

New quality-control investigations on vaccines: micro- and nanocontamination

Abstract

Vaccines are being under investigation for the possible side effects they can cause. In order to supply new information, an electron-microscopy investigation method was applied to the study of vaccines, aimed at verifying the presence of solid contaminants by means of an Environmental Scanning Electron Microscope equipped with an X-ray microprobe. The results of this new investigation show the presence of micro- and nanosized particulate matter composed of inorganic elements in vaccines' samples which is not declared among the components and whose unduly presence is, for the time being, inexplicable. A considerable part of those particulate contaminants have already been verified in other matrices and reported in literature as non biodegradable and non biocompatible. The evidence collected is suggestive of some hypotheses correlated to diseases that are mentioned and briefly discussed.

Keywords: vaccine, disease, contamination, protein corona, biocompatibility, toxicity, nanoparticles, immunogenicity, foreign body, environment, industrial process, quality control

Volume 4 Issue 1 - 2017

Antionietta M Gatti,^{1,2} Stefano Montanari³

¹National Council of Research of Italy, Institute for the Science and Technology of Ceramics, Italy

²International Clean Water Institute, USA

³Nanodiagnostics srl, Italy

Correspondence: Dr. Antionietta Gatti, National Council of Research of Italy, c/o Nanodiagnostics Via E. Fermi, 1/L, 41057 San Vito (MO), Italy, Tel 059798778, Email gatti@nanodiagnostics.it

Received: November 30, 2016 | **Published:** January 23, 2017

Introduction

Vaccines are one of the most notable inventions meant to protect people from infectious diseases. The practice of variolation is century-old and is mentioned in Chinese and Indian documents dated around 1000 A.D. Over time, variolation has been replaced by vaccination, vaccines have been enhanced as to technology, and the vaccination practice is now standardized worldwide.

Side effects have always been reported but in the latest years it seems that they have increased in number and seriousness, particularly in children as the American Academy of pediatrics reports.^{1,2} For instance, the diphtheria-tetanus-pertussis (DTaP) vaccine was linked to cases of sudden infant death syndrome (SIDS);³ measles-mumps-rubella vaccine with autism;^{4,5} multiple immunizations with immune disorders;⁶ hepatitis B vaccines with multiple sclerosis, etc.

The notice of Tripedia DTaP by Sanofi Pasteur reports "Adverse events reported during post-approval use of Tripedia vaccine include idiopathic thrombocytopenic purpura, SIDS, anaphylactic reaction, cellulitis, autism, convulsion/grand mal convulsion, encephalopathy, hypotonia, neuropathy, somnolence and apnea". The epidemiological studies carried out did not show a clear evidence of those associations, even if in 2011 the National Academy of Medicine (formerly, IOM) admitted: "Vaccines are not free from side effects, or adverse effects".⁷

Specific researches on components of the vaccines like adjuvants (in most instances, Aluminum salts) are already indicated as possible responsible of neurological symptoms⁸⁻¹⁰ and in some cases, in-vivo tests and epidemiological studies demonstrated a possible correlation with neurological diseases.^{10,11} Neurological damages induced in patients under hemodialysis treated with water containing Aluminum are reported in literature.¹²

Recently, with the worldwide-adopted vaccines against Human Papillomavirus (HPV), the debate was reawaken due to some adverse effects reported by some young subjects.

Specific studies communicated the existence of symptoms related to never-described-before syndromes developed after the vaccine

was administered. For instance, Complex Regional Pain Syndrome (CRPS), Postural Orthostatic Tachycardia Syndrome (POTS), and Chronic Fatigue Syndrome (CFS).¹³ The side-effects that can arise within a relatively short time can be local or systemic.

Pain at the site of injection, swelling and uncontrollable movement of the hands (though this last symptom can also be considered systemic) are described. Among the systemic effects, fever, headache, irritability, epileptic seizures, temporary speech loss, lower limbs dysaesthesia and paresis, hot flashes, sleep disorders, hypersensitivity reactions, muscle pain, recurrent syncope, constant hunger, significant gait impairment, incapacity to maintain the orthostatic posture are reported.¹⁴

It is a matter of fact that every day millions of vaccine doses are administered and nothing notable happens, but it is also irrefutable that, regardless of the amount of side effects that are not recorded and the percentage of which remains in fact unknown, in a limited number cases something wrong occurs. No satisfactory explanation or, in many cases, no explanation at all has been given and it seems that those adverse effects happen on a random and stochastic basis.

Those situations induced us to verify the safety of vaccines from a point of view which was never adopted before: not a biological, but a physical approach. So, we developed a new analysis method based on the use of a Field Emission Gun Environmental Scanning Electron Microscope investigations to detect possible physical contamination in those products.

Materials and methods

44 types of vaccines coming from 2 countries (Italy and France) were analyzed. Table 1 groups them in terms of name, brand and purpose.

Some vaccines, in fact a minority, are meant to deal with a single bacterium or virus, while others are multi-valent. The list of vaccines we analyzed may contain repeated names, because we considered different batches and years of production of the same vaccine: the ones against influenza in particular.

Table 1 List of vaccines analyzed, according to their purpose

N	Name	Brand Name, Country of Distribution	Description	Production Batch, Expiry Date
1	Vivotif Berna	Berna Biotech SA, Italy	Anti-Thyphoid Vaccine (Live), group Ty21a	3000336 [2004]
2	Typhim Vi	Aventis Pasteur MSD, Italy	Anti-Salmonella typhi Vaccine	U1510-2 [2004]
3	Typherix	GlaxoSmithKline S.p.a., Italy	Anti-Thyphoid Vaccine (polysaccharide Vi)	ATYPB061BB [2009]
4	Anatetall	Chiron (now Novartis) Italy	Adsorbed anti-Tetanus Vaccine	030106 [2004]
5	Anatetall	Novartis Vaccines and Diagnostics, Italy	Adsorbed anti-Tetanus Vaccine	060510 [2009]
6	Tetabulin	Baxter AG, Italy	Adsorbed anti-Tetanus Vaccine	VNG2G006A [2009]
7	Dif-Tet-All	Novartis Vaccines and Diagnostics, Italy	Adsorbed anti-Tetanus and diphtheria Vaccine	070501 [2009]
8	Infanrix	GlaxoSmithKline S.p.a., Italy	Anti-Diphtheria, tetanus and pertussis vaccine	AC14B071AJ [2009]
9	Infanrix hexa	GlaxoSmithKline Biologicals s, Italy	Anti-diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and disease caused by Haemophilus influenzae type b	A21CC512A [2017]
10	Infanrix hexa	GlaxoSmithKline Biologicals s.a. France	Anti-diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and disease caused by Haemophilus influenzae type b	A21CC421A [2017]
11	M-M-R vaxPro	Sanofi Pasteur MSD, Italy	M-M-R vaxPro (measles, mumps, and rubella) analyzed in Cambridge	L012437 [2017]
12	Repevax	Sanofi Pasteur MSD, France	Anti-diphtheria-tetanus-pertussis-polio-vaccine	L0362-1 [2017]
13	Repevax	Sanofi Pasteur MSD SNC France	Anti-diphtheria-tetanus-pertussis-polio-vaccine	L0033-1 [2016]
14	Priorix	GlaxoSmithKline S.p.a., Italy	Anti--measles-mumps, and rubella (MMR) vaccine	A69CB550A [2009]
15	Morupar	Chiron (now Novartis,), Italy	Anti-measles- mumps, and rubella (MMR) vaccine	7601 [2004]
16	Varilrix	GlaxoSmithKline S.p.a., Italy	Anti-Chicken pox vaccine (group OKA)	A70CA567A [2009]
17	Stamaril Pasteur	Sanofi Pasteur MSD, Italy	anti-yellow fever vaccine	A5329-6 [2009]
18	Allergoid-Adsorbat 6-Graser Starke B.	Allergopharma, Germany	Antiallergic vaccine	Ch-B.:30005999-B [2006]
19	Engerix-B	GlaxoSmithKline S.p.a., Italy	Adsorbed anti-hepatitis B vaccine	AHBVB468BD [2009]
20	Prenevar 13	Pfizer, Italy	Antipneumococcal vaccine	G79324 [2013]
21	Prevenar 13	Pfizer, France	Antipneumococcal vaccine	N27430 [2018]
22	Mencevax Acwy	GlaxoSmithKline, Italy	anti-Neisseria meningococcal group A, C, W135 and Y vaccine	N402A47B 12 [2004]
23	Meningitec	Pfizer, Italy	(group C 10) (adsorbed on Al-Phosphate)	H92709 [2015]
24	Meningitec	Pfizer-Italy	Anti-meningococcus (group C 10) vaccine (adsorbed on Al-Phosphate)	H20500 [2014]
25	Meningitec	Pfizer-Italy	Anti-meningococcus vaccine sequestred by Procura della Repubblica	G76673 [2014]
26	Meningitec	Pfizer-Italy	Anti-meningococcus vaccine sequestred by Procura della Repubblica	H99459 [2015]
27	Meningitec	Pfizer-Italy	Anti-meningococcus vaccine sequestred by Procura della Repubblica	H52269 [2015]
28	Menjugate	Novartis Vaccines and Diagnostics	Anti-meningococcus group C	YA0163AB [2010]
29	Menveo	Novartis Vaccines and Diagnostics	Antimeningococcus groups A, C, W135, Y	A15083 [2017]
30	Meningitec	Wyeth Pharmaceutical - France	Anti-meningococcus group C vaccine	E83920 [2011]
31	Inflexal V	Berna Biotech	Anti-flu vaccine 2008/2009	3001463-01 [2009]
32	Vaxigrip	Sanofi Pasteur MSD	Anti-flu vaccine 2008/2009	D9703-1 [2009]
33	Vaxigrip	Sanofi Pasteur	Anti-flu vaccine 2012/2013	J8401-1 [2013]
34	Vaxigrip	Sanofi Pasteur, Italy	Anti-flu vaccine, with inactivated and split virus	M7319-1 [2016]
35	Focetria	Novartis Vaccines and Diagnostics	Anti-pandemic flu H1N1 vaccine	0902401 [2010]
36	Agrippal	Novartis	Anti-flu vaccine 2012/2013	127002A [2013]
37	Agrippal	Novartis vaccines, Italy	Anti-flu vaccine with inactivated and split virus 2015/2016 -	152803 [2016]
38	Agrippal SI	Novartis Vaccines and Diagnostics	Anti-flu inactivated/superficial antigene v - 2014/2015	147302A [2015]
39	Fluarix	GlaxoSmithKline - GSK	Anti-flu vaccine 2013	AFLUA789AA [2014]
40	Fluad	Novartis Vaccines and Diagnostics	Anti-flu inactivated/superficial antigene vaccine - 2014/2015	142502 [2015]

Table Continued...

N	Name	Brand Name, Country of Distribution	Description	Production Batch, Expiry Date
41	Gardasil	Sanofi Pasteur MSD, Italy	Anti-HPV types 6,11,16,18 vaccine	NP01250 [2012]
42	Gardasil	Sanofi Pasteur MSD, Italy	Anti-HPV (types 6,11,16,18) vaccine	K023804 [2016]
43	Cervarix	GlaxoSmithKline Biological, Italy	Anti-HPV (type 16,18)	AHPVA238AX [2017]
44	Feligen CRP	Virbac S.A. - Carros - Italy	anti-panleucopenia, infectious rhinotracheitis and infections by Calicivirus, veterinary Vaccine for cats	3R4R [2013]

Table 2 List of the vaccines according to their manufacturers with the chemical composition of the debris identified in each sample. The elements most represented are reported

N	Company	Name	Alluminum	Elements Identified
1	Allergopharma - Germany	Allergoid	yes	Al
2	Aventis Pasteur MSD Lyon - Francie	Typhim Vi	no	BrKP, PbSi, FeCr, PbClSiTi
3	Baxter AG	Tetabulin	no	SiMg, Fe, SiTiAl, SBa, Zn
4	Berna Biotech	Vivotif Berna	no	FeAl, ZrAlHf, SrAl, BiAlCl
5	Berna Biotech	Inflexal V	no	CuSnPbZn, Fe, CaSiAl, SiAl, NaPZn, ZnP, AlSiTi
6	Chiron	Anatetall	Al(OH) ₃	FeAl, SZnBaAl
7	Chiron	Morupar	no	/
8	GlaxoSmithKline- Belgium	Mencevax ACWY	no	FeCrNi, ZrAl, FeCrNiZrAlSi
9	GlaxoSmithKline	Infanrix	Al(OH) ₃	Al, AlTi, AlSi
10	GlaxoSmithKline Biologicals	Infanrix hexa	Al(OH) ₃	SBa, FeCu, SiAl, FeSi, CaMgSi, AlCaSi, Ti, Au, Sca, SiAlFeSnCuCrZn, CaAlSi
11	GlaxoSmithKline Biologicals	Infanrix hexa	Al(OH) ₃ , AlPO ₄ . 2H ₂ O	W, FeCrNi, Ti
12	GlaxoSmithKline	Typherix	no	Ti, TiW, AlSiTiWCr, SBa, W, SiAl, AlSiTi
13	GlaxoSmithKline	Priorix	no	WCa, WFeCu, SiAl, SiMg, PbFe, Ti, WNiFe
14	GlaxoSmithKline	Engerix-B	no	Al (precipitates)
15	GlaxoSmithKline	Varilrix	no	FeZn, FeSi, AlSiFe, SiAlTiFe, MgSi, Ti, Zr, Bi
16	GlaxoSmithKline	Fluarix	no	AlCu, Fe, AlBi, Si, SiZn, AlCuFe, SiMg, SBa, AlCuBi, FeCrNi, SPZn
17	GlaxoSmithKline Biologicals	Cervarix	Al(OH) ₃	AlSi, FeAl, SiMg, CaSiAl, CaZn, FeAlSi, FeCr, CuSnPb
18	Novartis Vaccines and Diagnostics	Anatetall	Al(OH) ₃	Al, FeCrNi, AlCr, AlFe, BaS, ZnAl
19	Novartis Vaccines and Diagnostics	Dif-Tet-All	Al(OH) ₃	Fe, SBa, SiSBa, AlZnCu, AlZnFeCr
20	Novartis Vaccines and Diagnostics	Menjugate kit	Al(OH) ₃	SiAl, Ti, FeZn, Fe, Sb, SiAlFeTi, W, Zr
21	Novartis Vaccines and Diagnostics	Focetria	no	Fe, FeCrNiCu, FeCrNi, SiFeCrNi, Cr, SiAlFe, AlSiTiFe, AlSi, SiMgFe, Si, FeZn
22	Novartis	Agrrippal S1	no	Ca, Fe, SBa, SBaZn, Cr, Si, Pb, Bi, e FeSiAlCr, SiAlSBaFe, CaAlSi, Zn, CeFeTiNi, FeCrNi
23	Novartis Vaccines and Diagnostics	Agrrippal S1	no	SiAlK, Si, SiMgFe, CaSiAl, SBaZn
24	Novartis vaccines	Agrippal	no	Cr, Ca, SiCaAl, ZrSi, SBa, CuZn, Sca
25	Novartis Vaccines and Diagnostics S	Fluad	no	CaSiAl, FeSiTi, SiMgAlFe, SBa
26	Novartis Vaccines and Diagnostics	Menveo	no	CaSiAl, SiAlFe, FeCrNi, Fe, Al, SBa
27	Pfizer	Prenevar 13	no	FeCr
28	Pfizer	Prevenar 13	no	W, CaAlSi, Al, CaSiAlFe, FeS, FeCr, FeCrNi, Fe, , CaP, FeTiMn, Ba, SiMgAlFe
29	Pfizer	Meningitec - ctrl	no	Cr, Si
30	Pfizer	Meningitec - ctrl	no	FeCrNi, W
31	Pfizer	Meningitec	no	CaSiAl, CaSi, SiAlFeTi, FeCrNi, W, Fe, Pb
32	Pfizer	Meningitec	no	Cr (precipitates), Ca, AlSi
33	Pfizer	Meningitec	no	W, SiCa, CaSi, Pb, FeCrNi, Cr
34	Wyeth Pharmaceutical - UK	Meningitec	no	SiAlFe, SiAlTi, SiMgFe, W, Fe, Zr, Pb, Ca, Zn, FeCrNi
35	Sanofi Pasteur MSD-France	Vaxigrip	no	Fe, FeCrNi, SiAlFe, AlSi, SiAlFeCr
36	Sanofi Pasteur MSD	Stamaril Pasteur	no	CaSiAl, AlSi, Fe, SiMgFe, SiMgAlFe, CrSiFeCr, CrSiCuFe
37	Sanofi Pasteur MSD	Gardasil	AlPO ₄ . 2H ₂ O	AlCuFe, PbBi, Pb, Bi, Fe
38	Sanofi Pasteur MSD	Gardasil	AlPO ₄ . 2H ₂ O	CaAlSi, AlSi, SiMgFe, AlFe, AlCuFe, FeSiAl, BiBaS, Ti, TiAlSi
39	Sanofi Pasteur	Vaxigrip	no	Ca, CrFe, FeCrNi, CaSZn, CaSiAlTiFe, Ag, Fe
40	Sanofi Pasteur	Vaxigrip	no	SiMgFe, CaSiAl, AlSiFe, AlSi, FeCr, FeZn, Fe
41	Sanofi Pasteur MSD	Repevax	AlPO ₄ . 2H ₂ O	Bi, Fe, AlSiFe, SiMg, SBa, Ca
42	Sanofi Pasteur MSD S	Repevax	AlPO ₄ . 2H ₂ O	Ti, Br, AuCuZn, Ca, SiZn, SiAuAgCu, SiMgFe, FeCrNi, AlSiMgTiMnCrFe, SiFeCrNi, FeAl
43	Sanofi Pasteur MSD	M-M-R vaxPro	no	Si, SiFeCrNi, FeCrNi, FeNi, Fe, Sca, AlSiCa, CaAlSiFeV, SBa, Pt, PtAgBiFeCr
44	Virbac S.A. - Carros - France	Feligen CRP	no	Ca, SiAl

Table 3 List of the debris' number identified in each vaccine as single particle, clusters and aggregates. Characterization is made by shape, size range and variability of the number of particles counted in each aggregate [in brackets]

Name	Total Debris n.	Size Range in mm	Cluster n.	Size Range in mm	Aggregate n.(Range of Particles)	Size Range mm
Allergoid	NaCl precipitates	/	/	/	/	/
Typhim Vi	394	0,1-2,5			3[9-350]	Feb-35
Tetabulin	519	0,1-15			3[100-180]	25-60
Vivotif Berna	4	1,5-15				
Inflexal V	103	0,1-17	1	20	3[35-45]	25-Oct
Anatetall	2	3-Jan				
Morupar	/		/		/	
Mencevax ACWY	13	0,2-5				
Infanrix	3	5-Jan	1	25		
Infanrix hexa	1821	0,1-15			15[1820]	20-140
Infanrix hexa	162	0,3-7	12	60	2[7 debris]	3.5-44
Typherix	8	0,2-8	1	15		
Priorix	641	0,05-30	1	10	3[600]	20-70
Engerix-B	precipitates			/		
Varilrix	2723	0,1-4			36 [120-2000]	15-40
Fluarix	1317	0,1-40			3[83]	30-Jul
Cervarix	1569	0,2-3	2	10-May	4[1400]	30-Aug
Anatetall	47	0,05-40				
Dif-Tet-All	111	0,2-3				
Menjugate	73	0,1-5				
Focetria	35	0,7-10				
Agrippal SI	430	0,2-6	13	0.2-80	5[410]	20
Agrippal SI	1029	0,1-12			9[1025]	35-80
Agrippal	480	0,1-6			11[460]	Feb-80
Fluad	605	0,2-15	4	25-Dec	6[600]	70
Menveo	452	0,1-13			4[430]	30-110
Prenevar 13	precipitates + 5 debris	7-Jan				
Prevenar 13	precipitates + 81 debris	0,2-50	3	May-40	1 [60]	25
Meningitec	3	20-Oct				
Meningitec	24	Aug-60				
Meningitec	673	0,1-20	1	7	9[624]	5-110
Meningitec	precipitates + 40	0,1-3,5			2[40]	25-70
Meningitec	177	0,2-10			3[165]	15-100
Meningitec	241	0,1-15	1	50	2[230]	50
Vaxigrip	86	0,1-7			2[50]	2-2,5
Stamaril Pasteur	152	0,1-7	2	7-May	3[145]	20-Apr
Gardasil	304	0,05-3			1[300]	15
Gardasil	454	0,1-30	2	20-Jul	9[445]	May-60
Vaxigrip	304	0,1-10	1	13	2[300]	35
Vaxigrip	674	0,3-25	2	12-Feb	10[660]	9-150
Repevax	137	0,1-20			2[130]	40-50
Repevax	214	0,1-10			6[150]	30-May
M-M-R vaxPro	93	0,1-15			2[50]	15-Oct
Feligen CRP	92	0,1-12	1	12	1 (40 debris)	25

The study was aimed at verifying a possible physical contamination. To do that, we performed a new kind of investigation based on observations under a Field Emission Gun Environmental Electron Scanning Microscope (FEG-ESEM, Quanta 200, FEI, The Netherlands) equipped with the X-ray microprobe of an Energy Dispersive Spectroscopy (EDS, EDAX, Mahwah, NJ, USA) to detect the possible presence of inorganic, particulate contaminants and identify their chemical composition.

A drop of about 20microliter of vaccine is released from the syringe on a 25-mm-diameter cellulose filter (Millipore, USA), inside a flow cabinet. The filter is then deposited on an Aluminum stub

covered with an adhesive carbon disc. The sample is immediately put inside a clean box in order to avoid any contamination and the box is re-opened only for the sample to be inserted inside the FEG-ESEM chamber. We selected that particular type of microscope as it allows to analyse watery and oily samples in low vacuum (from 10 to 130 Pa) at a high sensitivity.

When the water and saline the vaccine contains are evaporated, the biological/physical components emerge on the filter and it is then possible to observe them. This type of microscope (low-vacuum observations) prevents the possible sample contamination and the creation of artefacts. The observations are made with different sensors

(SE: secondary-electron sensor and BSE: backscattered-electron sensor), and are performed at a pressure of 8.9 e-1 mbar, at energies ranging from 10 to 30kV to detect the particulate matter's size, morphology and its elemental composition. The method identifies clearly inorganic bodies with a higher atomic density (looking whiter) than the biological substrate. So, organic entities are visible and easy to distinguish from inorganic ones. The method cannot distinguish between proteins and organic adjuvants (e.g. squalene, glutamate, proteins, etc.) or viruses, bacteria, bacteria's DNA, endo-toxins and bacteria's waste, but their comparatively low atomic density allows us to identify these entities as organic matter. In some vaccines, the organic matter contains white-looking debris named aggregates, while a high concentration or inorganic debris is called a cluster.

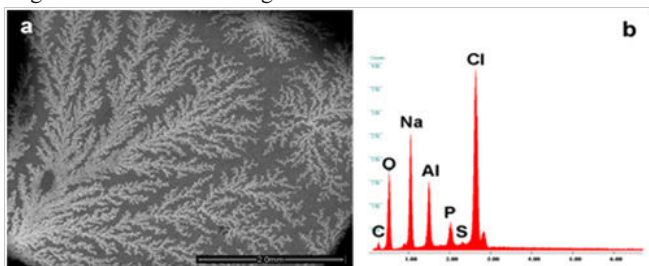


Figure 1 Crystals of saline solution and Aluminum Phosphate and corresponding EDS spectra.

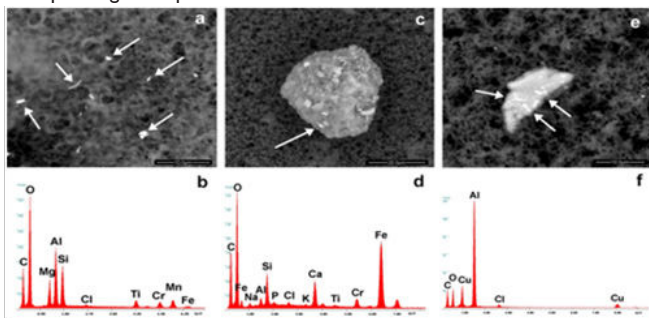


Figure 2 Images of single particles, cluster of micro- and nanoparticles (<100nm) and aggregates with their EDS spectra. They are respectively composed of (a,b) Aluminum, Silicon, Magnesium, Titanium, Chromium, Manganese, Iron, (c,d) Iron, Silicon, Calcium Titanium, Chromium, (e,f) Aluminum, Copper. The arrows show the points where EDS spectra were taken.

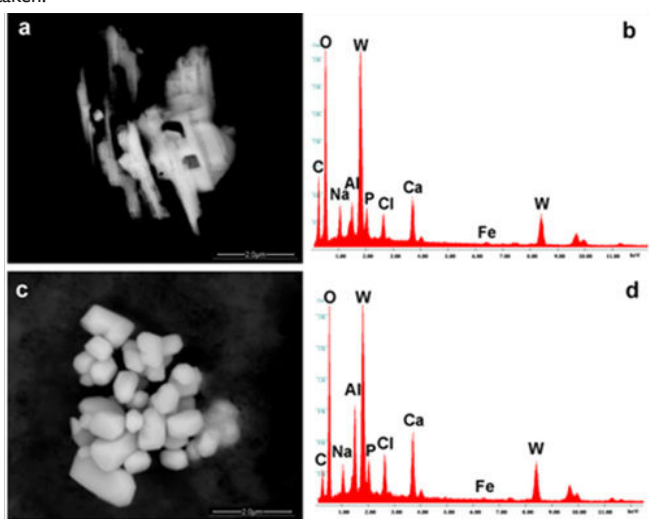


Figure 3 Images of Tungsten particles identified in drops of Prevenar and Infarix. They are composed respectively of Tungsten, Aluminum, Iron but in different concentrations. The arrows show the points where EDS spectra were taken.

Single inorganic particles or organic-inorganic aggregates are identified, evaluated and counted. The counting procedure is repeated three times by three different operators, with an error lower than 10%. When a layer of salts (Sodium chloride or Aluminum) is detected, we record the situation but we do not do body count.

Results

The investigations verified the physical-chemical composition of the vaccines considered according to the inorganic component as declared by the Producer. In detail, we verified the presence of saline and Aluminum salts, but further presence of micro-, sub-micro- and nanosized, inorganic, foreign bodies (ranging from 100nm to about ten microns) was identified in all cases, whose presence was not declared in the leaflets delivered in the package of the product (Table 2). Play Video

Figure 1a shows a layer of crystals of Sodium chloride (NaCl) embedding salts of Aluminum phosphate ($AlPO_4$) in a drop of Gardasil (anti-HPV vaccine by Merck) as the EDS spectrum (Figure 1b) shows. Saline is the fluid base to any vaccine preparation and Aluminum salts or Aluminum hydroxide $Al(OH)_3$ are the adjuvants which are usually added.

Looking at the area outside these precipitates but inside the liquid drop, we identified other things: single particles, clusters of particles and aggregates (organic-inorganic composites) that are due to an interaction of the inorganic particulate matter with the organic part of the vaccine.

Figure 2a-2f shows the different typology of entities identified in the vaccines (Repevax, Prevenar and Gardasil); single particles, cluster of micro- and nanoparticles (<100nm) and aggregates with their EDS spectra (Figure 2d-2f). The images (Figure 2a & 2d) show debris of Aluminum, Silicon, Magnesium and Titanium; of Iron, Chromium, Silicon and Calcium particles (Figure 2b & 2e) arranged in a cluster, and Aluminum -Copper debris (Figure 2c & 2f) in an aggregate.

As can be seen, the particles are surrounded and embedded in a biological substrate. In all the samples analyzed, we identified particles containing: Lead (Typhim, Cervarix, Agrippal S1, Meningitec, Gardasil) or stainless steel (Mencevax, Infarix Hexa, Cervarix, Anatell, Focetria, Agrippal S1, Menveo, Prevenar 13, Meningitec, Vaxigrip, Stamaril Pasteur, Repevax and MMRvaxPro).

Figure 3a-3d show particles of Tungsten identified in drops of Prevenar and Infarix (Aluminum, Tungsten, Calcium chloride).

Figure 4a-4d present singular debris found in Repevax (Silicon, Gold, Silver) and Gardasil (Zirconium).

Some metallic particles made of Tungsten or stainless steel were also identified. Other particles containing Zirconium, Hafnium, Strontium and Aluminum (Vivotif, Meningetec); Tungsten, Nickel, Iron (Priorix, Meningetec); Antimony (Menjugate kit); Chromium (Meningetec); Gold or Gold, Zinc (Infarix Hexa, Repevax), or Platinum, Silver, Bismuth, Iron, Chromium (MMRvaxPro) or Lead, Bismuth (Gardasil) or Cerium (Agrippal S1) were also found. The only Tungsten appears in 8/44 vaccines, while Chromium (alone or in alloy with Iron and Nickel) in 25/44. The investigations revealed that some particles are embedded in a biological substrate, probably proteins, endo-toxins and residues of bacteria. As soon as a particle comes in contact with proteic fluids, a nano-bio-interaction⁶ occurs and a "protein corona" is formed.⁷⁻¹⁰ The nano-bio-interaction generates a bigger-sized compound that is not biodegradable and can induce adverse effects, since it is not recognized as self by the body.

Priorix contain Tungsten. Tungsten was also identified in Menjugate kit by Novartis, and Prevenar, Meningitec by Pfizer and Meningitec by Wyeth).

Feligen, the only veterinary vaccine tested, proved to be the only sample free from inorganic contamination, while Allergoid generates a layer of inorganic salts so thick that it does not allow to detect other particulate contaminants.

Discussion

The quantity of foreign bodies detected and, in some cases, their unusual chemical compositions baffled us. The inorganic particles identified are neither biocompatible nor biodegradable, that means that they are biopersistent and can induce effects that can become evident either immediately close to injection time or after a certain time from administration. It is important to remember that particles (crystals and not molecules) are bodies foreign to the organism and they behave as such. More in particular, their toxicity is in some respects different from that of the chemical elements composing them, adding to that toxicity which, in any case, is still there, that typical of foreign bodies. For that reason, they induce an inflammatory reaction.

After being injected, those microparticles, nanoparticles and aggregates can stay around the injection site forming swellings and granulomas.¹⁷ But they can also be carried by the blood circulation, escaping any attempt to guess what will be their final destination. We believe that in many cases they get distributed throughout the body without causing any visible reaction, but it is also likely that, in some circumstances, they reach some organ, none excluded and including the microbiota, in a fair quantity. As happens with all foreign bodies, particularly that small, they induce an inflammatory reaction that is chronic because most of those particles cannot be degraded. Furthermore, the protein-corona effect (due to a nano-bio-interaction¹⁸ can produce organic/inorganic composite particles capable of stimulating the immune system in an undesirable way.¹⁹⁻²² It is impossible not to add that particles the size often observed in vaccines can enter cell nuclei and interact with the DNA.²³

In some cases, e.g. as occurs with Iron and some Iron alloys, they can corrode and the corrosion products exert a toxicity affecting the tissues.²⁴⁻²⁶

The detection of presence of Aluminum and NaCl salts is obvious as they are substances used by the Producers and declared as components, but other materials are not supposed to be in the vaccine or in any other injectable drug, at that, and, in any case, Aluminum has already been linked with neurological diseases.²⁷⁻²⁹

Given the contaminations we observed in all samples of human-use vaccines, adverse effects after the injection of those vaccines are possible and credible and have the character of randomness, since they depend on where the contaminants are carried by the blood circulation. It is only obvious that similar quantities of these foreign bodies can have a more serious impact on very small organisms like those of children. Their presence in the muscles, due an extravasation from the blood, could heavily impair the muscle functionality.^{30,31}

We come across particles with chemical compositions, similar to those found in the vaccines we analyzed, when we study cases of environmental contamination caused by different pollution sources. In most circumstances, the combinations detected are very odd as they have no technical use, cannot be found in any material handbook and look like the result of the random formation occurring, for example, when waste is burnt. In any case, whatever their origin, they should

not be present in any injectable medicament, let alone in vaccines, more in particular those meant for infants.

Other forms of so-far unknown contaminations have recently been observed and, in any case, vaccines contain components that could themselves be the cause of adverse effects. It is a well-known fact in toxicology that contaminants exert a mutual, synergic effect, and as the number of contaminants increases, the effects grow less and less predictable. The more so when some substances are unknown.

As a matter of fact, no exhaustive and reliable official data exist on the side-effects induced by vaccines. The episodic evidence reported by people allegedly damaged by vaccines is twofold: some say the damage occurred and became visible within a few hours from administration, and some maintain that it was a matter of some weeks. Though we have no indisputable evidence as to the reliability of those attestations, we can put forward a hypothesis to explain the different phenomena. In the former case, the pollutants contained in the drug have reached the brain and, depending on the anatomical site interested, have induced a reaction. If that is the case, the whole phenomenon is very rapid. In the latter circumstance, the pollutants reached the microbiota, thus interfering with the production of enzymes necessary to carry out neurological functions.³²⁻³⁵ That possibility takes time, as it involves the production of chemical compounds in a sufficient quantity, and an elapse of some weeks between injection and clinical evidence is reasonable. Of course, ours is no more than a hypothesis open to discussion and in need of proof, hoping that a chance of further investigation is allowed.

Conclusion

The analyses carried out show that in all samples checked vaccines contain non biocompatible and bio-persistent foreign bodies which are not declared by the Producers, against which the body reacts in any case. This new investigation represents a new quality control that can be adopted to assess the safety of a vaccine. Our hypothesis is that this contamination is unintentional, since it is probably due to polluted components or procedures of industrial processes (e.g. filtrations) used to produce vaccines, not investigated and not detected by the Producers. If our hypothesis is actually the case, a close inspection of the working places and the full knowledge of the whole procedure of vaccine preparation would probably allow to eliminate the problem.

A further purification of the vaccines could improve their quality and could probably decrease the number and seriousness of the adverse incidental effects.

Acknowledgments

The Authors are indebted to Dr. Federico Capitani, Dr. Laura Valentini and Ms. Lavinia Nitu for their technical assistance. The opinions and conclusions are not necessarily those of the Institution.

Conflicts of interest

Author declares there are no conflicts of interest.

Funding

None.

References

1. Healthy Children.org
2. US Dpt of health and human services. Report Update: Vaccine Side Effects, Adverse Reactions, Contraindications, and Precautions. *CDC* . 1996;45(RR-12):1-35.

3. Ottaviani G, Lavezzi AM, Matturri L. Sudden infant death syndrome (SIDS) shortly after hexavalent vaccination: pathology in suspected SIDS? *Virchows Arch*. 2006;448(1):100–104.
4. Taylor B, Miller E, Farrington CP, et al. Autism and measles, mumps, and rubella vaccine: no epidemiological evidence for a causal association. *Lancet* 353(9169): 2026–2029.
5. Demicheli V, Rivetti A, Debalini MG, Di Pietrantonj C (2012) Vaccines for measles, mumps and rubella in children. *Cochrane Database Syst Rev*. 1999;15(2):CD004407.
6. Carola Bardage, Ingemar Persson, Åke Örtqvist, et al. Neurological and autoimmune disorders after vaccination against pandemic influenza A (H1N1) with a monovalent adjuvanted vaccine: population based cohort study in Stockholm, Sweden. *BMJ*. 2011;343:d5956.
7. Johann Liang R. Updating the Vaccine Injury Table following the 2011 IOM Report on Adverse Effects of vaccines. *HRSA*. 2012. p.1–27.
8. L Tomljenovic, CA Shaw. Aluminum Vaccine Adjuvants: Are they Safe? *Current Medicinal Chemistry*. 2011;18(17):2630–2637.
9. Shaw CA, Petrik MS. Aluminum hydroxide injections lead to motor deficits and motor neuron degeneration. *J Inorg Biochem*. 2009;103(11):1555–1562.
10. Authier FJ, Sauvat S, Christov C, et al. AlOH₃-adjuvanted vaccine-induced macrophagic myofasciitis in rats is influenced by the genetic background. *Neuromuscul Disord*. 2006;16(5):347–352.
11. Exley C, Esiri MM. Severe cerebral congophilic angiopathy coincident with increased brain aluminium in a resident of Camelford, Cornwall, UK. *J Neurol Neurosurg Psychiatry*. 2006;77(7):877–879.
12. Wills MR, Savory J. Water content of aluminium, dialysis dementia, and osteomalacia. *Environ Health Perspect*. 1985;63:141–147.
13. Brinth L, Pors K, Theibel AC, et al. Suspected side effects to the quadrivalent human papilloma vaccine. *Danish Medical J*. 2015;62(4):1–12.
14. Palmieri B, Poddighe D, Vadalà M, et al. Severe somatoform and dysautonomic syndromes after HPV vaccination: case series and review of literature. *Immunol Res*. 2016.
15. Visani G, Manti A, Valentini L, et al. Environmental nanoparticles are significantly over-expressed in acute myeloid leukemia. *Leuk Res*. 2016;50:50–56.
16. Artoni E, Sighinolfi GL, Gatti AM, et al. Micro and nanoparticles as possible pathogenetic co-factors in mixed cryoglobulinemia. *Occupational Medicine*. 2016.
17. T Hansen, L Klimek, F Bittinger, et al. Mast cell reiches Aluminium granuloma Pathologie. 2008;29(4):311–313.
18. Gatti AM, Manti A, Valentini L, et al. Nano biointeraction of particulate matter in the blood circulation. *Frontiers*. 2016;30:3.
19. Tenzer S, Docter D, Rosfa S, et al. Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: a comprehensive quantitative proteomic analysis. *ACS Nano*. 2011;5(9):7155–167.
20. Radauer Preiml, Andosch A, Hawranek T, et al. Nanoparticle-allergen interactions mediate human allergic responses: protein corona characterization and cellular responses. *Fibre toxicology*. 2015;13:3.
21. Cedervall T, Lynch I, Lindman S, et al. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *PNAS*. 2016; 104 (7):2050–2055.
22. Lynch I, Cedervall T, Lundqvist M, et al. The nanoparticle-protein complex as a biological entity; a complex fluids and surface science challenge for the 21st century. *Advances in Colloid and Interface Science*. 2007;134–135:167–174.
23. Gatti AM, Quaglino D, Sighinolfi GL. A Morphological Approach to Monitor the Nanoparticle-Cell Interaction. *International Journal of Imaging and Robotics*. 2009;2:2–21.
24. Urban RM, Jacobs JJ, Gilbert JL, et al. Migration of corrosion products from modular hip prostheses. Particle microanalysis and histopathological findings. *The Journal of Bone and Joint Surgery*. 1994;76(9):1345–1359.
25. Kirkpatrick CJ, Barth S, Gerdes T, et al. Pathomechanisms of impaired wound healing by metallic corrosion products. *Mund Kiefer Gesichtschir*. 2002;6(3):183–190.
26. Lee SH, Brennan FR, Jacobs JJ, et al. Human monocyte/macrophage response to cobalt-chromium corrosion products and titanium particles in patients with total joint replacements. *J Orthop Res*. 1997;15(1):40–49.
27. Shaw CA, Seneff S, Kette SD, et al. Aluminum-Induced Entropy in Biological Systems: Implications for Neurological Disease. *Journal of Toxicology*. 2014;2014:491316.
28. Shaw CA, Kette SD, Davidson RM, et al. Aluminum™s Role in CNS-immune System Interactions leading to Neurological Disorders. *Immunome Research*. 2013;9:069.
29. Seneff S, Swanson N, Chen Li. Aluminum and Glyphosate Can Synergistically Induce Pineal Gland Pathology: Connection to Gut Dysbiosis and Neurological Disease. *Agricultural Sciences*. 2015;6(1):42–70.
30. Pegaz B, Debeve E, Ballini JP, et al. Effect of nanoparticle size on the extravasations and the photothrombic activity of meso(p-tetracarboxyphenyl)porphyrin. *J Photochem Photobiol B*. 2006;85(3):216–222.
31. Brinth LS, Pors K, Hoppe AG, et al. Is Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis a Relevant Diagnosis in Patients with Suspected Side Effects to Human Papilloma Virus Vaccine? *International Journal of Vaccines and Vaccination*. 2015;1(1):1–5.
32. Moos WH, Faller DV, Harpp DN, et al. Microbiota and Neurological Disorders: A Gut Feeling. *Biores Open Access*. 2016;5(1):137–145.
33. Sekirov I, Russell SL, Caetano L, et al. Gut Microbiota in Health and Disease. *Physiological Rev*. 2010;90(3):859–904.
34. Umbrello G, Esposito S. Microbiota and neurologic diseases: potential effects of probiotics. *J Transl Med*. 2006;14(1):298.
35. Kinoshita T, Abe RT, Hineno A, et al. Peripheral sympathetic nerve dysfunction in adolescent Japanese girls following immunization with the human papillomavirus vaccine. *Intern Med*. 2014;53(19):2185–2200.