procedure was adopted in which the DNA was made single-stranded by means of treatment with a low concentration of formamide, at low ionic strength. In this way (Table), exposure of single-stranded DNA to ≥10 nM sulfur mustard could be detected in the ELISA, whereas the same detection limit now holds for double-stranded DNA, when unwound in the proper way.

Table - Detection limits of competitive ELISA for calf-thymus DNA alkylated with 1 μM sulfur mustard

DNA sample	Detection limit N7-guanine monoadduct (adduct 1) at 50% inhibition point (fmol/well)
Single stranded	3.8
Double stranded	45
Double stranded/ unwound	2.9

In order to detect DNA adducts of nucleated cells in sulfur mustard-treated blood, white blood cells had to be isolated. These were lysed and a procedure for isolation of DNA was adopted, which included treatment with a proteinase in order to remove proteins around the DNA. Subsequently, the double-stranded DNA could be converted into single-stranded material. The detection limit in white blood cells in blood is ca. 2 μM, i.e., two orders of magnitude higher than for purified DNA. This is understandable since the proteins in blood bind several orders of magnitude more sulfur mustard than DNA. A detection limit of 2 μM sulfur mustard in blood is considered to be at the lower limit of toxicological relevance, since this is approximately the minimal concentration of the agent that inhibits proliferation of cells.

c. Detection of DNA adducts in human skin

Recently, experiments were performed to detect local DNA damage in skin samples. Pieces of human skin obtained from cosmetic surgery were exposed to air saturated with mustard vapor (at 30 °C, i.e., at a vapor concentration of 1260 $mg.m^{-3}$) for periods ranging from 0.25 to 10 min. The pieces of skin were then frozen to cut 5 μM slices, which were fixed on glass slides. Proteins and RNA were degraded enzymatically on the slide, and DNA was unwound. Subsequently, the preparation was treated with the monoclonal antibody against the N7-guanine adduct. Next, the antibody residing on the DNA adducts was allowed to bind to goat-anti-mouse antibodies. The latter antibodies contained a covalently attached fluorescent group emitting green light. The preparation was also treated with propidium iodide which intercalates with DNA and emits red light when properly irradiated. The coupes were analyzed under a laserscan fluorescence microscope. The red fluorescence from the propidium group was recorded which serves to locate exactly the nuclei of the epidermal cells in general. Scanning for the green light emitted by the fluorescent antibody locates the DNA that has reacted with sulfur mustard. In a slice of skin exposed for ≥ 1 min to sulfur mustard vapor it was observed that many of the nuclei of the epidermal cells showed this fluorescence. At this stage of the investigations the detection limit is at an exposure time of 0.5 min exposure, which amounts to a Ct-value of sulfur mustard (630 $mg.min.m^{-3}$) that would cause erythema, but not blisters, on human skin (12).