5 SUMMARY

Endotoxin, a lipopolysaccharide component of the cell wall of gram negative bacteria, may play a major role in the development of the Sick Building Syndrome. Filter of heating-, ventilation- and air-conditioning systems can represent possible source of endotoxin contamination of indoor air. Measurements of endotoxin on filters cannot be compared between investigators because of different retrieving conditions. This study was inaugurated to determine the most effective method and their recovery rate for endotoxin retrieval. Determination of endotoxin was performed with a biological test (Limulus Amoebocyte Lysate). In this investigation the most common filter types of HVAC systems used in Germany were examined. These are cellulose-, glass- and synthetic fiber filters that were inoculated with four different types of endotoxins LPS in two different activities, *Escherichia coli*, *Pseudomonas aeruginosa* and dust out of an HVAC system). Filters were brand new to prevent pre-contamination because of impracticable detoxification of the material. Different contamination fluids were used to imitate most realistic (dust) circumstances on one and to perform most standardized (LPS) conditions on the other hand. To prepare endotoxin suspension by extracting the inoculated filters these were shaken or sonicated in a Tween 20 or Triethylamine buffer (TAP) solution. For illustration see Figure 5-1. A total number of 360 samples were measured.

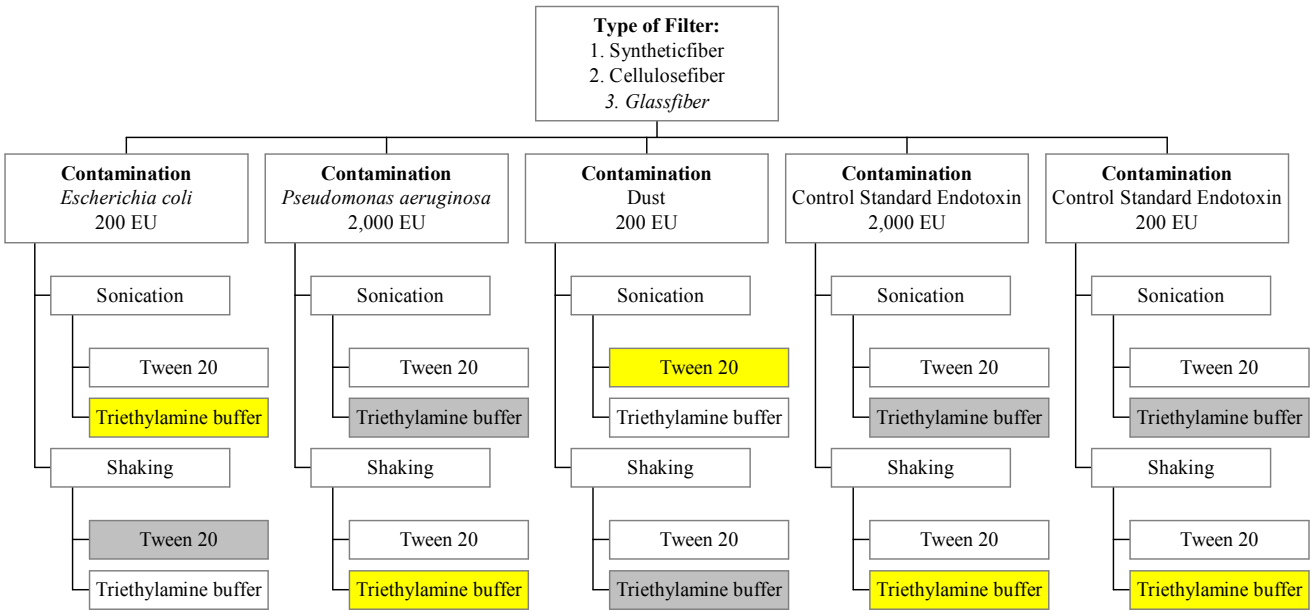

FIGURE 5-1 SETTING OF THE INVESTIGATION FOR CONTAMINATING 3 DIFFERENT TYPES OF FILTERS WITH 5 DIFFERENT TYPES OF ENDOTOXIN AND THE ENDOTOXIN RETRIEVAL WITH 2 EXTRACTION METHODS AND 2 SOLUTION MEDIA. DARK BACKGROUND INDICATES HIGHEST DEVIATION FROM "TRUE VALUE", BRIGHT BACKGROUND INDICATES SMALLEST DEVIATION FOR GLASSFIBER FILTERS.

In extensive preinvestigations optimal settings and conditions were drawn up (see 2.1.1.2.1, 2.1.1.2.2, 2.2.4.3, 2.2.1). Detoxification with γ-radiation (2.2.4.4) was checked, improvement of the test system, times of extraction and optimal concentration of solution media were worked out. Strict prevention of endotoxin contamination and a partly blinding were performed. For statistical evaluation descriptive data and data calculated with "Generalized Linear Models" were shown. Because of inhomogeneous distribution of *Pseudomonas aeruginosa* endotoxin contaminated filters and its enormous influence on the results this strain was determined separately.

When we analyzed data of all contaminations except *Pseudomonas aeruginosa* glass fiber filter showed a deviation from 100% recovery rate of 2.6%, cellulose fiber of 276.2% and synthetic fiber filter with 51.0%. Sonication revealed as best solution media with 18.9% difference ahead of shaking with 190.8%. TAP was supreme of Tween 20 with 53.3% difference and 160.6%, respectively. As expected LPS, it represents the most standardized endotoxin**,** showed best recovery of all examined endotoxins with a mean square fractional difference (MSFD) (2.1.8.2) of 70.1% for the activity of 200 EU and 48 % for 2,000 EU, respectively. Looking at the most standardized conditions with contamination of well defined endotoxin (LPS 2,000 EU) and a combination of the extraction method it was shown for cellulose fiber filter: Best extraction is performed with sonication in TAP with recovery of - 6.1% and an MSFD of 14.1%. In worst case if sonicated in Tween difference will present with -91.4% and an MSFD of 91.6%. Extraction of glass fiber filter is best if shaken in TAP (Figure 5-1) with MSFD of 19.2% and a difference of -16.7%. Almost as good as was the removal with sonication and TAP is with MSFD of 29.2% and an overestimation of 27.9%. Synthetic fiber filter revealed almost same results for all four combinations. Smallest difference for Tween and sonication with an overestimation of 5.5% is shown. MSFD for these combinations was between 20.3% and 24.1%.

When calculating data with "Generalized Linear Models" it revealed no significant differences for any constellation. This might be due to differences of sample conditions and their relatively small number of five samples per unique method.

The *extraction methods* are slightly but not statistical significant (n.s.) different (p=0.323).
Sonication showed a mean difference of 2 (CI_{95} –370 to 374) compared with the TA and was better than shaking which showed a mean difference of 263 (CI_{95} –109 to 635).

Solution media (n.s.) (p=0.327).
TAP showed a mean difference of 3 (CI_{95} –369 to 375) from the TA and was more potent than Tween 20 that showed a mean difference of 262 (CI_{95} -110 to 634).
Type of filter (n.s.) revealed a "p" of 0.375.
Glass fiber filters showed a mean difference of 0.5 (CI_{95} –455 to 456) that was better than synthetic fiber filters that showed a mean difference of 2.6 (CI_{95} –453 to 458) and was better than cellulose fiber filters which showed a mean difference of 394 (CI_{95} –62 to 850).

The *contamination fluids* reported a "p" of 0.393 and were not statistical significant.
LPS 200 showed a mean difference of 0.5 (CI_{95} –525.8 to 526.8) and was not as close as LPS 2,000 that showed a mean difference of 0.2 (CI_{95} –526.1 to 526.6) which was better than Dust that showed 1.0 for mean difference (CI_{95} –525 to 527) and
Escherichia coli which showed 528 (CI_{95} of 2 to1,054) for mean difference.

Although using a sophisticated statistical analysis, precise preparation of sample contamination with well defined endotoxin solutions, sample extraction and accurate assay work with partly blinding it was not possible to find one significant, most reliable and precise method for endotoxin recovery on HVAC systems filter. There were major differences between filter material and types of endotoxin. Tendencies of better methods were observed and need to be confirmed in larger extent. Especially the recovery rate has to be determined prior to the investigation and should be labeled because of its major impact for the results. A preinvestigation study of the interesting filter type with LPS contamination and approximately 50 reiterations could attain the recovery rate. Most important differences can arise if the standard curve is not prepared with the same dilution media like used for endotoxin recovery. As a general recommendation it is mentioned to use endotoxin free material wherever possible, to check water for absence of endotoxin, to perform very precise work, to verify nonattendance of interference for every single probe, to use same lot number of test kits for all tests and to do the assay work immediately following the extraction. Especially the samples should be dried after sampling when immediately extraction and determination is not possible, because of uncertain recovery for suspended samples.