INTRODUCTION:
2,4-D (2,4-dichlorophenoxy acetic acid) is widely used as a selective herbicide to protect grain crop against broadleaf weed. Less known is the fact that 2,4-D is pre- and post-harvestly applied during citrus fruit production. During cultivation citrus trees are treated with 2,4-D in order to delay abscission of mature fruits. When applied post-harvest 2,4-D induces healing of injuries, retards senescence and aids in controlling post-harvest decay. For post-harvest application citrus fruits are immersed in water-based wax emulsions containing 2,4-D as acid, salt or ester.

A total of 196 citrus fruit samples were analysed in our laboratory between June 1996 and April 1999. 2,4-D residues were found in 71 samples (37%). The method used for analysis involves Supercritical Fluid Extraction (SFE) and is published in J. Chromatogr. 825 (1998) 45-54. In accordance to German regulations all samples were analysed as a whole.

BOUND 2,4-D RESIDUES:
A great part of 2,4-D residues on citrus fruits is chemically combined to the matrix and therefore not extractable by organic solvents. Nature and mechanisms of binding were not investigated in this study. However, it is assumed that the non-extractable 2,4-D residues are mainly bound covalently (by ester bonds) to the cuticle of citrus fruit and to waxes used as coatings of citrus fruit after harvest. Residues bound in such a way can be released by alkaline hydrolysis. The effect of alkaline hydrolysis was studied on real samples and samples treated with 2,4-D in our laboratory. The results achieved when alkaline hydrolysis was performed were up to six times higher than those obtained when samples were extracted directly. This indicates that total 2,4-D concentrations are systematically underestimated when no alkaline treatment is performed.

RELEASE OF BOUND* 2,4-D:
Investigations were made to estimate the extent to which bound 2,4-D residues can be released during processing and consumption of citrus fruits. Commercial lemon samples containing 2,4-D residues were used for these experiments. As an example for food processing a citrus jam was prepared. During this process 20% of the bound 2,4-D-residues in the sample were released. Intestinal and gastric digestion were simulated separately in vitro using pepsin and intestinal enzymes and bile of swine.

DISCUSSION:
2,4-D is widely used on citrus fruits. Great parts of 2,4-D residues are covalently attached to matrix components and are therefore non-extractable by organic solvents. In this study it was shown that non-extractable 2,4-D residues can be released easily under simulated conditions of intestinal digestion and can therefore be considered as potentially bioavailable and relevant to human. They should therefore be taken under consideration during analysis.