Natural Rubber Latex Protein Allergy:

A literature review and a survey of the major allergens in household products taken from the German retail market

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August 2009 – Edited February 2010

Contents

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Overall Summary and Conclusions

This report comprises two parts:

Part 1: Literature Review. The literature on natural rubber latex and latex protein allergy has been reviewed. From relatively humble beginnings, the latex rubber industry now plays an important and ubiquitous part in life; natural rubber latex (NRL) is used in 40,000 products.

However, from the mid 1980's there has been an increasing number of reports indicating that natural rubber latex is a significant source of allergy, particularly amongst health care workers and children with spina bifida and/or subjected to multiple surgical procedures. Amongst the general population however, the prevalence of latex protein allergy is certainly no higher than 1% and there are encouraging signs that the prevalence of new cases, even in the "high risk" groups is diminishing. However this current review has highlighted difficulties of diagnosing NRL protein allergy and in the epidemiological assessment of both sensitised groups and those suffering from allergy symptoms.

A detailed review of reports suggesting a link between latex soother and bottle teats would indicate that allergic reactions from NRL soothers and teats are relatively rare and we would also question whether contact with these products results in initial sensitisation.

The tests for total protein, antigens and allergens in latex products have been reviewed as have the relationship between the various methods. It is concluded that measurement of four major allergens provides an excellent correlation to the overall potential allergenicity of the product.

It is considered that reduction as far as is practicable of the proteins and allergens in the latex product, rather than removal of the NRL product itself is the medium to long term answer to the problem of latex allergy.

An outline of common methods of manufacturing dipped natural latex products has been given together with the effects these production techniques have on residual allergens.

Part II: A survey of major allergens in household products. Samples of a number of NRL products used in the home: household gloves, toy balloons, soother and bottle teats, were taken from the German market.

Four major allergens were analysed by the FITkit® test, extractable proteins by a Modified Lowry Assay and extractable antigens by a LEAP technique.

The results tend to confirm previous reports that the correlation between these three methods is rather poor.

The total allergen content of the gloves tested are significantly less than previously reported. However, 42% of the samples had total allergen contents in excess of the 1µg/g widely considered to be the threshold for allergic reactions

Only one out of 24 samples of soothers and bottle teats had a detectable allergen (Hev b 3) and accordingly all the samples were well below the 1µg/g threshold. Products having an allergen content of below 15µg/g are also considered by one research group to be unlikely to sensitize people or to cause reactions in already sensitized individuals; all the soothers and bottle teats were well below this level.

Comparison of boiled and un-boiled soother and bottle teats showed a small but insignificant effect on total allergens determined.

It is noted that other unrelated regulations have caused manufacturers of NRL soother and bottle teats to adopt leaching techniques greatly in excess of those used for gloves and balloons, which, it is surmised, has resulted in the negligible allergen levels found.

Given these data and the evidence of the literature review we are of the opinion that it is highly unlikely for NRL soother and bottle teats to promote sensitisation. In addition, a sensitised child within the general population is unlikely to suffer allergic reactions from any of the samples tested. This does not imply that “at-risk” paediatric groups - spina bifida sufferers and others receiving multiple surgical procedures – should diverge from a latex-free environment.

Part I - Literature Review

Introduction and Methodology

This review confines itself to Natural Rubber Latex Protein Allergy, sometimes called Latex Protein (immediate) Allergy and which is defined by the World Allergy Organisation [15] as an immediate allergic reaction caused by IgE-antibodies to latex, which develops in minutes after contact with latex by those who have developed IgE-sensitization to latex proteins.

Searches were made of the Internet, Pubmed and the ICMRS database using key words: *latex, allergy, protein, allergens, gloves, balloons, soothers/pacifiers and teats*, either singularly or in combination.

For completeness, a brief history of natural rubber latex and an outline of the manufacture of dipped latex products have been included.

A Brief History of Natural Rubber Latex (NRL)

The major commercial source of natural rubber latex is the Para rubber tree (*Hevea brasiliensis*), a member of the spurge family, Euphorbiaceae which responds to wounding by producing more latex. Latex is often described as the sap of the Hevea tree. This is not an accurate description. The sap runs deeper inside the tree, beneath the cambium; latex runs in the latex ducts which are in a layer immediately outside the cambium.

The first use of natural rubber latex from the Hevea Tree is considered to be by the Ancient Mayans in 1600 BC who boiled the harvested latex to make a ball for sport [132].

In 1496 Christopher Columbus returned from his second voyage and brought back the first rubber balls from the West Indies. There was, Spanish observers said: *nothing comparable in the world to the way that the balls bounced*. Previously only packed leather balls had been known in Europe, and this new material was a total novelty.

Then the Spaniards discovered in 1615 that latex could be used for the water proofing of leather and fabrics. Because no method of preserving latex was known at that time, a thriving fabric proofing industry grew up in Mexico and the finished product exported.

In 1735 the French mathematician Charles Marie de La Condamine joined an expedition to Peru to measure the length of a degree of meridian at the equator. Soon after his arrival in Quito, in 1736, la Condamine sent a package of rubber to the Académie Royale des Sciences of France with a memorandum describing many aspects of its origins and production. These included the words “Hévé” as the name of the tree from which the milk or “latex” flowed and the name given to the material by the Maninas Indians: “cahuchu” or “caoutchouc”.

“Latex”, the word used by la Condamine to describe the juice of the tree, was derived from the Spanish word for milk and remains in use to this day.

Before returning to France, la Condamine met François Fresneau who was a trained engineer and amateur botanist. Fresneau caught la Condamine's enthusiasm for rubber and was the first person to consider it as a potential industrial material rather than as a novelty.

In 1751 la Condamine presented a paper by Fresneau to the Académie (eventually published in 1755) which described many of the latter’s findings and this can truly be called the first scientific paper on rubber.

When samples of rubber first arrived in England, in 1770, Joseph Priestley observed *that a piece of the material was extremely good for rubbing out pencil marks on paper*, hence the name “rubber”.

Perhaps the rubber industry in Europe really started with Charles Macintosh in 1818. He exploited the discovery by James Syme, that coal tar naphtha was a good solvent for rubber and used this naphtha-based rubber solution as a waterproofing layer between 2 fabrics. Hence the 'macintosh' was born.

South America remained the main source of what limited amount of latex rubber was consumed during much of the 19th century. However in 1876, Henry Wickham gathered thousands of seeds from Brazil, and these were germinated in Kew Gardens, UK. The seedlings were then sent to Ceylon (Sri Lanka), Indonesia, Singapore and British Malaya (Malaysia). Today, over 90% of natural rubber latex is produced in Asia, especially Indonesia, Malaysia and Thailand. .

With the invention of the vulcanization process in the mid 1800's, the utilisation of latex became wide-spread, resulting in its use in the manufacture of over 40,000 products today.

These include:

- **Surgical and Household gloves**

The first suggestion of using gloves to reduce the risk of infection was made by Adam Elias von Siebold in 1813 [133]. However his suggestion was made to reduce the risk of the physician acquiring infections from the patient rather than the other way round[134].

Thomas Hancock received patents for clothing items, including gloves made of rubber, as early as 1830. But the instability of rubber remained a problem until 1843 when Charles Goodyear and Nathaniel Hayward in the USA and Thomas Hancock in England almost simultaneously discovered the process of vulcanization.

By 1852, a French surgical catalogue listed rubber anatomic gloves to prevent infection and in 1878 the first patent for rubber surgical gloves was granted to T Forster.

Although he may not have actually been the inventor of the surgical glove, William Halstead MD first introduced the sterile latex reusable glove into the operating room in 1889 and is thus considered by many to be the father of the surgical glove – certainly in the USA.

As scientific knowledge of infectious agents and their transmission grew, it became evident that extra precautions were necessary to guard against the spread of disease. Latex quickly was recognized as an effective substance to provide a barrier to prevent infections.

A significant development occurred in 1928, when the dipping of latex resulted in a thinner, stronger glove. These thinner gloves provided enhanced tactile qualities, which in turn allowed surgeons to perform more delicate surgeries. The gloves' increased strength and elasticity increased their barrier qualities.

Gloves originally were intended to be multiple-use items. They were washed, lubricated, re-sterilized, and used as long as they appeared to be intact. Single-use gloves increasingly became the norm by 1966 and are now standard practice rather than a preference because it is more economical to discard the gloves after each use than risk inadvertent transmission of infections.

By the early to mid 1980's single-use gloves had achieved a "new" role, that is at the commencement of the HIV/AIDS pandemic; gloves were being used by health providers and other sections of the population (such as the emergency services, the Police etc) as a protection for the caregiver rather than for the patient. This resulted in a dramatic expansion of the manufacture of natural rubber latex gloves. Industry estimates of the United States market in 1991 for exam gloves varied between 12 billion and 5.5 billion. The US Department of Health estimated the usage of latex gloves in 1997 at 20 billion pairs [13].

Synthetic alternatives to natural latex gloves, certainly in the mid 1990's, were not advantageous because neoprene gloves were not acceptable to surgeons and vinyl gloves were thought to be permeable to viruses.

The first commercial introduction of natural rubber latex gloves into the home is unknown. However, a number of companies in the USA set up glove dipping factories between the two World Wars. The majority of these companies were involved with dipping cotton gloves in latex rubber to make them waterproof. There is no doubt that true exploitation of the household market began after 1945, when glove companies had surplus capacity and with the advent of the “affluent society”.

- **Balloons**

The first rubber balloons were made by Professor Michael Faraday in 1824 for use in his experiments with hydrogen at the Royal Institution in London.

Toy balloons were introduced by pioneer rubber manufacturer Thomas Hancock the following year in the form of a do-it-yourself kit consisting of a bottle of rubber solution and a condensing syringe. Vulcanized toy balloons, which unlike the earlier kind were unaffected by changes in temperature, were first manufactured by J.G. Ingram of London in 1847 and can be regarded as the prototype of modern toy balloons.

In 1931, balloon technology advanced significantly when, Neil Tillotson dipped the first modern latex balloon.

- **Soothers and Feeding Bottle Teats**

The first patent for a process involving a medical application made from latex – dentures - was granted in 1851 to Charles Goodyear [2]. However the first use of latex to make a soother predated that by several years; a patent was granted to the British Company Maws, in 1845 for a soother which used an “Indian Rubber” nipple [1].

By the late 1800’s rubber soother teats were very common and in 1894 the English company Allen & Hanburys invented the first “banana” shape feeding bottle with a removable latex teat. 'The Allenbury Feeder' brought out in 1910 remained unchanged for the next 50 years.

Soothers were settling into their modern form around 1900 when the first latex rubber teat, shield and handle design was patented in the US as a “baby comforter” by C W Meinecke.

Mass-produced dipped teats for soothers and bottles paralleled in the time-frame with both gloves and balloons. Natural rubber latex enjoyed a virtual monopoly in the soother and bottle teats market until the mid 1980’s, when synthetic silicone rubber began to be introduced for these applications; 20 years later, natural rubber latex is used in less than 50% of teats.

Manufacture of dipped latex products

Processing of natural rubber latex (NRL) involves harvesting, anti-coagulation, concentration, and compounding.

NRL is harvested from Hevea brasiliensis trees, primarily in Southeast Asia. This latex contains 30 – 45% rubber, cytosolic organelles, nucleic acids, and proteins typically found in plant cells. Keeping the latex liquid is essential for the manufacture of ‘‘dipped’’ rubber products (gloves, condoms, catheters, balloons, pacifier and bottle teats etc.). Generally only a minority of the tapped rubber is used for dipping. The majority is processed by acid coagulation and drying into “dry rubber” for products such as tyres, hoses, belts, and gaskets by acid coagulation and drying.

At tapping, chemicals such as ammonium hydroxide, formaldehyde, tetramethylthiuram disulfide (TMTD), and zinc oxide are added to prevent coagulation of the rubber and to inhibit bacterial or fungal growth [17]. Latex destined for dipping is centrifuged to concentrate the total solids content to approximately 60%.

Although the production of gloves, balloons and teats varies somewhat, certain aspects are not dissimilar. Prior to manufacturing, the latex is formulated (compounded) by the addition of a number of chemicals, including surfactants, sulphur compounds, zinc oxide, accelerators (thiurams,

carbamates, and/or mercapto compounds), and anti-oxidants. Dipped latex products are produced in assembly line fashion. Product shaped moulds ("formers") are immersed in tanks of coagulant (calcium nitrate) or heated and then dipped in the compounded latex (dipping) to produce a thin coating of latex on the surface of the mould.

The device can then be leached while the latex is still moist (pre-vulcanized leaching), passed through ovens (vulcanization), and leached post-vulcanization, before the addition of lubricants if required (powders or polymers). Vulcanization is a key step that produces the cross-linking of the isoprene molecules resulting in an effective elastomeric barrier. In the case of gloves, powder (cornstarch) was often used to facilitate donning and removal of gloves, but as the powder has been implicated in latex protein allergy, powder-free gloves are more commonly manufactured, using for example a chlorination process to reduce the tackiness of the surface.

A Glossary of Terms related to Natural Rubber Latex Protein Allergy

Latex Protein (immediate) Allergy: An immediate allergic reaction caused by IgE-antibodies to latex, which develops in minutes after contact with latex by those who have developed IgE-sensitization to latex proteins [15,16].

Non-allergic hypersensitivity: An irritant reaction; not involving IgE antibodies

IgE: Immunoglobulin class E. An antibody class concerned with immediate hypersensitivity reactions.

IgE-sensitization to latex: The presence in the body of specific IgE antibodies against latex protein.

Urticaria: A skin rash, also called hives, or nettle rash, which is often accompanied by swelling and itching of the skin.

Angioedema: (previously termed giant urticaria or angioneurotic edema) is a condition involving swelling in the deeper layers of the skin, caused by a build up of fluid leaking from thin-walled blood vessels.

Contact Dermatitis: An inflammation of the skin characterized by redness, itching, blistering and, in chronic cases, flaking of scales of skin, resulting from exposure of the skin to substances in the environment. The site and shape of the affected areas of skin are directly related to the area that has been exposed to the causative substance.

Rhinosinusitis: Inflammation of the sinuses and nasal passages.

Allergic conjunctivitis: A broad group of allergic conditions involving inflammation of the conjunctiva, the thin membrane that covers the inside of the eyelids and the eye, up to the cornea.

Anaphylaxis: An acute, possibly life-threatening hypersensitivity reaction, involving the whole body.

Symptoms of Anaphylaxis: Anaphylaxis is a medical emergency that develops rapidly and can be fatal. A few or all of the following symptoms, often developing in this order, may be experienced:

- Itching of the lips, tongue and palate, swelling of the lips, tongue and throat
- Swelling of the eyelids, itchy, watery eyes
- Generalized itching, flushing, swelling of the skin, and hives (urticaria)
- Increased heart rate
- Abdominal cramps, nausea, vomiting, diarrhoea
- Difficulty in breathing due to throat swelling, wheezing and asthma
- A sense of impending doom
- Collapse, loss of consciousness, weakness and faintness caused by a drop in blood pressure.

Severe initial symptoms can develop within minutes following an encounter with an allergen, and usually reach peak severity within 3-30 minutes. Sometimes there can be a second phase reaction, 1–8 hours after the initial anaphylaxis.

A Brief History of Natural Rubber Latex Protein Allergy

One of the first cases of urticaria to latex was reported in 1927 - a reaction to a dental plate [3] and the first reported case of contact urticaria to latex household gloves was described in 1979 [4]. A year later, Forstrom [5] reported contact urticaria caused by surgical gloves.

The first report of anaphylactic reaction to surgical gloves was in an abstract by Dr Kristina Turjanmaa in 1984 [135]. She described two female nurses with systemic allergic reactions. In the same year additional reports of contact urticaria caused by latex exposure came from Sweden and Germany [136,137].

It was not until 1986 that it was determined that the reactions to latex products were IgE-mediated [6] and that the allergens were latex proteins remaining in the finished products [7].

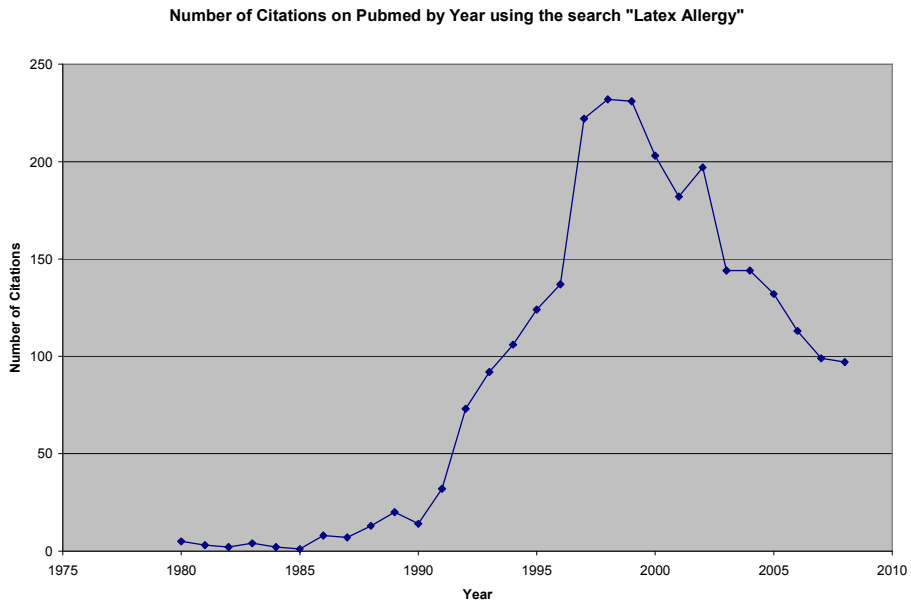
Between 1989 and 1992, there were over 1100 cases of allergic responses to latex products and 15 deaths resulting from latex protein-induced anaphylaxis reported to the US FDA [9]. An additional 1200 allergic reactions and 13 deaths had been reported by 1997 [10].

Over 9.9 million people were employed by the health care industry in the US in the late 1990's [11], and of these, 2.9 – 12.1% were estimated to be latex-allergic. Additionally, approximately 1% of the general population may be sensitized to latex [12].

Although the reasons underlying the increase in latex allergy are unknown, it has been suggested that several factors are involved. As has been noted there was a tremendous increase in glove use world wide and particularly in the US. This increase in demand for gloves resulted in changes in latex harvesting and manufacturing practices that may have altered the protein content and allergenicity of the gloves. However, there are no reported results of allergen assays on large numbers of gloves manufactured before (say) 1987, making it impossible to refute or substantiate this theory.

One important factor related to the increasing recognition of latex allergy was the discovery that it actually existed. Once a new disease has been identified and criteria for the diagnosis have been formulated, it is much easier for others to recognise similar cases; a phenomenon termed *a bias of ascertainment.* Of course an alternate view is that it is highly unlikely that so many reactions could have gone unrecognised prior to the early 1980's.

In any event the recognition and interest in latex allergy can be demonstrated by the number of citations on Pubmed found per year using the search: *Latex allergy*:



Although the vast majority of these papers involve gloves within the medical sector, the problem has not been confined to these parameters.

For example, a summary of the Annual meeting of the American Academy of Allergy, Asthma and Immunology in 2000 stated: *latex sensitivity amongst the general population is about 1% and that primary exposure is from balloons, pacifiers, shoes, elastic straps, adhesive mattresses, latex gloves, and condoms* [14].

Although the number of large-scale epidemiological surveys is limited, there is no evidence that the incidence of latex allergy is currently increasing. Indeed there is good evidence that latex allergy has decreased significantly in the last decade – by as much as 80% in Germany for example [111-116] and continues to decrease in many other countries [117, 120, 164, 165,].

Diagnosis of NRL allergy

In spite of many position papers and other recommendations, there appears to be a universal lack of consensus on how to diagnose allergy to NRL [138]. Even the criteria employed for positivity and negativity of tests are not uniform, although, at least in Europe, clear guidelines have been issued by the European Academy of Allergology and Clinical Immunology (EAACI) [139]. However the lack of overall consensus has led to many controversies in scientific papers and confusion among doctors and nurses not experienced in allergic diseases. Comparison of published results is therefore often impossible.

Even the terms “sensitisation” and “allergy” are widely confused within the research papers reviewed in this current paper.

Therefore it is difficult to give a definitive method for the diagnosis of NRL allergy. But perhaps in general it is based on the presence of a compatible clinical history and documentation of IgE-sensitization to NRL. A compatible clinical history would consist of symptoms typical of IgE-mediated reactions such as of urticaria, rhinitis, conjunctivitis, asthma, or anaphylaxis in association with exposure to NRL [22]. Documentation of sensitization in the US is generally done using serological testing, as no approved diagnostic extract for use in latex skin prick test (SPT) is currently available. Published sensitivities relative to SPT for the three FDA-cleared serological tests range between 73% and 92%, while specificities are between 73% and 97% [36].

In countries where approved allergenic extracts exist, SPT would be the first choice for documentation of sensitization. In some cases, clinical history and tests for IgE-sensitization to NRL give discordant results. In these cases, provocative challenges with NRL allergens can be useful to establish or rule out the diagnosis of NRL allergy [59].

Mechanism of sensitisation and latex allergy

It is known that latex allergy is mediated through IgE mechanisms, however, the immunopathogenesis of the disease is not completely understood [62]. Furthermore, many questions remain including the relative importance of individual proteins, the role of the route of exposure, and the potential for concurrent exposure to other chemicals/contaminants in the environment to modulate the immune response to latex proteins. Animal models of latex allergy have been developed in order to begin to investigate these questions and it has been shown that numerous aspects of the immunopathogenesis of latex allergy are similar between animals and man. [63-68].

Little is known concerning the role of the route of exposure in the development of latex sensitization. However animal models have demonstrated the potential for the development of sensitization following subcutaneous (SC), intratracheal (IT), intranasal (IN), and topical exposure to latex proteins [67]. In vitro penetration studies using hairless guinea pig skin and human surgical specimens demonstrated that the amount of protein penetrating into and through the skin was positively correlated to the degree of dermal abrasion [69].

However, sensitization occurs when exposure to latex proteins causes the body's immune system to develop antibodies to these proteins. Because the body perceives the protein as a threatening foreign substance, it prepares to launch a defence against it in future encounters. Therefore, people may have been sensitized to latex without having yet shown external allergic symptoms. They are, however, at risk of becoming increasingly sensitized and eventually symptomatic if exposure to latex continues/repeated.

Thus the events leading to an allergic state in susceptible individuals result form exposure to the allergens via the skin and/or mucous membranes, with repeated contact over an undetermined period of time that may extend over a number of years. They culminate in the production of several classes of antibodies, including IgE, directed against the allergens. Specific IgE antibody is present in the serum and on the surface of the connective tissue mast cells of latex-allergic individuals [154].

In conclusion therefore, sensitization to natural rubber latex is a prerequisite to immediate hypersensitivity reactions (urticaria, angioedema, anaphylaxis, and allergic rhinitis) that result from subsequent latex exposure [34].

Epidemiology of Natural Rubber Latex Allergy

Some general assessment considerations

The epidemiology of NRL allergy, particularly in health care workers, as well as the general population, has been the subject of some controversy [12,34]. Perhaps this is because identification of NRL allergy on a population-wide basis is not straightforward. In the clinical setting, as has been mentioned, NRL allergy is usually diagnosed based on a compatible clinical history and documentation of IgE-sensitization. Thus, in a population study, documentation of IgE-sensitization without clinical symptoms is not equivalent to identifying clinical NRL allergy [22].

Another major problem is that tests intended for clinical use may not perform optimally when applied to screening low prevalence populations for a condition such as IgE-sensitization to NRL. Clinical tests are optimized for use in evaluating patient populations where there is usually a high pre-test probability for the condition of interest. Assuming constant sensitivity and specificity, the higher the true prevalence of a condition, the more accurately a test will identify the prevalence of that condition in a population. If a condition is present at low prevalence, a larger proportion of test positives will be false-positives, resulting in poor positive predictive value of the test and overestimation of prevalence [35].

Tests for IgE-sensitization to NRL (including the often used ALSTAT assay), have reported specificities of 97%, 97%, and 73%, respectively, relative to the skin-prick test (SPT) [36]. Thus, even if the true prevalence of IgE-sensitization to NRL was zero, these tests would identify 3%, 3%, and 27%, respectively, of a population as being sensitized. Other studies suggest similar performance for serological assays [37]. In contrast, Liss and Sussman [12] reviewed available studies using SPT to estimate the prevalence of NRL sensitization in general population groups and found that estimates from such studies were in the range of 1%.

Another important issue affecting epidemiological studies is that of study design. Most published studies evaluating prevalence and risk factors for NRL allergy are cross-sectional (that is, they evaluate a population at a single point in time). Cross-sectional study designs fail to evaluate individuals who have already suffered adverse effects and left the population. Thus, cross-sectional studies can underestimate the prevalence of a condition in an exposed population by failing to evaluate those individuals most susceptible or most exposed.

NRL allergy and sensitisation in the general adult population

The prevalence of latex allergy in the general population is reported to be about 1% [89,90].

In addition a number of studies have examined the prevalence of IgE-sensitization to NRL (as opposed to clinical evidence of allergy) in the adult general population. A study screening sera from

1000 US blood donors for anti-NRL IgE using the AlaSTAT assay reported a prevalence of positive tests of 6.4% [38]. Another study using three separate laboratories to screen 1997 US blood donors with the AlaSTAT assay reported prevalence rates of positive tests ranging between 5.4% and 7.6% [39].

A study of British blood donors using the AlaSTAT assay found prevalence rates for NRL sensitization of approximately 4% in the winter and 7% in the summer. Cross-reactivity was noted between NRL and grass allergens [40].

These prevalence rates are not much different from the background false-positive rates that would be predicted from the reported specificity of the AlaSTAT assay relative to the skin-prick test.

NRL allergy in health care workers (HCW)

Over the past two decades, clinical NRL allergy has been an important occupationally related disease of health care workers [48]. Exposure of latex-sensitized health care workers to NRL can lead to a range of allergic symptoms including contact urticaria, rhinoconjunctivitis, asthma, or even anaphylaxis. Atopic health care workers have consistently been found to be at increased risk for both sensitization and clinical disease [49-53].

Historically, among health care workers, the prevalence of latex sensitization has been estimated at 3–22 % (11,49,52,155-161), depending on the population studied, the definition of sensitization used, and the selection criteria for inclusion of subjects in the study.

Despite the clear importance of latex allergy as an occupational disease of HCW, the relationship between such employment and IgE-sensitization to NRL has been controversial [12,34]. Some have argued, based on cross-sectional studies evaluating prevalence of IgE-sensitization to NRL, that employment in the health care industry is not a significant risk factor for NRL sensitization [34].

Despite these dissenting reports, several cross-sectional studies have found relationships between employment in the health care industry and IgE-sensitization to NRL. Baur et al. [50,54], in two cross-sectional studies have shown relationships between objective measurements of airborne NRL allergen and risk of sensitization in German health care workers. Levy et al. [55] reported a cross-sectional evaluation of graduating French and English dental students using SPT to evaluate for NRL sensitization. It was reported that students who had used powdered gloves were more likely to be sensitized than students who had used non-powdered gloves.

Current evidence from cohort studies, evaluating the incidence of health effects over time, strongly implicates employment in the health care industry as a risk factor for both latex sensitization and latex-induced allergic disease [56-58].

NRL allergy in children with spina bifida (SB) and others receiving multiple surgical procedures

Children with spina bifida (congenital spinal cleft) are clearly at increased risk for both NRL sensitization and anaphylactic reactions to NRL during surgery [43,44]. However, Porri et alia [124] considered that it is the number of surgical procedures rather than spina bifida per se that is related to sensitization to latex. Nevertheless, within spina bifida children, the number of operations experienced is a main risk factor for latex-related symptoms in sensitised children [125].

NRL sensitization rates ranging between 34% and 65% have been reported [44–47]. In this population, direct internal and mucosal contact with NRL medical devices may be the route of sensitization, as factors such as number of operations [79], and use of NRL devices such as catheters or ventriculoperitoneal shunts were associated with increased risk of NRL sensitization and allergy. Atopy (an underlying or familial tendency to become sensitised and produce IgE antibodies) has also been documented to be an important risk factor [45–47]. Atopic children with a history of three or more surgical procedures have a high risk of sensitisation to latex [75].

Nakamura et alia [78] showed that children receiving home mechanical ventilation also had a high incidence of latex allergy (circa 30%).

Dehlink et alia [126] have also showed that children suffering from chronic renal failure (CRF) are at increased risk of latex-hypersensitivity. Significant associations with atopy and repeated surgeries were observed.

Clearly, children with spina bifida have to live in a latex-free environment, both in the medical [127,128,130] and home settings. For example, Michael et alia [96] showed that 14 spina bifida patients out of 165 (8.5%) had clinical symptoms of latex allergy whilst inflating a latex balloon. There are indications that reducing latex proteins in all medical items and creating latex-free zones are resulting in a reduced number of SB patients sensitised to latex [131].

There is also some evidence that non-spina bifida children admitted to hospital for elective surgery have an increased risk of latex sensitisation [129].

NRL allergy in the general infant population

Two 1997 European studies evaluated the prevalence of NRL allergy in all children presented for evaluation in allergy clinics. In one study, 2.2% of 453 children had positive SPT to an NRL extract [41]. Half of the sensitized children reported symptoms related to NRL exposure, mostly triggered by contact with balloons and gloves. In another study [42], 3269 children undergoing allergy evaluations were screened for NRL allergy by SPT with 1.7% positive, and 1.1% confirmed by re-examination. One percent exhibited a combination of positive SPT, RAST and glove challenge. Balloons, followed by gloves, were the most important latex products causing symptoms.

Another study involving 1175 children (mean age +/- SD, 105 +/- 17.5 months) in 11 elementary schools in Tuscany (Italy) showed 0.7% had positive SPT responses to latex. No children showed allergic reactions to latex. However, 28.9% had one or more positive SPT responses to aeroallergens and 2.2% had responses to food allergens, indicating that latex allergy in the general infant population was rarer than other allergic reactions [91].

A relatively recent study shows that the prevalence of latex allergy and sensitization in Portuguese atopic and non-atopic children is similar to that observed in other countries [105].

However a UK study of 1877 children aged 7-years has suggested lower figure of 0.2% for the prevalence of latex sensitisation and clinical latex allergy in the general childhood population [140].

NRL allergy in children using NRL soothers or bottle teats

In 1988 Axelsson et alia showed that three atopic children first experienced their symptoms when blowing up rubber balloons. They argued that sensitisation in these patients must have been induced by repeated exposure to natural rubber products and pointed out that most soother and bottle teats (at that time) are made of natural rubber. Although they stated that none of the patients had experienced discomfort from using soothers or teats, they concluded, without further evidence, that: *we find it likely that these products might have induced the sensitization*. [8]. All the children had other sensitisations such as to peanuts, eggs, pollen and Alternaria fungi.

Wrangsjo et al. [83] found two adults, with SPT and RAST positive for *Hevea* brasiliensis, who reacted to extracts of rubber teats or pacifiers, and suggested that this may indicate a possible exposure of latex allergens earlier in life (without any other supporting evidence) and warned against the use of these products.

Makinen-Kiljunen et alia [88] reported on three Finnish children aged 0.7 to 2.7 years, who were admitted to an allergy clinic for severe atopic eczema. All the families had a positive history of atopy, and all had food allergy (cow's milk, cereals and other). They all used latex pacifiers. After testing positive to latex allergy, the latex pacifiers were removed from use and the children were reported to improve.

Novembre et alia [84] in a series of 326 allergic outpatient children, found 10 children with NRL-positive SPT. Among them, the frequency of use of rubber teats or pacifiers was comparable to that of atopic latex-SPT negative children or nonatopic children. They concluded that their use does not affect sensitization to latex.

Niggemann et al. [85] made a retrospective analysis of the risk of latex allergy development in a population of 5-year-old children. Among 149 atopic children, sensitization to latex was found in 20 (13.4%). The researchers were unable to discover relevant lifestyle factors inducing sensitization such as cross-reacting foods, the composition of mattresses, and the use of pacifiers. Retrospective study of the sera of 13/ 20 sensitized children showed that none of them had detectable specific IgE at the age of 1 year. Sensitization started around the second year of life, but most of the children appeared to be sensitized when between 3 and 5 years of age, which is generally after the use of pacifiers has ceased.

Venuta et alia [86] described the case of an 11 month old child who developed a cough. The child was negative to fruit allergy tests. The patient had used a rubber pacifier from birth. When replaced with a silicone pacifier the cough disappeared. The child tested positive to natural rubber latex allergy.

Freishtat and Goepp [87] reported on an atopic 2-month-old infant who experienced the previously unreported reaction of repeated stridor (noisy breathing, caused by narrowing or partial obstruction of the larynx or trachea) on exposure to a latex bottle teat while feeding. It was also reported that the mother had a history of atopy, including an episode of anaphylaxis to latex in a dental office. Substitution of the latex with a silicone bottle teat appeared to cure the child of these stridor symptoms.

Kimata [82] in 2004 reported on nine cases of latex allergy in Japanese infants younger than 1 year. None of the patients had any abnormality or had had any surgical operations. The author presented clear evidence that the latex allergic symptoms were caused by:

Patient No.	Major symptoms	Caused by
1	Wheezing	Teat
2	Wheezing	Teat
3	Swelling of face	Nose cleaner
4	Wheezing	Pacifier
5	Facial rash	Teat
6	Swelling of face	Pacifier
7	Swelling of lips	Teether
8	Anaphylaxis	Enema tube
9	Swelling of face	Balloon

All of the nine patients had positive responses to latex and extracts from latex-containing products.

It was also reported that seven of the patients had sensitisation to various foods and eight of their mothers also had food sensitisations and/or house dust mite. In addition four fathers and four mothers had previous allergic symptoms induced by latex.

In summary, these reports spanning 17 years from a number of countries would indicate that allergic reactions from NRL soothers and teats are relatively rare and might also question whether contact with these products were causal to the initial sensitisation.

Prevention of NRL allergy

In 1997, the National Institute for Occupational Health and Safety issued an alert entitled ‘‘Preventing Allergic Reactions to Natural Rubber Latex in the Workplace’’ [60], in which a variety of measures were recommended to lower exposures to NRL allergens. However these measures were in the main related to the use of gloves. For example, substitution of non-powdered, low-protein gloves to lower aeroallergen levels in a medical centre [61] and non-latex devices substituted for NRL devices.

Although some commentators – mostly Internet-based – advocate latex-free “zones” for the general population and even suggest the banning of all latex products, it is clear from all the research evidence presented in this report that reducing the allergen levels in NRL products is a most efficacious long-term objective.

For example condoms, widely used as a means of contraception and/or to prevent sexually transmitted diseases, were among the first latex products reported to cause an allergic reaction — from genital urticaria to anaphylaxis — in individuals who had become sensitised to latex [107,108]. This is not surprising given that classic latex condoms in the 1990’s may have contained as much latex allergen as latex gloves [73,109].

Levy et alia [110] investigated the use of deproteinised latex condoms by tests on nineteen adults with documented latex allergy who were unable to use classic latex condoms because they had previously had one or more allergic reactions when using them. Each patient was given 12 deproteinised condoms, to be used over a period of 6 weeks, and a diary form to be filled out each time a condom was used. All 19 patients used all 12 condoms during the 6 week study period and none had an allergic reaction or any other adverse event on or after contact with these condoms. Subsequently, the patients were given an additional supply of these deproteinised condoms and they used them without incident for at least an additional 6 months providing further evidence that they are safe for use in latex allergic patients.

The present author of this review believes that this simple example demonstrates that reduction as far as is practicable of the proteins and allergens in the latex product, rather than removal of the NRL product itself is the medium to long term answer to the problem of latex allergy.

There is good evidence that this suggested policy is indeed working efficaciously. In Germany, a revised version of the compulsory technical regulations for dangerous substances (TRGS 540) has been enforced since 1998. This explicitly states that only low-allergen, powder-free NRL gloves are allowed at workplaces [111]. This led to an immediate decrease in the use of powdered NRL gloves and, with a time lag of about two years, the number of latex allergies in the healthcare service has also decreased by as much as 80%. [111-116].

In the USA, the number of workers' compensation claims for latex-related illness declined significantly between 1997 and 2005 [164] as have the reported allergic symptoms in operating room staff [165].

A report from France [117] found that the incidence of latex anaphylaxis is decreasing as a result of the identification of at-risk patients, improved testing, and preventive measures.

In addition many manufacturers are successfully decreasing the level of latex allergens in a number of products [118-119].

In the UK a National Guideline issued in 2008 by the Royal College of Physicians and the NHS Plus Project [120] stated:

- *The use of powder-free, low protein latex gloves as an alternative to powdered latex gloves significantly reduces the incidence of latex allergy and latex-induced asthma, as well as the prevalence of latex-related symptoms.*

- *No reports of new cases of latex allergy arising from non-powdered low protein latex glove use were found.*

- *The evidence does not therefore support a complete ban on the use of latex gloves.*

In 2002 the EU Medical Devices Experts Group issued guidelines on the interpretation of the Essential Requirements of the Medical Devices Directive, as they relate to the risks of natural rubber latex (NRL). It was concluded that the appropriate way to manage the risk is to reduce allergenic protein levels to a level as low as reasonably practicable and provide warnings about the residual risks (i.e. the presence of latex and powder) [123].

Characterization and relative reactions of latex allergens

Currently, there are 13 *Hevea* latex allergens recognized by the IUIS Allergen Nomenclature Committee [80].

Allergen	Biochemical or Trivial Name
Hev b 1	Rubber elongation factor
Hev b 2	beta-1,3-glucanase
Hev b 3	Small rubber particle protein
Hev b 4	Lecithinase homologue
Hev b 5	Acidic NRL protein
Hev b 6	Hevein precursor **
Hev b 7	Patatin-like protein
Hev b 8	Profilin
Hev b 9	Enolase
Hev b 10	Superoxide dismutase (Mn)
Hev b 11	Class I chitinase
Hev b 12	Non-specific lipid transfer protein
Hev b 13	Esterase

** Hev b 6 is the 20 kD precursor protein of Hev b 6.02 (4.72 kD polypeptide known as mature hevein).

The allergens found in latex include proteins involved in the biosynthesis or coagulation of polyisoprene (Hev b 1, 3, 6), pathogenesis related proteins (Hev b 2, 6, 7, 11), structural proteins (Hev b 4, 5, 8), and housekeeping enzymes (Hev b 9, 10).

The reaction to specific latex proteins varies quite considerably but there appears to be preferential reactivity to certain proteins among different patient populations [23]. For example, Hev b 1 and 3 are more common allergens for patients with medical exposure, such as spinal bifida or other urogenital malformations [24], while other proteins (Hev b 5, 6, 13) are more common allergens for health care workers with occupational exposures [25, 81].

However Pamies et alia [141] found in the serum of a study group of children that IgE antibodies rHev b 1, rHev b 5 and rHev b 6.01 were the most common and rHev b 3 rHev b 7 in the minority of cases. All the children were sensitised to NRL allergy and the majority had been subjected to at least two surgical operations.

It has been suggested that Hev b 5 is the cause for the high frequency of fruit sensitivity in latex-allergic patients [145].

Testing for latex protein content

It is commonly held that the amount of residual protein in the latex product determines the allergenic potency of the product. Thus, it is necessary to accurately measure the protein levels of latex products in order to evaluate allergic potential, and subsequently, to choose products with low protein/allergen contents. There are three major approaches to evaluating the amount of protein on rubber products: total protein, antigenic protein, and allergenic protein. Each approach has its merits and its technical problems [22].

Total proteins

Proteins can be routinely measured by relatively simple, chemical methods. These methods rely on the dye-binding properties of certain amino acids but cannot distinguish between allergens, antigens or sources of the protein. Certain compounding chemicals interfere and falsely elevate the readings in the total protein assays [26,142].

The method generally involves extraction of the water soluble proteins in a buffer solution and then precipitation with acids in the presence of deoxychlorate to concentrate and separate from other water soluble substances which may interfere with the determination. The extracted proteins are redissolved in alkali and quantified colorimetrically by a modified Lowry method. This assay is based on the reaction of proteins with copper and Folin reagent to give a characteristic blue colour. Spectrophotometric measurements are performed at a fixed wavelength in the range 600 nm to 750 nm.

The Lowry method was established as a standardized assay by the American Society of Testing and Materials (ASTM D5712) in 1995. The protein precipitation steps in the procedure reduce interferences, but the method still suffers from these problems.

The US FDA allows manufacturers to make low protein claims if their product contains less than 50 mg/gm by this method. ASTM standards for surgical and exam gloves (D3577 and D3578) recommend < 200 mg/dm2 on all exam and surgical gloves. In Germany, the Institution of Statutory Accident Insurance in the Health and Welfare Service (Berufsgenossenschaft für Gesundheitsdienst und Wohlfahrtspflege-BGW) recommends a limit of 30 µg/g by the Lowry Modified Test Method EN 455:3 [103] for medical gloves.

The Lowry method generally shows good correlation with the equivalent ASTM method [104], but neither discriminates between allergenic and non-allergenic proteins.

There are no International standards for the protein content of balloons or soother and bottle teats.

Antigens

An antigen is a substance that prompts the generation of antibodies and can cause an immune response. ELISA (Enzyme-linked immunosorbent assay) methods use antibodies to measure antigenic latex proteins.

The antigen level is thought to estimate potential allergenic protein content because the immunological processes, whereby proteins are recognized as antigens or allergens, are similar. The antigen assays have been criticized because they use animal sera, however, data indicate that antigenic protein is a useful estimate of allergen content [20,27]. Additionally, ELISAs are not plagued by problems with chemical interferences. Technically, the assays have the advantage of having common antisera which can be produced and standardized to ensure that the same antigens are being measured by all laboratories performing the assay.

Latex ELISAs have been established in indirect and inhibition formats [20,27,28]. The LEAP (Latex ELISA for Antigenic Proteins) assay is an indirect ELISA that has been used by manufacturers since 1993. More recently an inhibition format ELISA has been established as an ASTM standard (D6499-03 updated to D6499-07) [152,153]. Both assays use rabbit anti-ammoniated latex antisera and ammoniated latex (AL) protein as their reference material. Currently, ASTM is considering a recommendation of < 10 mg/dm^2 of antigenic protein on gloves [22].

The test result does not directly correlate with the allergen content of the end product because it measures NRL antigenic proteins not allergens.

Allergens

The amount of allergenic protein in natural rubber latex products is determined using IgE inhibition methods.

These methods use pooled sera from latex-allergic patients in an inhibition assay format [29-33]. The inhibition format involves a solid phase allergen on disks, tubes, or microtiter plates. A glove extract is mixed with patient sera (latex-specific IgE) and then added to the solid phase allergen. The soluble allergen competes for antibody binding with the solid phase allergen and the resulting inhibition is

used to determine the amount of soluble antigen in the samples. In testing the allergenicity of product extracts, allergen assay test methods have been found to best correlate with skin prick test (SPT) in latex-allergic patients [20,29], but these methods are difficult to standardize between laboratories because different pools of patient sera are used and there is a scarcity of patient sera.

The most recent assay, which is has been incorporated into the standard ASTM D7427, is the two-site immunoenzyometric assay (IEMA) which uses an insolubilized capture antibody to bind one of Hev b allergenic proteins from a latex product extract, and a second enzyme labeled antibody to detect bound allergens. Optical density responses are interpolated from reference curves constructed with known allergens, Hev b 1, 3, 5 and 6.02.

The first and perhaps best known of the IEMA methods is the FITkit® technology developed by FIT Biotech Ltd, Tampere, Finland which predates the ASTM International standard D7427-08. The FITkit® technology is currently owned by Icosagen AS (formerly Quattromed AS), Tartu, Estonia.

Relationship between extractable proteins and allergens, and skin-prick test reaction

Several studies have documented considerable differences between the allergen content of latex gloves made by different manufacturers, and even between gloves of different batches from a single manufacturer [29,72-74]. Allergen levels in toy balloons were comparable to those in powdered gloves; but much lower allergen levels have been measured in pacifier and bottle teats [29], as demonstrated in the following table taken from Yunginger et alia [73]:

It should be pointed out that these data were reported in 1994, probably prior to some of the changes and improvements in manufacturing techniques discussed elsewhere in this current report. Undoubtedly, the allergen level of many of the products have reduced dramatically, but the comparative differences between soother and bottle teats and the other products may still be relevant.

Extractable Latex Allergens in Some Medical and Consumer Products measured by inhibition immunoassay after Yunginger et alia [73]

Product	**Allergen (AU/ml)**
Condom	50
Anaesthesia re-breathing bag	50
Balloon	4,700
Pacifier teats	<5
Bottle teats	<5
Non sterile examination gloves	2,856 - 31,673

AU = allergy unit

Baur et alia [29] and Tomazic-Jezic et alia [94] found a poor correlation between extractable protein content and allergen content, but other teams have shown a reasonable correlation [73,74, 143].

However, the consensus of opinion is that the protein content in latex products should only be used for orientation purposes only and that the allergen content gives more detailed information and allows cogent decisions on preventive measures [95].

Even so, Yip et alia [77] demonstrated that higher extractable protein content of NRL gloves, determined by the modified Lowry microassay procedure, are always associated with positive SPT allergic responses, while very low protein contents tend to exhibit weak or no allergic reaction.

They also carried out similar studies with dry natural rubbers of various commercial grades and dry rubber products manufactured via processes quite different from those of latex-dipped articles. Their findings reveal that they not only have extremely low extractable protein contents, but also show negligible or no allergic responses when skin-prick tested on latex hypersensitive persons. The authors conclude that dry natural rubber products are free from the protein allergy problem reported for some latex products. This may well be true, but further corroborative research is difficult to find. In addition, a majority of the general public, let alone clinicians and researchers would have difficulty in distinguishing products made from dry rubber with similar products made from dipped latex.

Audo et alia [97] demonstrated that the modified Lowry method for the measurement of total extractable proteins (ASTM D 5712-99 in the USA and EN 455-3 in the European Union) [98,99] offered reasonable correlation with allergen-specific methods, such as IgE Elisa inhibition when the total protein content of gloves was ≥ 50 µg/g of glove, but that below this level the modified Lowry assay does not yield reliable estimates of allergenicity levels. This problem using the modified Lowry assay has also been documented by Beezhold et alia [144].

A significant dose-response relationship between the allergen load on the one hand and the risk of sensitization on the other hand has been found [70,73-74].

Palosuo et alia in 2002 [71] stated that minimizing allergen concentration in latex goods to prevent sensitization to natural rubber latex (NRL) and thereby the development of clinical allergy is acknowledged as of mutual interest for rubber manufacturers and regulatory health authorities. Using a quantitative capture-ELISA for the measurement of Hev b 6.02 and Hev b 5, the two major allergens for NRL-allergic adults, and Hev b 3 and Hev b 1, the two major allergens for children with spina bifida, they showed good limits of detection and also repeatability and reproducibility.

Palosuo et alia [71] also showed an excellent correlation between the sum of these four allergens and skin-prick test (SPT) reactivity. They concluded that *in general, when the sum of the four allergens exceeded 1 microg/g, most NRL-allergic patients showed positive skin prick test reactions against them. Using these new methods assessment of threshold levels that could in due course become guidelines for the rubber industry and regulatory health authorities is becoming possible. Eventually, this progress is expected to lead to a declining incidence of latex allergy.*

In a later study Palosuo et alia [106] compared the sum of these four major allergens (Hev b 1, 3, 5, 6.02) as determined by the capture EIA kit (Fitkit™) with SPT-validated IgE-based ELISA-inhibition. Excellent correlations were found. By comparing the sum concentration of these four selected NRL allergens with results obtained in human IgE-ELISA inhibition, the authors set a cut-off level (0.15 µg/g) below which virtually all gloves contain low or insignificant amounts of allergens, and can be considered as low allergenic.

Koh et alia [76] also used the FITkit to analyse surgical and examination gloves for the specific allergens, Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02. Examination gloves had higher NRL allergen content compared with surgical gloves, and powdered gloves had higher allergen content compared with non-powdered gloves. Among the various allergens, Hev b 5 and Hev b 6.02 were present in larger quantities than Hev b 1 and Hev b 3. Only two of 19 (11%) surgical gloves had the sum of the four allergens (Hev b 1, Hev b 3, Hev b 5, Hev b 6.02) in excess of the “threshold” 1 microg/g, whilst among the examination gloves, 25 of 30 (83%) exceeded this level.

Crippa et alia [92] carried out Radio allergosorbent tests (RAST) on a number of products; the results were expressed in terms of percentage inhibition. However the same test also produced up to 11% inhibition on latex-free products, probably mainly due to unspecific binding. In addition, Hev b1, Hev b5, and Hev b 6.02 were determined using the FITkit.

No attempt was made to correlate percentage inhibition with allergen content. However in respect of those products with low percentage inhibitions (which include a latex soother) the authors remarked that contact with devices with a low percentage of inhibition cannot cause sensitization in non-allergic subjects but could be a risk for sensitized subjects.

Yeang et alia [93] carried out various assays to estimate the amount of residual allergenic proteins extractable from latex gloves and to identify appropriate protein markers to assess the allergenic potential of latex gloves.

The presence of 6 latex allergens--Hev b 1, 2, 3, 5, 6, and 13 was measured in a cross-section of commercial latex medical gloves by using monoclonal and polyclonal antibody-based 2-site immunoenzymetric assays. The overall allergenic potential of these gloves was assessed by IgE-inhibition assay. All 6 latex allergens were detected in at least some of the glove samples. Hev b 5 and Hev b 13 were identified as the marker allergens that combined best to explain the variation in the glove allergenicity. The significant multiple correlation (R=0.855) between these 2 markers and

glove allergenic potency could form the basis of an assay to gauge latex glove allergenicity. The correlation between glove allergenicity and the level of these allergens was maintained for low-protein gloves (<200 microg/g). This estimation of glove allergenicity was superior to that obtained by using total protein readings.

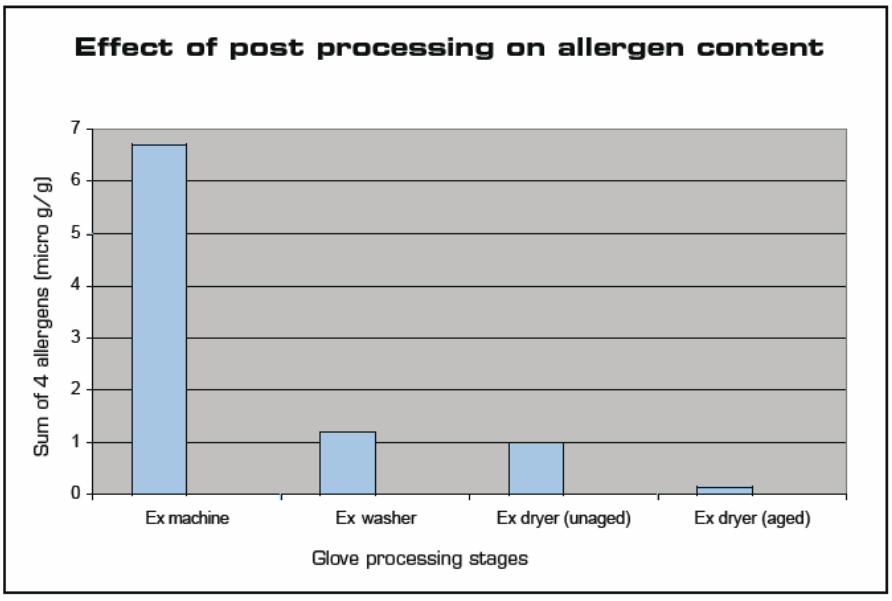
In conclusion this review of the literature indicates that it is not enough to measure the total protein content because (a) only some proteins are allergenic and (b) the current methods (such as the Modified Lowry Assay) certainly do not give reliable information or correlate with the allergenicity of a NRL product particularly at the lower ends of detection.

At the opposite end of the spectrum, it is certainly not sufficient to measure only one allergen because individuals maybe become sensitised to more than one allergen. According to the current literature, four allergens: Hev b 1, Hev b 3 Hev b 5 and Hev b 6.02 have been shown to be in glove and other NRL product extracts and their quantification provides an excellent correlation to the overall potential allergenicity of the product [71,106].

Effects of manufacturing on residual allergens

Treatment of field latex with ammonia to prevent coagulation causes the latex proteins to hydrolyze. The hydrolyzed peptides and chemicals bloom to the surface during the drying and vulcanization stages. Once on the surface, most of the residuals can be removed by leaching. The coagulants and other chemicals are leached out of the wet gel films during pre-vulcanization leaching. However as long as moisture remains in the wet gels, protein can continue to migrate to the surface of the film. Thus, effective protein reduction is achieved by leaching post-vulcanization and before final drying.

According to Ansell Limited, a major glove manufacturer [121] there is a dramatic reduction in allergen content as the gloves pass through the production stages. The allergen content shown in the following graph was measured using the FITkit™.



Alternatively, enzyme treatment of NRL to reduce antigenic proteins has proven successful [100] and indeed this process has been commercialised [122] and a range of deproteinised gloves (DPNR) have achieved market success. It is claimed that these products have allergen levels below the level of detection [121].

Particularly in the case of soothers and bottle teats, leaching has become over the past 25 years extremely sophisticated initially due to the need to reduce other contaminants, such as N-Nitrosamines and N-Nitrosatables, to conform to a variety of National and International Directives and

standards [18, 150,151]. For these products, fresh warm water, surfactants, agitation and time (24 hours is not untypical) may all be adopted to ensure as complete leaching as possible.

For gloves, special attention has been paid to the slurry tanks for powder application, as they have been shown to be a major source of problems. As gloves are dipped into the slurry (typically 10% cornstarch), residual proteins and chemicals leach from the film. As millions of gloves are dipped into the slurry, proteins accumulate and reach a high concentration [19]. When the slurry dries onto the gloves, accumulated proteins then dry back onto the surface of the glove and the powder. When dry, the powder acts as a carrier for the residuals (proteins and chemicals) and becomes airborne during donning and removal of gloves.

Replacement of the powder slurry with a chlorination step to produce powder-free gloves drastically reduces the protein content and endotoxin levels of the gloves [20,21].

Washing of NRL gloves in potassium hydroxide solutions has been shown to reduce the level of antigenic proteins [101] as has treatment with non-ionic surfactants such as Triton X-100 [102].

As a final note it is perhaps worthwhile adding, that the processing of *Hevea* latex into dry rubbers and their products is different from that of latex products and perhaps only 10% of tapped rubber is made into latex products. Instead of dipping, latex is first converted into dry rubber usually by acid coagulation. This is followed by crumbling/creping with extensive washing in water, and then drying at 100 - 130^{o}C. Fabrication of dry rubber products often involves compounding and curing of the dry rubber at elevated temperatures, sometimes reaching as high as 160^{o}C. It is considered that in view of such processing steps, that extractable protein contents are relatively low and probably allergens – this is investigated in Part II.

Part II - A survey of the major allergens in household products taken from the German retail market

Introduction

In the light of a literature review on NRL protein allergy, it was decided to survey a number of natural rubber latex products currently used in the household - gloves, toy balloons, soothers and feeding bottle teats.

Sufficient samples were obtained to carry out quantification of the major NRL specific allergens: Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 and to determine total extractable proteins and extractable latex antigens.

Methods

In October 2007 twelve samples each of latex household gloves, toy balloons, latex soothers and latex bottle teats were purchased in stores in the Frankfurt area of Germany.

The samples of were sent to Quattromed Ltd Tartu, Estonia and analysed for specific natural rubber allergens by the FITkit® test.

The FitKit® is the first commercial test for the measurement of clinically relevant NRL allergens: Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02. It is based on the EIA (enzyme immunoassay) or IEMA (immuno-enzymometric assay) method, as is the ASTM International standard D7427-08. These novel tests overcome the significant limitations of other methods by using highly purified and characterized allergens, and specific monoclonal antibodies, against four major latex allergens known to be present in NRL products.

Both the soother and bottle teats were boiled for five minutes in distilled water prior to testing to conform to both manufacture's instructions and the requirements of EN 1400 and EN 14350 [148,149].

Three samples of soothers and three samples of bottle teats were also tested without the pre-boiling procedure.

The detection limits were: Hev b 1 <0.050 µg/g; Hev b 3 <0.050 µg/g; Hev b 5 <0.025 µg/g; Hev b 6.02 <0.025 µg/g and the total of the four allergens <0.15 µg/g.

Six samples from each product group were also analysed at Biologisches Mess- und Analyselabor GbR (BMA Labor), Bochum, Germany for total extractable proteins and for extractable latex antigen content. The extractions for both tests were carried out according to Recommendation XXI, 59th Announcement of the German Federal Institute for Risk Assessment (BfR) Bundesgesundheitsbl. 1999, 42: 814-816.

Determination of latex proteins was by the above Recommendation - the modified Lowry Assay. Tests were carried out at least in duplicate on each sample. The detection limit was 2µg/ml in the Lowry test solution, which is a protein concentration of between 4 and 16µg/g, depending on the weight of sample used and the extract volume.

Allergen concentration was determined by ASTM D 6499-03, a LEAP assay using an inhibition format ELISA. Tests were carried out at least in duplicate on each sample. The detection limit was 0.03µg/ml in the LEAP test solution, which is an allergen concentration of between 0.08 and 0.15µg/g, depending on the weight of sample used and the extract volume.

Results

FITkit® allergens test

The results for the four products tested in Tables 1a, 1b, 1c and 1d.

Hev b 1 was only detected at a relatively low level in 6 out of 12 gloves samples and not at all in toy balloons, soothers or bottle teats.

69% of allergens detected in household gloves were Hev b 5 and Hev b 6.02 and 84% in the toy balloons. Total allergens in 5 out of 12 (42%) household gloves exceeded 1 µg/g and in 10 out of 12 (83%) toy balloon samples.

No allergens were detected in the soother samples and only a small amount of Hev b 3 in one of the bottle teat samples.

Table 1a: Allergen Content of Household Gloves

Sample No	Hev b 1	Hev b 3	Hev b 5	Hev b 6.02	Total Allergens
	µg/g				
G1	UD	2.21	0.07	UD	2.28
G2	UD	UD	UD	UD	UD
G3	UD	UD	0.03	UD	0.03
G4	UD	0.12	0.22	0.04	0.38
G5	0.11	1.65	0.12	0.11	1.99
G6	UD	0.42	0.09	0.17	0.68
G7	0.29	0.34	0.03	0.03	0.69
G8	UD	0.20	0.21	0.05	0.46
G9	0.07	0.54	1.51	2.74	4.86
G10	0.06	2.91	2.25	6.2	11.42
G11	0.34	2.00	3.62	8.32	14.28
G12	0.06	0.33	0.07	UD	0.46
Averages	**0.08**	**0.89**	**0.69**	**1.47**	**3.13**
% Total Allergens	2.5	28.6	21.9	47.1	2.5

UD = Undetectable

Table 1b: Allergen Content of Toy Balloons

Sample No	Hev b 1	Hev b 3	Hev b 5	Hev b 6.02	Total Allergens
	µg/g				
B1	UD	0.62	0.08	0.72	1.42
B2	UD	0.24	0.08	0.66	0.98
B3	UD	0.16	UD	0.15	0.31
B4	UD	UD	8.52	50.72	59.24
B5	UD	6.34	0.31	UD	6.65
B6	UD	2.72	0.32	UD	3.04
B7	UD	1.08	0.39	2.44	3.91
B8	UD	0.95	0.10	0.24	1.29
B9	UD	0.41	0.52	0.13	1.06
B10	UD	1.68	0.67	4.89	7.24
B11	UD	UD	1.09	0.03	1.12
B12	UD	UD	0.24	3.95	4.19
Averages	UD	**1.18**	**1.03**	**5.33**	**7.54**
% Total Allergens	0.0	15.7	13.6	70.7	

UD = Undetectable

Table 1c: Allergen Content of Soothers

Sample No	Hev b 1	Hev b 3	Hev b 5	Hev b 6.02	Total Allergens
	µg/g				
S1	UD	UD	UD	UD	UD
S2	UD	UD	UD	UD	UD
S3	UD	UD	UD	UD	UD
S4	UD	UD	UD	UD	UD
S5	UD	UD	UD	UD	UD
S6	UD	UD	UD	UD	UD
S7	UD	UD	UD	UD	UD
S8	UD	UD	UD	UD	UD
S9	UD	UD	UD	UD	UD
S10	UD	UD	UD	UD	UD
S11	UD	UD	UD	UD	UD
S12	UD	UD	UD	UD	UD
Averages	**UD**	**UD**	**UD**	**UD**	**UD**
% Total Allergens	0.0	0.0	0.0	0.0	

UD = Undetectable

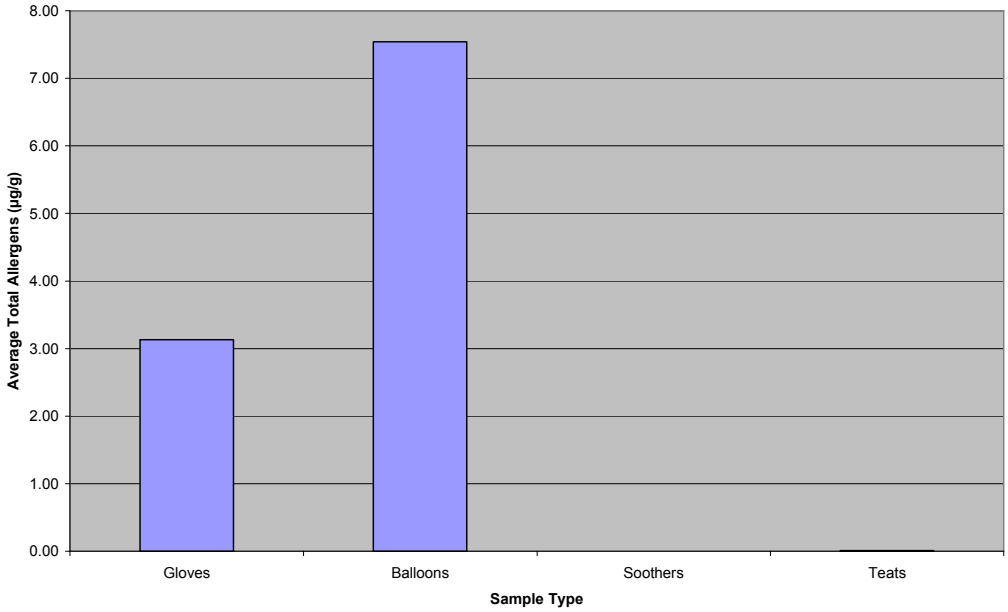
Table 1d: Allergen Content of Bottle Teats

Sample No	Hev b 1	Hev b 3	Hev b 5	Hev b 6.02	Total Allergens
	µg/g				
T1	UD	UD	UD	UD	UD
T2	UD	UD	UD	UD	UD
T3	UD	UD	UD	UD	UD
T4	UD	UD	UD	UD	UD
T5	UD	UD	UD	UD	UD
T6	UD	UD	UD	UD	UD
T7	UD	UD	UD	UD	UD
T8	UD	UD	UD	UD	UD
T9	UD	UD	UD	UD	UD
T10	UD	0.07	UD	UD	0.07
T11	UD	UD	UD	UD	UD
T12	UD	UD	UD	UD	UD
Averages	**UD**	**0.01**	**UD**	**UD**	**0.01**
% Total Allergens	0.0	100.0	0.0	0.0	

UD = Undetectable

Average total allergens are shown graphically in Figure 1.

Figure 1: Average Total Allergens by Sample Type



The difference between the average total allergens for gloves and balloons was not statistically significant using Student t test, but the differences between both the soother and bottle teats and gloves and balloons was significant ($p < 0.05$).

FITkit® test results for pre-boiled and un-boiled soother and bottle teat samples are shown in Table 2.

Table 2: Allergen Content of Soothers and Bottle Teats, with and without pre-boiling

Product	Sample No	Total Allergens	
		Pre Boiled	Not Boiled
Soothers	S1	UD	0.12
	S2	UD	UD
	S8	UD	0.05
Bottle Teats	T1	UD	UD
	T2	UD	UD
	T4	UD	UD

UD = Undetectable

Although in two of the soother samples, relatively low amounts of allergens (all Hev b 3) were found in the un-boiled samples, none was found in the un-boiled bottle teats.

Modified Lowry Assay for extractable proteins and LEAP allergen determination

For ease of comparison averages for the FITkit® test, Lowry, and the LEAP are shown together in Table 3.

Both the BfR and the Berufsgenossenschaft für Gesundheitsdienst und Wohlfahrtspflege (BGW) recommend a level of 30µg/g for extractable proteins by the Lowry method. It can be seen from Table 3 that Four out of six glove samples and all the balloon samples exceed this level. Four of the soother samples and 4 of the bottle teat samples also exceed the 30µg/g recommendation. However, only one balloon sample exceeded the “Richtwert” of 200µg/g set in 2002 by the BfR.

Table 3: Comparison of Results for Total Allergens, Extractable Proteins and Antigen Content

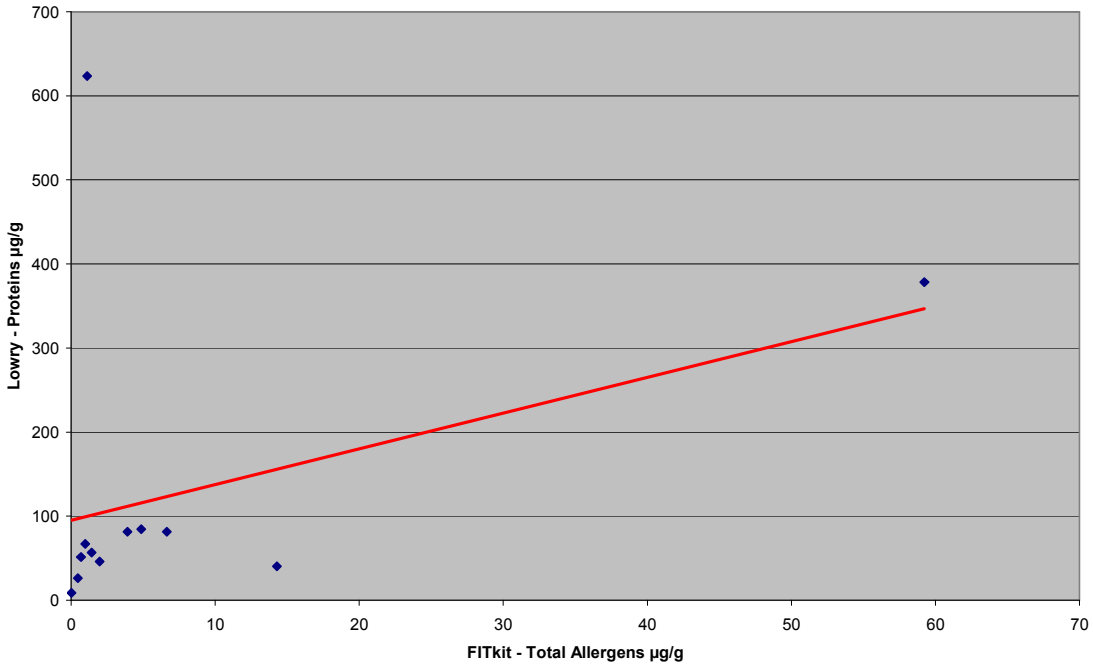
Product	Sample No	FitKit Total Allergens (µg/g)	Lowry Total Proteins (µg/g)	LEAP Antigen Content (µg/g)
Gloves	G3	0.03	8.9	UD
	G5	1.99	46.2	0.79
	G6	0.68	51.4	0.76
	G9	4.86	84.4	2.43
	G11	14.28	40.5	3.93
	G12	0.46	26.5	1.38
Averages for Gloves		**3.72**	**43.0**	**1.55**
Balloons	B1	1.42	56.7	0.97
	B2	0.98	67.1	1.30
	B4	59.24	378.4	11.42
	B5	6.65	81.7	8.35
	B7	3.91	81.5	3.41
	B11	1.12	623.6	7.56
Averages for Balloons		**12.22**	**214.8**	**5.50**
Soothers	S1	UD	29.4	3.10
	S2	UD	32.0	4.23
	S5	UD	UD	UD
	S8	UD	31.6	6.07
	S10	UD	72.9	2.34
	S11	UD	40.0	UD
Averages for Soothers		**0.00**	**34.3**	**2.62**
Teats	T1	UD	96.3	0.38
	T2	UD	UD	0.66
	T4	UD	42.3	2.84
	T8	UD	76.0	0.65
	T10	0.07	43.6	1.68
	T11	UD	UD	UD
Averages for Teats		**0.01**	**44.8**	**1.03**

UD = Undetectable

The ASTM recommendation of < 10 mg/dm^2 of antigenic protein for gloves as determined by the LEAP method [22] cannot be directly compared with the results in Table 4 as the surface area of the product samples is unknown.

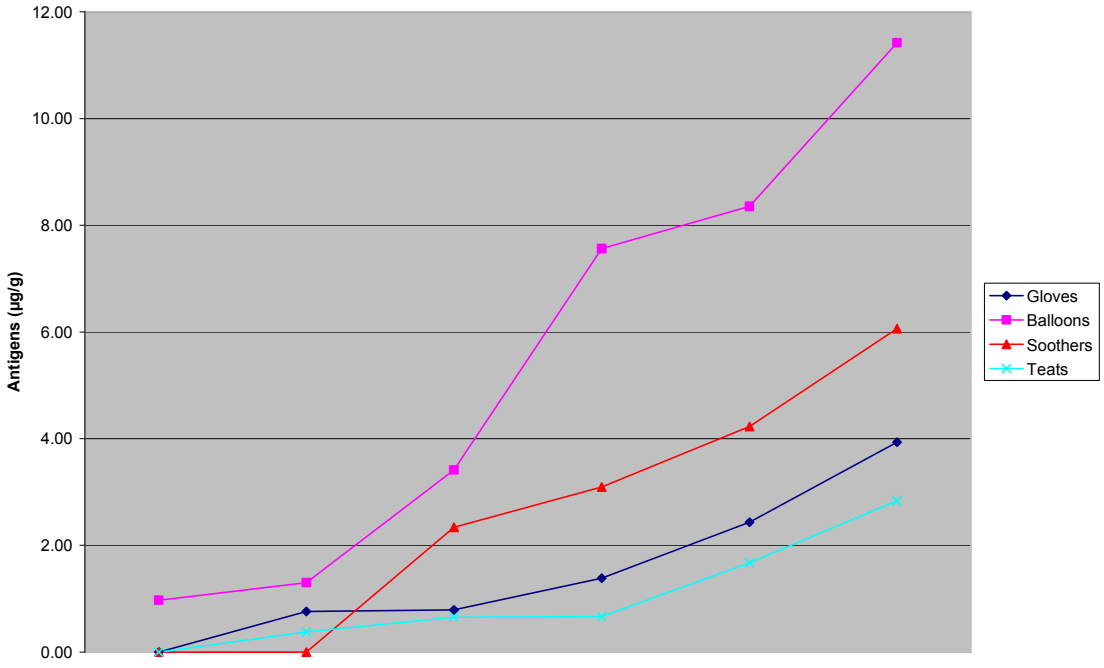
The correlation between total protein results and total allergen data was poor at r = 0.45, but statistically significant (p = 0.03) as shown in Figure 2:

Figure 2: Correlation of FITKit and Lowry Results



However it can be seen from Table 4 and Figure 3 that the highest antigen results are from the balloon samples, followed by soothers, then gloves and finally feeding bottle teats.

Figure 3: LEAP Results by Product



The LEAP results correlated with the FITkit tests and with the Lowry Assay to a similar order (r = 0. 681, p <0.001 and r =0 .650, p <0.001 respectively).

Discussion

All the products tested, household gloves, toy balloons, soothers and feeding bottle teats have been implicated in the sensitisation of natural rubber latex protein allergy and causing allergic symptoms [6,14,8,83 for example].

Further more, a significant dose-response relationship between the allergen load on the one hand and the risk of sensitization on the other hand has been found [70,73-74]. It has also been demonstrated that the sum of the total of four allergens, Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 is a good indicator of the allergenic potential of medical gloves [71,106].

We have taken samples from the German retail market and tested them for these four allergens using an EIA (FitKit®) technique specific to these four major latex allergens. A number of these samples were also tested for extractable proteins using a Modified Lowry Assay and also extractable antigens by an inhibition format ELISA to obtain an indication of the correlation between these methods and the EIA technique.

It is clear from the results of this present survey that the total allergen content of the gloves tested are significantly less than previously reported [92,76]. However, 42% of the samples had total allergen contents in excess of the 1µg/g considered widely to be the threshold for sensitisation [71,76] and 83% were above the 0.15µg/g limit considered by Palosuo et alia [106] to be level where gloves contain insignificant amounts of allergens.

As far as we are aware this is the first time that a survey of this size, measuring four major allergens in, toy balloons, soothers and bottle teats, has been carried out; although Crippa et alia did report that the total Hev b1, Hev b5, and Hev b 6, determined also by the FITkit® technique, of a toy balloon was found to be 214µg/g [92].

In the current survey the average sum of allergens for toy balloons was found to be 7.54µg/g, over twice as high as the average found in the household gloves. However this average was inflated by a single sample result of 59.24µg/g. Nevertheless, 83% of the samples exceeded the 1µg/g “threshold” and all were in excess of the Palosuo 0.15µg/g cut-off. It is perhaps apposite to mention that some authors have considered that toy balloons, followed by gloves, were the most important latex products causing symptoms [41,42].

In addition, there are several other reports indicating that contact with balloons induce allergic reactions, both in the “at risk” paediatric sector and the general infant population [8,75,82,96].

There are no equivalent historical results for soothers and bottle teats, although the indications are that allergen contents are relatively low [29,73]. For example, Yunginger et alia measured the total extractable allergen content in soothers and bottle teats using inhibition immunoassay. They found levels for both below 5 Allergen Units/ml as compared with a balloon which had 4700 AU/ml.

In the present survey only one out of 24 samples of soothers and bottle teats had a detectable allergen (Hev b 3) and accordingly all the samples were well below both the 1µg/g and the 0.15µg/g thresholds and cut-off points.

In Europe, all soothers have to include the following instruction on or within the packaging [148]: *Before first use place the soother in boiling water for 5 min. This is to ensure hygiene.* Similarly for bottle teats [149]: *Before first use place in boiling water for 5 minutes. This is to ensure hygiene.* Accordingly, prior to the enzyme immunoassay assay soothers and bottle teats were boiled in distilled water for 5 minutes.

To check whether this boiling regime had a measurable effect on total allergen content, we also carried out the assay on three un-boiled samples of soothers and three un-boiled samples of bottle teats. Lack of boiling had a small but insignificant effect on total allergens determined.

The poor correlations between the three methods FITkit, Lowry and LEAP perhaps confirms previous reports that the Lowry test does not give a reliable measure of the allergenicity of latex products, particularly at the lower levels [97,142,144]. Also, poor correlations have been found between the

LEAP test and Lowry assay [94]. In addition ASTM state in the Scope of D 6499-3: *Although the method detects antigenic proteins, it should not be considered as a measure of allergenic proteins. Correlation of protein/antigen levels with the level of allergenic proteins has not been fully established* [152]. This statement also appears in the updated version of the standard D 6499-7 [153].

There are very few reports implicating soothers and bottle teats in sensitisation for latex protein allergy [85] although some sensitised children have clearly suffered reactions to using these products [82,83,86,88].

Perhaps significantly, the last two decades have involved a change in the manufacture of NRL soothers and bottle teats. The 1993 Directive concerning the release of *N* Nitrosamines and *N* Nitrosatable substances from elastomer or rubber teats and soothers [18] and the advent of the European Standards EN 1400 and EN 14350 [146,147] have caused the manufacturers to utilise purer raw materials (such as double centrifuged latex) and also to incorporate extensive leaching techniques into their production. Therefore, it is surmised that a not altogether fortuitous spin-off from these changes is the virtual negligible levels of allergens found in these products.

Conclusions

It is concluded from these results that, as compared with generally recognised thresholds for allergens, there is still some room for improvements in the quality of household gloves and toy balloons.

However, the allergen contents of the NRL soother and bottle teat samples were mainly undetectable and certainly well below the cut-off point, indicating an insignificant amount of allergens.

We have confirmed previous reports that poor correlations exists between the allergen test results and extractable protein and antigens. We consider the EIA specific (FitKit®) due to its excellent repeatability and relative ease of use to be ideal for controlling allergen levels in natural rubber latex products, rather than for example the Modified Lowry Assay for extractable proteins.

Given these data and the evidence of the literature review we are of the opinion that it is highly unlikely for NRL soother and bottle teats to promote sensitisation. In addition, a sensitised child within the general population is unlikely to suffer allergic reactions from any of the samples tested.

This does not imply that “at-risk” paediatric groups - spina bifida sufferers and others receiving multiple surgical procedures – should diverge from a latex-free environment.

Acknowledgments

This report was written on behalf of the Wirtschaftsverband der deutschen Kautschukindustrie e.V. (WDK) – Frankfurt, Germany. The author also acknowledges the support of MAM Babyartikel GesmbH for whom he has acted as a medical and technical consultant.

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