CORVELVA
insieme dal 1993

First revision December 16, 2018

Vaccinegate:

Study on the chemical composition profile of Infanrix Hexa

Translated by team
CLiVa
clivatoscana.com

CORVELVA
corvelva.it
info@corvelva.it
facebook.com/corvelva

CORVELVA
insieme dal 1993

First revision December 16, 2018

Brief presentation of the results

When we started these analysis, from the metagenomics to the chemical ones, we had a lot of questions and we were only looking for answers...
After these first results, more questions have arisen and so did the concerns!
The quali-quantitative analysis of organic compound is of great importance in the pharmacological field, as potential safety problems arise from the new production processes of biological drugs and from the complex structural and biological characteristics of these products.

In Infanrix Hexa we found:

- **chemical contamination from the manufacturing process or cross-contamination with other manufacturing lines;**
- **chemical toxins;**
- **bacterial peptide toxins;**
- **insoluble and indigestible macromolecule that reacts to the protein assay, but cannot be recognized by any protein databases.**

We have not found:

- **Protein antigens of diphtheria toxoids, tetanus, pertussis, hepatitis B, haemophylus influenzae B, Poliomyelitis 1-2-3;**
- **Formaldehyde and glutaraldehyde, phenoxyethanol, antibiotic residues indicated in the composition;**

In Infanrix Hexa there are six antigens

Tetanus, diphtheria and pertussis toxoids, D antigens of Poliomyelitis 1-2-3, hepatitis B proteins obtained with genetic engineering and Haemophylus polysaccharides chemically linked to tetanus toxoid as carrier. Toxoids are created by treatments with formaldehyde and glutaraldehyde that should remove toxicity keeping intact their ability to stimulate protective antibodies against original toxins.
We were expecting to find the three toxoids and the other antigens not modified by treatment with formaldehyde and glutaraldehyde, to separate the antigens from each other and to be digestible by the enzyme specific for proteins (trypsin). **We have found instead a real polymer, insoluble and indigestible, that we supposed to be the set of antigens chemically bound together (has to be defined if this is present as an aggregate of the individual antigens or a single macromolecule), on which we can find in literature partial information regarding the single antigens.**

This macromolecule could not be recognized in any way by the protein databases, and in fact it turned out to be a solid compound of an unknown chemical structure.

Proteins solubility and their digestion (i.e. the capacity to divide them into small peptide fragments) are two typical proteins characteristics that not only makes it possible to study them through some specific analysis methods **but are also fundamental for the interaction with the immune system to create protective antibodies**, because if the protein structure is heavily altered from the original one, the new antibodies result completely different from those that are able to attack the original antibodies causing illnesses.

Since this polymer we have encountered, derived from the antigenic mix, is not only different for its spatial conformation but it's chemically different, so **we can state that we are not facing antigens similar to the original ones but in the form of a compound with an unknown and unpredictable toxicity and efficacy.**

Not only vaccine antigens have been not detected, there were also 65 signs of chemical contaminants of which only 35% is known, there are among these various processing residues and cross-contaminations from other manufacturing lines, and their identification will be checked during the second level of the analytical study (i.e. with standard controls).

7 chemical toxins among these signals have also been identified, probably deriving from chemical contaminants of the manufacturing process or other manufacturing lines at the vaccine manufacturing site; these toxins have a structure that could probably be partially derived from the formaldehyde, glutaraldehyde and cyanogen bromide reaction with other chemical contaminants in the vaccine. We'd like to point out that the toxicity of many of these toxins have been confirmed and published in Pubchem or Toxnet and **this poses important safety problems, issues and concerns.**

From the protein and peptide fraction study, various free peptides of bacterial origin have been obtained probably coming from the bacterial culture cells used for the antigen extraction. **Literature reports bacterial peptides as potential allergens 5 and also as capable of inducing autoimmune**

reactions 6 and these too put a safety issue that needs to be further clarified with the regulatory bodies.

Coming back to the two basic principles that have been our topic on this analysis path, we reaffirm what we have said in the recent interview on the scientific journal Nature: we are inquiring the vaccines efficacy and safety and we can't quite understand how it is possible to claim that this vaccine is even able to generate the 6 protective antibodies - reason why it is designed for - and furthermore to understand how this cluster made of 6 neurotoxic antigens bound together can be claimed as not toxic for newborns.

Infanrix Hexa hexavalent, as for the method we have commissioned, casts major doubts on both its effectiveness and on its safety...
One thing is for sure: we will not stop to proceed.

CORVELVA
insieme dal 1993

First revision December 16, 2018

Study on the chemical composition profile of Infanrix Hexa

Introduction and description of the need

The quali-quantitative analysis of organic compounds is of great importance in the pharmacological field [1], as potential safety problems arise from the new production processes of biological drugs and from the complex structural and biological characteristics of these products. [2]
The review of the registration dossiers for military vaccines that we find in the final report[3] of the Parliamentary Commission of Inquiry "Depleted Uranium"[4] revealed the presence of protein-chemical contaminants and impurities, which required further analytical study. Our association has decided to take charge of it, as far as possible.
This project is part of the above-mentioned insights. It has been therefore necessary to develop a technology capable of analyzing a wide spectrum of molecules of chemical, metabolic and protein origin in order to evaluate the quality of the obtained results.
A method has been therefore developed, based on SANIST technology to test vaccines for purity and safety (further information below).

Results and Discussion

1. Analysis of the composition declared in the vaccine leaflet

Compound	Presence	Ionic species
Amino acids	YES	$[M+H]^+$
Formaldehyde[5]	Not detected	-
Lactose anhydrous	YES	$[M+H-H_2O]^+$
Vitamins	Not detectable	-
Water	YES	$[M+H]^+$
Neomycin	Weak signal	$[M+2H]^{2+}$
Diphtheria Toxoid[6]	Not detected	$[M+nH]^{n+}$
Tetanus Toxoid[7]	Not detected	$[M+nH]^{n+}$
Pertussis Toxoid[8]	Not detected	$[M+nH]^{n+}$
Filamentous Haemagglutinin Adhesin (FHA)	Not detected	$[M+nH]^{n+}$
Pertactin (PRN)	Not detected	$[M+nH]^{n+}$
Haemophilus Influenzae B polysaccharide[9]	Not detected	$[M+nH]^{n+}$
Polyribosylribitol Phosphate (PRP) [10]	Not detectable	-
Polymyxin[10]	Non rilevabile	-

[1] Lett Appl Microbiol. 2015 Feb;60(2):174-80. doi: 10.1111/lam.12355 - https://www.ncbi.nlm.nih.gov/pubmed/25376111
[2] Fuchs F., Biochimie. 2002 Nov;84(11):1173-9 - https://www.ncbi.nlm.nih.gov/pubmed/12595146
[3] http://www.camera.it/leg17/491?idLegislatura=17&categoria=022bis&tipologiaDoc=documento&numero=023&doc=pdfel
[4] http://www.camera.it/leg17/436?shadow_organo_parlamentare=2588
[5] https://pubchem.ncbi.nlm.nih.gov/compound/formaldehyde
[6] https://www.who.int/biologicals/vaccines/diphtheria/en/
[7] https://www.who.int/ith/vaccines/tetanus/en/
[8] http://www.who.int/biologicals/vaccines/pertussis/en/
[9] https://www.who.int/biologicals/areas/vaccines/haemophilus/haemophilus_influenzae_typeb_Hib/en/
[10] https://www.sciencedirect.com/topics/neuroscience/polymyxin

2. Protein fraction analysis

According to the manufacturer, Infanrix Hexa vaccine contains some proteins. The sample has been analyzed for the identification of these proteins. At a visual analysis, the sample appears milky.

Different analysis have been conducted on the sample:

2.1 - 1st analysis: Digestion as it is

To start with, the sample has been subjected to an enzymatic digestion process: 10 µL of a raw sample has been treated with 50 µL Trypsin, left overnight in thermoblock at 37 ° C. A 1 mg / mL hemoglobin control has been prepared and treated as the sample.
This analysis revealed the absence of any proteins in the sample.

2.2 - 2nd Analysis: Digestion of Precipitate

The sample has been then subjected to further analysis by separating, by centrifugation, the liquid part from the solid part of the milky suspension. All the supernatant has been taken. The remaining precipitate has been treated with 30 µL Trypsin and left overnight in thermoblock at 37 ° C. A 1 mg / mL hemoglobin control has been prepared and treated as the sample. After digestion, the sample and the control have been centrifuged. The supernatant has been taken and placed in vials for analysis. 20 µL osmotized H_2O have been added in order to give enough volume for injection.
This analysis revealed the absence of any proteins in the sample.

2.3 - 3rd analysis: Bradford assay

To identify the actual presence of proteins, Infanrix Hexa vaccine has been subjected to the Bradford assay. 200 µL of the raw sample has been treated with 300 µL osmotic H_2O to obtain volume. Then 500 µL of Bradford reagent have been added. After a visual analysis, we can confirm the presence of proteins or peptide sequences given by the blue color (see Figure below):

Based on the calibration line, a protein concentration of 1.099 mg/mL was detected.

2.4 - 4th analysis: Digestion as it is at 57 ° C

After Bradford's assay, 20 µL of the raw sample has been treated with 80 µL of Trypsin. A 1 mg / mL hemoglobin control has been prepared and treated as the sample. They have been left in thermoblock at 37 ° C for 4 hours and then at 57 ° C for 30 minutes. The sample and the control have

then been subjected to centrifugation and the supernatant has been taken and placed in vial for analysis.

In order to process the data thus obtained, the Mascot[11] database has been initially used but **nothing has been found**. Therefore, the GMP has been used but also in this case **no protein sequences have been detected**. By the DeNovo research, the following peptide sequences that do not meet the trypsin cutting criteria and therefore potentially belong to free peptides have been identified. Below is the detected sequences list:

YLSA	YLSA	SLGS	HNLPFT
QLYTCC	CHFAHD	WRASST	SYLPFT
SAGE	HLLNMT	YSDDQC	NMAWW
DEV	CHPPYL	TDTENW	GPFRVW
AEYHW	TLAPRF	ALAPWF	RWGPLH
DEV	GSAAG	MNFHR	DSYWH
VLYACPP	DEV	NSNWW	WGC
	SNCGYY	VFHRF	

These sequences have been filed into the MS-BLAST [12] search engine obtaining the characterizations reported in Table 1. As can be seen, they have been potentially attributed by structural similarity to proteins of the bacterial world. **The proteins relating to the antigens present in the vaccine have not been detected.** This may be due to its extensive structural modification, introduced by formaldehyde and glutaraldehyde. In fact, the database research has been carried out Without considering the m/z variation introduced by the above-mentioned compounds

It is important to verify whether these changes have led to cross-linked macromolecular complexes shaping. In this regard, we will ask for further analysis using MALDI-TOF-MS [13] technology widely acknowledged in clinical practice for the study of high weight macromolecules,

Table 1 - Batch #1 (A21CD072D)

Name of Protein	Organism	Total Score
▪ hypothetical protein CALCODRAFT_501505	▪ Calocera cornea HHB 12733	159
▪ erg4/erg24 family protein	▪ Dictyostelium lacteum	154
▪ 3-phenylpropionic acid transporter	▪ Rhodopseudomonas palustris	154
▪ hypothetical protein LAESUDRAFT_731137	▪ Laetiporus sulphureus 93-53	149
▪ aldehyde ferredoxin oxidoreductase ▪ aldehyde ferredoxin oxidoreductase	▪ Alkaliphilus oremlandii ▪ Alkaliphilus oremlandii OhILAs	138
▪ hypothetical protein KAFR_OH02570	▪ Kazachstania africana CBS 2517	136
▪ hypothetical protein	▪ Pseudoxanthomonas mexicana	136
▪ hypothetical protein	▪ Endozoicomonas elysicola	135
▪ Homeodomain-like DNA binding domain-containing transcription factor	▪ Phycomyces blakesleeanus NRRL 1555(-)	110
▪ transcriptional regulator ▪ DNA-binding protein	▪ Streptomyces clavuligerus ▪ Streptomyces clavuligerus ATCC 27064	109
▪ glycosyl hydrolase family 3 N-terminal domain protein	▪ Firrmicutes bacterium CAG:56	108
▪ PREDICTED: zinc finger CCCH domain-containing protein 69-like	▪ Pyrus x bretschneideri	108
▪ hypothetical protein CC1G_06886	▪ Coprinopsis cinerea okayama7#130	108
▪ hypothetical protein FIBSPDRAFT_917685	▪ Fibulorhizoctonia sp. CBS 109695	107
▪ uncharacterized protein ▪ unnamed protein product	▪ Blastocystis hominis ▪ Blastocystis hominis	104
▪ hypothetical protein J132_09024	▪ Termitomyce ssp. J132	104

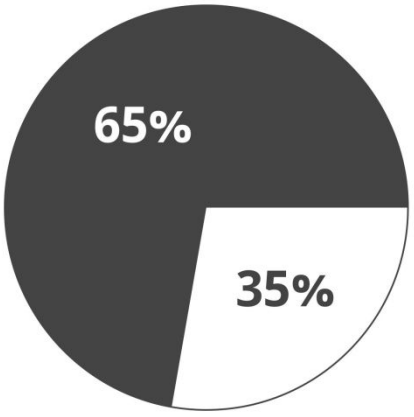
[11] http://www.matrixscience.com/help/seq_db_setup_db_gui.html
[12] http://genetics.bwh.harvard.edu/msblast/
[13] https://it.wikipedia.org/wiki/MALDI

3. Metabolic fraction Analysis

It should be emphasized that this screening study provides a semi-quantitative data which correspond to a range **from nanograms to micrograms** as an indicative order of magnitude. To obtain accurate quantitative data, it will be necessary to proceed using certified analytical standards of known strength.

Below, we report the identification screening results obtained in the two batches under examination:

Batch #1 (A21CD072D)



Unknown compounds

Known compounds

In Batch # 1 we have 65 signals of which only 35% are known

It has not been possible to carry out the analyzes on different batches because since all the Infanrix Hexa we have purchased for more than a year on the national territory, in different regions and in different periods, belong to batch A21CD072D.

NOTES FOR UNDERSTANDING: this is a first level analysis, that is an identification based on molecular weight. If the result is univocal, (ie at a measured molecular weight only one compound is associated as a structure) it is more likely the right one, but absolute certainty is not possible in this phase. As you will notice, as far as a certain number of compounds is concerned, different substances correspond to a particular molecular weight.

3.1. Infanrix Hexa Vaccine - Batch # 1 (A21CD072D)

65 signals were detected, among which only the **35%** gave a potential classification (**Table 2**).

It must be specified that compound identity is not certain and it should be confirmed by a second level screening carried out with certified analytical standards.

In fact, during screening level, the device measures a particular data by its accurate molecular weight (measurement error <10 ppm). The empirical formula is calculated on the basis of these measures. Some formulas might correspond to several compounds having the same molecular weight but different chemical identity.

NOTES FOR UNDERSTANDING: in essence, the certain data is that we have 65 chemically different substances among which only 35% is known.

Molecules potentially belonging to toxin category have been researched. They have been suggested on the basis of accurate m / z mode research (error <10 ppm) using the toxic compounds database in the Metlin search engine. Table 3 shows the candidates obtained.

It is emphasized that different detected compounds have a candidate empirical formula containing sulfur compounds or sulfur in the form of various functional groups. Furthermore, the presence of formic acid in the form of sodium salt and a polymer deriving from contaminations of Poly Ethylene Glycol (PEG)[14] with an average molecular weight equal to 1340 Da have been detected.

5. Final considerations

Most of the contaminants and impurities detected were not characterized using the metabolic and protein reference databases (KEGG, NCBI-Prot e SwissProt). [8-9]
There is a critical issue in the contamination of various compounds potentially or definitely harmful to human health.

In short, the first questions we asked ourselves, and the relative answers obtained, are the following:

1. Are the chemical substances listed in the data sheet present?	**in part**
2. Are there any chemical and protein contaminations?	**YES**
3. How many contaminating compounds are there?	**From 65**
4. What are they?	**Chemical toxins, chemical compounds, peptides**

Next analysis

1. First of all, it is necessary to identify with certainty the most interesting probable compounds	
2. Then to determine the exact amount of each contaminant	
3. Finally to determine the structure of the macromolecule constituted by the set of antigens	

6. Future research developments

Confirmation and identity analysis will be performed using the **"Tandem Mass Spectrometry (MS / MS)"** technique associated with the aid of certified analytical standards. The analysis will be performed in compliance with the European directives (EU directive 2002/657 / EC) useful for the identification of compounds.

In particular, the investigation will have the objective of confirming those substances whose toxicity and allergenicity is known and the 7 toxins identified will be an element of careful study.

7. Description of the SANIST technology

The innovative internationally renowned SANIST platform, through publications in peer-reviewed scientific journals[15]-[16] - was used to perform a first identification screening on the vaccines of interest.

[14] https://www.sciencedirect.com/topics/materials-science/polyethylene-glycol
[15] Albini A. et al., Rapid Commun Mass Spectrum. 2015 Oct 15;29(19):1703-10. doi: 20.1002/rcm.7270. (https://onlinelibrary.wiley.com/doi/full/10.1002/rcm.7270)
[16] Cristoni S. et al., J Mass Spectrom. 2017 Jan;52(1):16-21. doi:10.1002/jms.3895. (https://www.ncbi.nlm.nih.gov/pubmed/27776380)

8. Details related to the analytical method

SANIST technology consists of:

a) a **kit** for the extraction of analytes (the unknown substances to be determined);

b) the **LC-SACI / ESI-MS** analysis system which allows to reduce the chemical noise of mass spectrometers and obtain a better detection of instrumental signals;

c) the **SANIST data processing system** consisting of a local bioinformatics and network platform capable of processing data using dedicated databases and customized algorithms. It is specified that, during the screening phase, the recognition is made in the context of scientific research and through research in official banks (KEGG, NCBI-Prot and SwissProt)[17,18] without the aid of certified analytical standards. **It is therefore necessary to perform a second level analysis with certified analytical standards to confirm their identity.**

9. Areas of application of SANIST technology

To date, the **SANIST platform** is applicable in the following fields:

a. In **clinical research** of disease markers and their direct application in the diagnostic field.
b. **Food services**, food traceability. Comparative studies to determine the quality of products based on their complex molecular composition. Control of food counterfeiting.
c. **Nutraceutical sector**, development of the nutritional value of a food supplement based on its molecular composition. Forged search (for example: added drugs).
d. **Pharmaceutical sector**, drug control and research of active biomolecules.
e. **Cosmetic industry**: the molecular composition of cosmetic products can be carefully monitored and correlated with the quality of the product.

10. How to read the tables

This is a screening phase; the instrument measures a particular data by its accurate molecular weight (measurement error <10 ppm). On the basis of these measures a brute formula is calculated. Some formulas can correspond to several compounds having the same molecular weight but different chemical identity.

Example of a single associated component:

▪ Atovaquone	Medication for the treatment of malaria

In this example, the instrument detected a signal with a certain molecular weight. By inserting the brute formula in the databases, it was possible to associate **a probable component**.

Example of a number of associated components:

▪ Tetracenomycin F2 ▪ Decaketide tricyclic intermediate ▪ 1'-hydroxyversicolorone	▪ Monocarboxylic hydroxy acid ▪ Member of the anthracenes ▪ Antrafuran

In this example, the instrument detected a signal with a certain molecular weight. By inserting the brute formula in the databases, it was possible to associate **three probable components**.

11. Tables of contaminants

[17] Kanehisa M. et al., Nucleic Acids Res. 2017 Jan 4;45(D1):D353-D361. doi:10.1093/nar/gkw1092. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5210567/)
[18] Cristoni S. et al., Expert Rev Proteomics. 2004 Dec; 1(4):469-83. (https://www.ncbi.nlm.nih.gov/pubmed/15966842)

Table 2 - Batch #1 (A21CD072D)

▪ Tungsten carbide	▪Inorganic carbide industrially used to synthesize cemented carbides.
▪ Tetracenomycin F2 ▪ Decaketide tricyclic intermediate ▪ 1'-hydroxyversicolorone	▪ Monocarboxylic hydroxy acid ▪ Member of the anthracenes ▪ Antrafuran
▪ Lactose	▪ Added as a stabilizer.
▪ Sodium methallylsulfonate	▪ Monomer used in the polymer industry.
▪ Salicin 6-phosphate ▪ Pachyrrhizone ▪ Arnottin II ▪ Tetracenomycin F1	▪ Glycoside phosphate derived from salicin (anti-inflammatory agent) ▪ Member of the Rotenoni ▪ Member of 2-benzofurans ▪ Monocarboxylic hydroxy acid
▪ Octadecanamide	▪ Amide of stearic acid
▪ L-Leucine	▪ Amino acid
▪ Lichenin(altri 19 possibili candidati)	▪ Glucan. Study to use glucans as vaccine adjuvants
▪ Justicidin B	▪ Lignan
▪ Dihydrochelirubine ▪ 6-oxocheleritrhine ▪ 7,8-Didemethyl-8-hydroxy-5 -deazariboflavin	▪ Dihydrobenzophenantridinico Alkaloid ▪ Alkaloid ▪ Riboflavin
▪ Deamino-alpha-keto-demethylphosphinothricin	▪ -
▪ Cassythine ▪ (6-alpha-D-glucosaminyl)-1D-myo inositol	▪ Alkaloid ▪ A derivative of a D-glucosaminide and a monosaccharide.
▪ Carbenicillin sodium	▪ Bactericidal antibiotic
▪ bis-D-fructose 2', 1:2, 1'-dianhydride ▪ D-Fructofuranose 1,2':2,3'-dianhydride ▪ Prazepam ▪ 2,3-Dehydro-UWM6 / ▪ Levofuraltadone ▪ Mycocyclosin	▪ Dianhydride sugar ▪ Dianhydride sugar ▪ Benzodiazepine derivative / ▪ Member of the phenanthrenes ▪ Antibiotic that can be used in combination with a vaccine consisting of hybrid cells for cancer treatment ▪ Heterotetracyclic compound
▪ Atovaquone	▪ Medication for the treatment of malaria
▪ Amoxicillin ▪ Cephalexin monohydrate ▪ Cefroxadine ▪ CGP 28-392	▪ Antibiotic ▪ Antibiotic that decreases the effectiveness of vaccines ▪ Cephalosporin antibiotic ▪ Aromatic ether
▪ 7-deoxyloganate ▪ 8-epideoxyloganic acid ▪ LY395153 ▪ AL-294	▪ Metabolite of plants ▪ Metabolite of plants ▪ Member of the benzamides ▪ Alkylbenzene
▪ 4-Chloro-orto-phenylenediamine	▪ Member of the monochlorobenzene
▪ 2-N, 6-N-Bis(2,3-dihydroxy benzoyl)-L-Lysine amide	▪ -
▪ 2-Iodo-6-methoxyphenol	▪ Member of the phenols
▪ Tungstate	▪ Compound containing a tungsten oxoanion
▪ 2-amino-5-chloromuconate-6 -semialdehyde	▪ Semialdehyde
▪ 2,5-Dichloro-4-oxohex-2-enedioate	▪ Member of the family of medium-chain keto acids
▪ 1-Palmitoyl-2-(5-hydroxy-8-oxo-6-octenoyl)-sn-glycero-3-phosphatidylcholine	▪ 1,2-diacil-sn-glicero-3-fosfocolina

Table 3 - Batch #1 (A21CD072D)

Candidate compound	Empirical Formula
▪ Sodium formate	CHNaO2
▪ Hepta-2,3,4,5,6-pentaenenitrile	C7H3N
▪ (Methanesulfonyl)(dioxo)-lambda~5~-azane ▪ Oxaziridine-2-sulfonic acid	CH3NO4S
▪ 3,4-Dihydro-3-thioxo-1,2,4-triazin-5(2H)-one ▪ 5-Thioxo-4,5-dihydro-1,2,4-triazin-3(2H)-one ▪ 1,2,4-oxadiazole-3-carbothioamide ▪ 1,2,3-Thiadiazole-4-carboxamide ▪ 5-amino-1,3,4-thiadiazole-2-carbaldehyde ▪ 1,1-Difluoro-1-isocyanatoethane ▪ 1,1-Difluoro-2-isocyanatoethane	C3H3N3OS
▪ 3-(methylsulfonyl)-1H-1,2,4-triazole ▪ 1H-Imidazole-2-sulfonamide ▪ N-(4,5-Dihydro-1,3-thiazol-2-yl)nitramide ▪ 1H-Imidazole-5-sulfonamide ▪ 1H-Pyrazole-4-sulfonamide ▪ Undeca-2,4,6,8,10-pentaynenitrile	C3H3F2NO
▪ 2-Aminobenzenethiol ▪ 4-Methyl-5-vinylthiazole ▪ 2-Pyridinemethanethiol ▪ 2-Isopropenylthiazole ▪ 5,6-Dihydro-4H-cyclopenta[d][1,3]thiazole ▪ 4-Aminothiophenol 3-Pyridinemethanethiol ▪ Pyridine, 2-(methylthio) ▪ Pyridine, 3-(methylthio)-	C6H7NS
▪ Phenthiazamine	C9H8N2S
▪ 4-Chloro-N-hydroxybenzene-1-sulfonamide	C6H6ClNO3S
▪ Carbamodithioic acid, (4-hydroxyphenyl)-	C7H7NOS2