
40 C.F.R. § 79.67

Glial fibrillary acidic protein assay.

- (a) *Purpose.* Chemical-induced injury of the nervous system, *i.e.*, the brain, is associated with astrocytic hypertrophy at the site of damage (see O'Callaghan, 1988 in paragraph (e)(3) in this section). Assays of glial fibrillary acidic protein (GFAP), the major intermediate filament protein of astrocytes, can be used to document this response. To date, a diverse variety of chemical insults known to be injurious to the central nervous system have been shown to increase GFAP. Moreover, increases in GFAP can be seen at concentrations below those necessary to produce cytopathology as determined by routine Nissl stains (standard neuropathology). Thus it appears that assays of GFAP represent a sensitive approach for documenting the existence and location of chemical-induced injury of the central nervous system. Additional functional, histopathological, and biochemical tests are necessary to assess completely the neurotoxic potential of any chemical. This biochemical test is intended to be used in conjunction with neurohistopathological studies.
- (b) *Principle of the test method.* (1) This guideline describes the conduct of a radioimmunoassay for measurement of the amount of GFAP in the brain of vehicle emission-exposed and unexposed control animals. It is based on modifications (O'Callaghan & Miller 1985 in paragraph (e)(5), O'Callaghan 1987 in paragraph (e)(1) of this section) of the dot-immunobinding procedure described by Jahn *et al.* (1984) in paragraph (e)(2) of this section. Briefly, brain tissue samples from study animals are assayed for total protein, diluted in dot-immunobinding buffer, and applied to nitrocellulose sheets. The spotted sheets are then fixed, blocked, washed and incubated in anti-GFAP antibody and [I] Protein A. Bound protein A is then quantified by gamma spectrometry. In lieu of purified protein standards, standard curves are constructed from dilution of a single control sample. By comparing the immunoreactivity of individual samples (both control and exposed groups) with that of the sample used to generate the standard curve, the relative immunoreactivity of each sample is obtained. The immunoreactivity of the control groups is normalized to 100 percent and all data are expressed as a percentage of control. A variation on this radioimmunoassay procedure has been proposed (O'Callaghan 1991 in paragraph (e)(4) of this section) which uses a "sandwich" of GFAP, anti-GFAP, and a chromophore in a microtiter plate format enzyme-link immunosorbent assay (ELISA). The use of this variation shall be justified.

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