehydrogenase and ribulose-5-phosphate kinase [Asada1987].

There are studies confirming that some of these chemical agents and their degradation products may be cumulated in plant's tissues [Karnjanapiboonwong 2011] and significantly influence the reduce fatty acid biosynthesis and total lipid content by inhibiting the trans-2 enoyl- ACP reductase. Coogan et al. [2007] estimated coefficient BAF values for triclosan in algae at the level of 900–2100. The lower coefficient values BCF for soybeans (*Glycine max*) (2 and 6 respectively for 60 and 110 days' exposition) were reported by Wu et al. [2010].

There is a wide range of methods for testing phytotoxicity implemented by different authorities like: ASTM, OECD, ISO, US EPA and others. There are many various measured endpoints in these methods, like: fresh and/or dry weight of the shoot, the number of seedling emergence, the number of plants remaining at the harvest, the germination rate, a valuation (development of cotyledons, root – health and intensity, plant development), root weight, root length, development of the root system, germination rate, shoot/root ratio, plant abnormalities, mitotic and meiotic aberrations and others. **[ASTM E1841, ASTM E1598, OECD 208, OECD 227, ISO 11269-2:1995, ISO/CD 17126, ISTA 1976, ISTA 1999, US EPA 1996 a-e**, Kristen 1997].

The most commonly used tests in phytotoxicity assessment are Growth Test, Conductivity Test (based on measurement of electrical conductivity indicates the level of membrane permeability) [Vieira et al. 2004, Leeks 2006], Hiltner Test (growing test in brick gravels, weak seeds have no force to overcome the pressure of brick gravels), Paper Piercing Test (growing test in paper piercing, weak seedlings are not able to pierce the paper), Cold Test (the test differentiate between weak and vigorous seeds by subjecting them to low temperature before germination) and Accelerated Ageing Test (the seeds are subjected to high temperature and high humidity before germination) [Khan et al. 2010].

There is still very little information about impact of military decontaminants and their active substances on the environment.

To evaluate an impact of such compositions on phytotoxicity, early growth tests with *Sinapis alba, Lepidium sativum* and *Sorghum saccharatum* were used. Parallely with standard toxkit endpoints (seed germination and root elongation, data not presented here), seed vigor indexes and germination indexes were calculated. The seed germination test and seed vigor test were initially used in seed quality assessment, but they were also useful in ecotoxicity testing. According to the ISTA (International Seed Testing Association) [Hampton & Tekrony 1995] definition, vigor is "*the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence*". The main aim of this paper is to evaluate an impact of tested military decontaminants on plants, based on seed germination index (GI) and seed vigor index (SVI) in the Growth Test. The assumption of the Growth Test is that vigorous seeds grow faster than poor vigor seeds even if both of them grow up in favorable conditions. The vigorous seeds are characterized by faster germination, better metabolism and establishment in the field.

2. MATERIALS AND METHODS

Test substances:

Four powder disinfectants, containing an active chlorine-based component, sorbent and filler were chosen as model substances. These are:

1. Decontaminant I (IPP95): chloramine B (CAS: 127-52-6) – 38%, zinc oxide (CAS: 1314-13-2) – 5%, zeolite (CAS: 1318-02-1) – 50%, magnesium stearate (CAS: 557-04-0) – 7%.
2. Decontaminant II: chloramine T (CAS: 7080-50-4) – 30%, zinc oxide (CAS: 1309-48-4) – 65%, magnesium stearate (CAS: 557-04-0) – 5 %.
3. Decontaminant III: triclosan (CAS: 3380-34-5) – 30%, zinc oxide (CAS: 1309-48-4) – 65%, magnesium stearate (CAS: 557-04-0) – 5 %.
4. Decontaminant IV: halazone (CAS: 80-13-7) – 30%, zinc oxide (CAS: 1309-48-4) – 65%, magnesium stearate (CAS: 557-04-0) – 5 %.

Phytotoxicity assessment

The Phytotoxkit test with *Sorghum saccharatum*, *Lepidium sativum* and *Sinapis alba* seeds, was executed according to the SOP supplied by the producer of the test – Microbiotest (Belgium). The standard OECD soil was spiked by disinfectants in concentrations of 3–300 g/kg dw (with series of dilution at q = 2,5). The samples were incubated in the dark, for 72 h, at 25°C. After incubation, the number of germinated seeds was counted and the root's and stem's length were measured using UTHSCSSA ImageTool (ver. 3.0) image analysis software. All experiments were conducted in triplicate.

Germination index (GI) and seed vigor index (SVI) for each treatment were calculated using equations (1) and (2) respectively:

$$GI = N \cdot L \quad (1)$$

where: N is the number of germinated seeds, and L is the mean root length [mm].

$$SVI = G \cdot S \quad (2)$$

where: G is the percentage of the germination [%], and S is the mean length of the seedling [mm]. Inhibition of GI and SVI was calculated using equation 3.

$$Inhibition = (I_c - I_t) \cdot Ic^{-1} \cdot 100 \quad (3)$$

where: I_c is an GI or SVI in control samples, and I_t is GI or SVI in treated samples.

EC50–72h values were calculated using the probit method. Based on obtained EC50–72h values for GI and SVI, the predicted no effect concentrations (PNEC) for soils were calculated according to the TGD – assessment factor equal 1 000 **[Technical Guidance Document 2003]**.

3. RESULTS

Germination index in control samples ranged from 480 to over 600 for *Sorghum saccharatum* and *Lepidium sativum* respectively. The highest tested concentrations of selected decontaminants reduced the GI values by over 70% for all tested plants. EC50–72h values for GI and SVI are presented in Tables 1 and 2.

The decontaminants showed diverse ecotoxicity toward the tested plants. EC50-72h values for GI ranged from 8,9 mg as/kg dw for decontaminant III and *Sorghum saccharatum* to 42 mg as/kg dw for decontaminant I and *Sinapis alba*. *Sorghum saccharatum* seems to be the most sensitive species in terms of decontaminants' impact on GI.

Seed vigor index varied among tested species in control samples from 1240 for *Sorghum saccharatum* to 4250 for *Lepidium sativum*. The highest tested concentrations of selected decontaminants reduced the SVI values by over 60% for all tested plants in exception of decontaminant I and Sinapis alba.

EC50-72h values ranged from 12,2 mg as/kg dw for decontaminant III and *Lepidium sativum* to 265,4 mg as/kg dw for decontaminant I and Sinapis alba. Decontaminant I seems to be the least toxic of the tested compositions.

GI is a more sensitive endpoint in phytotoxicity testing than SVI. EC50-72h values based on GI are about 2-3 times lower than those based on SVI.

Based on EC50-72h values for GI and SVI, safety concentrations for decontaminants were calculated. Predicted no effect concentrations (PNEC) for selected decontaminants in soils are presented in Table 3.

Table 1. The EC50-72 h values for the tested decontaminants based on germination index

	Sinapis alba		*Lepidium sativum*		*Sorghum saccharatum*	
	EC50-72h [mg/kg dw]	EC50-72h [mg as*/kg dw	EC50-72h [mg/kg dw]	EC50-72h [mg as*/kg dw]	EC50-72h [mg/kg dw]	EC50-72h [mg as*/kg dw]
Decontaminant I	110,4 [77,6-160,2]	41,95 [29,48-60,88]	25,9 [13,0-57,6]	9,84 [4,94-21,88]	70,9 [47,3-106,3]	26,94 [17,97-40,39]
Decontaminant II	50,1 [27,2-92,5]	15,3 [8,16-27,75]	55,3 [15,7-181,3]	16,59 [4,71-54,39]	32,8 [26,8-40,2]	9,84 [8,4-12,06]
Decontaminant III	83,0 [30,0-299,9]	24,9 [9,0-68,97]	122,9 [107,9-140,2]	36,87 [32,37-42,03]	29,7 [13,2-66,7]	8,91 [3,96-20,01]
Decontaminant IV	72,2 [30,4-171,5]	21,66 [9,12-51,45]	111,9 [98,4-138,6]	33,57 [27,12-41,58]	78,6 [66,5-92,9]	23,58 [19,95-27,87]

* - active substance

Table 2. The EC50-72 h values for the tested decontaminants based on seed vigor index

	Sinapis alba		*Lepidium sativum*		*Sorghum saccharatum*	
	EC50-72h [mg/kg dw]	EC50-72h [mg as*/kg dw]	EC50-72h [mg/kg dw]	EC50-72h [mg as*/kg dw]	EC50-72h [mg/kg dw]	EC50-72h [mg as*/kg dw]
Decontaminant I	698,5 [523,7-931,7]	265,43 [199,0-354,05]	52,2 [21,4-127,1]	19,83 [8,13-48,3]	250,6 [113-556]	95,23 [42,94-211,28]
Decontaminant II	78,2 [63,4-96,5]	23,46 [19,02-28,95]	66,4 [47,3-93,2]	19,92 [14,19-27,96]	44,9 [37,7-51,2]	13,17 [11,31-15,36]
Decontaminant III	214,7 [147,3-312,9]	64,41 [44,19-93,87]	40,8 [22,7-64,8]	12,24 [6,81-19,44]	68,6 [57,2-82,2]	20,58 [17,16-24,66]
Decontaminant IV	136,6 [37,8-493,8]	40,98 [11,34-148,14]	141,2 [123,6-161,2]	42,36 [37,08-48,36]	244,0 [214,6-277,5]	73,2 [64,38-83,25]

* - active substance

Table 3. PNEC soil values for the tested decontaminants

	PNEC soil [mg as*/kg dw]
Decontaminant I	0,00984
Decontaminant II	0,00984
Decontaminant III	0,00891
Decontaminant IV	0,02167

* - active substance

4. CONCLUSION

Chlorine-based decontaminants may pose significant hazard to soil biota, especially plants. These oxidizing agents released to the environment may contribute to disturbance of many physiological processes in plant cells.

The decontaminants show diverse ecotoxicity toward the tested plants. The results obtained in this research show that the tested decontaminants have an influence on GI and SVI. The highest tested concentrations of selected decontaminants reduced GI and SVI by over 70% and 60% respectively, for all tested plants. Additionally, GI is a more sensitive endpoint in phytotoxicity testing than SVI.

Very low PNEC soil values indicate that wide usage of such compositions in the case of CWA release will cause damage to vegetation in the environment. Although European legislation allows usage of single test result as a base in PNEC calculation, it should be noted that such estimations may be unrealistic. It must be pointed out that further tests especially long term studies conducted with/on other organisms e.g. consumers and decomposers will allow/enable us to reduce very conservative assessment factor used in this case, and therefore may provide more realistic values of hazardous concentrations of military decontaminants.

REFERENCES AND LEGAL ACTS

AL-SAFI S.A.J. 2011. Study of halazone stability and methods of its removal from aqueous solutions. Eur. J. Sci. Res. 54(2), 311-324.

ANTKOWIAK O., BRYTAN M., ZDANOWSKI R., KALINOWSKI R. 2011. Współczesne metody dekontaminacji bojowych środków trujących. Lek. Wojsk. 89(2), 111-115.

ASADA K, TAKAHASHI M. 1987. Production and scavenging of active oxygen in photosynthesis. In: *Photoinhibition*, ed. DJ Kyle, CB Osmond, CJ Arntzen, pp. 227–87. Amsterdam: Elsevier.

ASTM E1841 - 04(2012) Standard Guide for Conducting Renewal Phytotoxicity Tests With Freshwater Emergent Macrophytes.

ASTM E1598-94 (1994): Practice for Conducting Early Seedling Growth Tests.

BOONE C.M. 2007. Present state of CBRN decontamination methodologies. Report TD2007-0020. TNO Defence, Security and Safety, Holandia, Rijswijk.

BREEN A.P., MURPHY J.A. 1995. Reactions of oxyl radicals with DNA. Free Rod. Biol. Med. 18, 1033-1077.

CANOSA P., MORALES S., RODRÍGUEZ I., RUBÍ E., CELA R., GÓMEZ M. 2005. Aquatic degradation of triclosan and formation of toxic chlorophenols in presence of low concentrations of free chlorine. Anal. Bioanal. Chem. 383, 1119–1126.

CHAUHAN S., CHAUHAN S., D'CRUZ R., FARUQI S., SINGH K.K., VARMA S., SINGH M., KARTHIK V. 2008. Chemical warfare agents. Environ Toxicol Pharmacol. 26(2), 113-22.

COOGAN M.A., EDZIYIE R.E., LA POINT T.W., VENABLES B.J. 2007. Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in a North Texas wastewater treatment plant receiving stream. Chemosphere. 67(10), 1911-1918.

FRIDOVICH I. 1985. Cytochrome c. In: CRC handbook of methods for oxygen radical research. CRC Press, Boca Raton.

HAMPTON J.G., TEKRONY D.M.. 1995. Handbook of vigour test methods. Intl. Seed Testing

Assn., Zurich, Switzerland.

ISTA. 1976. International rules of seed testing. Rules and annexes. International Seed Testing Association. Seed Sci. Technolog. 4,3–177.

ISTA. 1999. International rules of seed testing. Rules 1999. Seed Sci. Technol. 27, 1–303.

ISO 11269-2:1995 Soil Quality – Determination of the effects of pollutants on soil flora – Effects of chemicals on the emergence and growth of higher plants.

ISO/CD 17126 SOIL QUALITY – Determination of the effects of pollutants on soil flora – Seedling emergence, screening test with lettuce (*Lactuca sativa* L.).

JANG H. Organic chloramines formation and its disinfection efficacy. A Dissertation Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy. Arizona State University 2009.

KARNJANAPIBOONWONG A., CHASE D.A., CANAS J.E., JACKSON W.A., MAUL J.D., MORSE A.N., ANDERSON T.A. 2011. Uptake of 17a-ethynylestradiolandtriclosaninpintobean, *Phaseolus vulgaris*. Ecotoxicol. Environ. Safety. 74, 1336–1342.

KHAN A.Z., SHAH P., MOHD F., KHAN H., PERVEENA.S., NIGAR S., KHALIL S.K., ZUBAIR M. 2010. Vigour tests used to rank seed lot quality and predict field emergence in wheat. *Pak. J. Bot*. 42(5), 3147-3155.

KOOKANA R.S., YING G.G., WALLER N.J. 2011. Triclosan: its occurrence, fate and effects in the Australian environment. Wat. Sci. Tech. 3(4), 598-604.

KRISTEN U. 1997. Use of Higher Plants as Screens for Toxicity Assessment. Toxicol. In Vitro. 181-191.

LATCH D.E., PACKER J.L., ARNOLD W.A., MCNEILL K. 2003. Photochemical conversion of triclosan to 2,8-dichlorodibenzop-dioxin in aqueous solution. J. Photochem. Photobiol. 158, 63–66.

LEEKS C.R.F. 2006. Determining seed vigour in selected Brassica species, Master thesis, Lincoln University.

LOVE A.H., BAILEY C.G., HANNA M.L., HOK S., VU A.K., REUTTER D.J., RABER E. 2011. Efficacy of liquid and foam decontamination technologies for chemical warfare agents on indoor surfaces. J. Hazard. Mater. 196, 115-122.

MANSOUR R.S., SALAMATI P., SAGHAFINIA M., ABDOLLAHI M. 2012. A review on delayed toxic effects of sulfur mustard in Iranian veterans. Daru. 20(1), 51.

SANDERSON H.; THOMSEN M.; FAUSER P.; SØRENSEN P.B. 2007. PBT screening profile of chemical warfare agents (CWAs). J. Hazard. Mater. 148, 210–215.

Szinicz L. 2005. History of chemical and biological warfare agents. Toxicol. 214, 167–181.

TERNAY JR A.L., SMART J.K., SALEM H. Brief History and Use of Chemical Warfare Agents in Warfare and Terrorism, in: Chemical Warfare Agents. Chemistry, Pharmacology, Toxicology, and Therapeutics. CRC Press 2007.

OECD-Guideline for the testing of chemicals: Proposal for updating Guideline 208, 2000. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test.

OECD- Guideline for the testing of chemicals: Proposal for a new Guideline 227, 2003. Terrestrial Plant Test: Vegetative Vigour Test.

STADTMAN E.R. 1992. Protein oxidation and aging. Science. 257, 1220-1224.

Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances; Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances; Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. 2003. Luxembourg.

US EPA, 1996 a. OPPTS Ecological Effect Guideline, 850.4025: Target Area Phytotoxicity.

US EPA, 1996 b. OPPTS Ecological Effect Guideline, 850.4100: Terrestrial Plant Toxicity, Tier I (Seedling Emergence).

US EPA, 1996 c. OPPTS Ecological Effect Guideline, 850.4200: Seed Germination/Root Elongation Toxicity Test.

US EPA, 1996 d. OPPTS Ecological Effect Guideline, 850.4225: Seedling Emergence, Tier II.

US EPA, 1996 e. OPPTS Ecological Effect Guideline, 850.4230: Early Seedling Growth Toxicity Test.

VIEIRA R.D., NETO A.S., MUDROVITSCH DE BITTENCOURT S.R., PANOBIANCO M. 2004. Electrical conductivity of the seed soaking solution and soybean seedling emergence. Sci. Agric. 61(2).

WU C., SPONGBERG A.L., WITTER J.D., FANG M., CZAJKOWSKI K.P. 2010. Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated water. Environ. Sci. Tech. 44(16), 6157-6161.

WAYSBORT D., MCGARVEY D.J., CREASY W.R., MORRISSEY K.M., HENDRICKSON D.M., DURST H.D. 2009. A decontamination system for chemical weapons agents using a liquid solution on a solid sorbent. J. Hazard. Mater. 161, 1114–1121.

YANG Y., BAKER J.A., WARD R. 1992. Decontamination of chemical warfare agents. Chem. Rev. 92(8), 1729-1743.

ZAFARGHANDI M.R., SOROUSH M.R., MAHMOODI M., NAIENI K.H., ARDALAN A., DOLATYARI A., FALAHATI F., MIRMOHAMMADKHANI M., MOUSAVI B., GHANEI M. 2013. Incidence of cancer in Iranian sulfur mustard exposed veterans: a long-term follow-up cohort study. Cancer Causes Control. 24(1), 99-105.