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Trifluoroacetic Acid (TFA) Use in Pharmaceutical Manufacturing and Potential Environmental Implications

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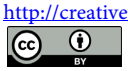

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Abstract

Trifluoroacetic acid (TFA) is a persistent pollutant that has been increasing in concentrations in aqueous environments over the past few decades. Anthropogenic sources of TFA include atmospheric degradation of certain volatile fluorinated compounds, degradation of fluorinated pesticides, including plant protection products (PPPs), and biotransformation of fluorinated pharmaceuticals in wastewater treatment plants (WWTPs) and other environments. Another significant potential source of TFA in the environment that has received less attention is its use in the manufacture of therapeutic peptides, such as insulin and GLP-1 agonists (large-scale weight loss and diabetes drugs). Large volumes of wastewater containing low concentrations of TFA can be generated from some of the GLP-1 manufacturing processes. Moreover, the production of some therapeutic peptides is expected to increase several-fold during the next few years. Herein, we review the manufacturing protocols for key therapeutic peptides and other drugs requiring TFA during manufacture and estimate the potential quantities of TFA released to the environment from these processes. Overall, a conservative estimate of total TFA used in the FDA-approved therapeutic peptide industry (excluding tirzepatide) was 555 - 979 metric tonnes per year, which gives an estimated 11,100 - 19,600 metric tonnes of accumulated TFA usage over the two decades ending in 2024. For comparison, this amount is equivalent to 33% to 58% of the global production of TFA in 2023 and is likely to increase substantially in the coming years. The large quantities of TFA currently used, which may be discarded as waste in the pharmaceutical industry, and the potential for large increases in coming decades, may result in significant point source discharges to WWTPs. Estimates of these discharges

and potential emissions are not yet possible owing to the lack of available data necessary for such determination. Treatment systems deployed at manufacturing facilities, which utilize reverse osmosis and/or ion exchange among other unit processes, are most likely to be effective for TFA removal from these complex waste streams.

Keywords

Trifluoroacetic Acid (TFA), Therapeutic Peptides, Solid-Phase Peptide Synthesis, Semaglutide, Tirzepatide, Pharmaceutical Manufacturing, Wastewater Treatment

1. Introduction

Anthropogenic trifluoroacetic acid (TFA) is the primary source of TFA found in rivers, ground waters, surface waters, and precipitation, with the oceans and other terminal water bodies (endorheic basins) acting as sinks for TFA (Albers & Sültenfuss, 2024; Nielsen et al., 2001). While the occurrence of natural TFA has been the subject of significant debate for many years (e.g., Joudan et al., 2021), a recent paper by Lindley indicates that a large natural source is required to account for the total mass of TFA in the Atlantic Ocean (Lindley, 2025). Recent publications have reported TFA concentrations in a wide range of water types, foodstuffs, and beverages (Garavagno et al., 2024; Moscato et al., 2025). A major anthropogenic source of TFA in the atmosphere is the degradation of certain volatile fluorinated compounds, including hydrochlorofluorocarbons (HCFCs), hydrofluorocarbons (HFCs), hydrofluoro-olefins (HFOs), hydrochlorofluoro-olefins (HCFOs) and fluoroethers, used mainly in refrigeration and air-conditioning applications and as foam blowing agents, and anaesthetics (Hart et al., 2026; Madronich et al., 2023; Solomon et al., 2016; Sulbaek Andersen et al., 2026). Degradation of plant protection products (PPP) containing the C-CF_3-moiety is another major source of TFA; over cropland, PPPs can be the dominant source of TFA to environmental aqueous phases (rivers, lakes and groundwater) (Henne et al., 2025). The yield of TFA from pesticides is uncertain, with an average TFA formation in metabolism studies of approximately 0.3 molar yield per CF_3 moiety being reported (Joerss et al., 2024). A more recent study (Johnsen et al., 2026) indicated lower TFA yields during 52-week laboratory studies for seven pesticides.

Pharmaceuticals and other per- or polyfluoro substances with a C-CF_3-moiety are additional potential sources of TFA in the environment. For example, the biodegradation of fluoxetine (a widely used antidepressant) by microorganisms is reported to yield TFA as a terminal degradation product (Khan & Murphy, 2021). In addition, the concentration of TFA has been observed to increase from the influent to the effluent of some wastewater treatment plants (WWTPs) employing ozonolysis (oxidation) (Scheurer et al., 2017), with the extent dependent on the composition of fluorinated compounds in the influent (potential sources of TFA).

This is a clear indication of oxidative decomposition of fluorinated organics in the WWTP treatment process to produce TFA. This is significant in that many fluorinated drugs ultimately end up in waters treated by WWTPs, which are then typically discharged back into surface water bodies. In sum, over 2000 chemicals are reported to produce TFA in the environment (Adlunger et al., 2022; Behringer et al., 2021).

Due to its physicochemical properties, including high water solubility and low K_{ow}, as well as its resistance to typical metabolic reactions, TFA is not expected to bioaccumulate or biotransform in most animals (Boutonnet et al., 1999; Hanson et al., 2024), although accumulation in plants has been reported (Chen et al., 2018; Freeling et al., 2022; Lan et al., 2020). Despite increasing exposure to aquatic and terrestrial organisms, our understanding of the potential ecotoxicological effects of TFA remains incomplete (Joerss et al., 2024). The UNEP Environmental Effects Assessment Panel (EEAP), in its assessments published in 2016 (Solomon et al., 2016), 2023 (Madronich et al., 2023), 2024 (Madronich et al., 2024), and 2025 (Neale et al., 2025), reviewed the known risks of TFA in the environment to ecosystems and human health. They concluded that the effects of TFA on marine organisms and chronic exposures in freshwater organisms have the greatest knowledge gaps. However, EEAP also concluded that, with current information, concentrations of TFA pose a *de minimis* risk in aquatic ecosystems, which is consistent with the conclusions of Boutonnet et al. (1999). EEAP also concludes that the risk to humans from chronic exposures to TFA in surface waters remains *de minimis* at current concentrations.

Hanson et al. (2024) identified several research gaps that should be addressed to improve our understanding of TFA sources, fate and ecotoxicity in the environment, as core uncertainties remain. One of the principal sources of uncertainty is the distribution and magnitude of TFA released from anthropogenic sources other than CFC replacements, such as manufacturing of fluorinated chemicals and the degradation of pharmaceuticals and pesticides that contain C-CF_3 moieties.

Although it has been reported (Lindley, 2025) that global manufacture of TFA has increased, reaching 34,000 tonnes in 2023 and in total about 350,000 tonnes in the period 2000 to 2020, there is relatively little published information on the current and future use, amounts recovered or discarded as waste, and potential emissions of manufactured TFA. Lindley (2025) estimated potential emissions using the 6.1% upper limit of the Medical and Chemical Technical Options Committee to the Montreal Protocol (MCTOC) most likely emission factor range for feedstock use (Technology and Economic Assessment Panel [TEAP], 2024), but comments that given its wide use, including in relatively small-scale processes, an estimate of TFA emissions from its production, use, and waste discharge is uncertain. TFA is widely used in the pharmaceutical industry for organic synthesis as a trifluoromethylating agent, as a reagent and solvent (Alam et al., 2024; Tan & Li et al., 2025; López & Salazar, 2013), and in separations of analytes in high-perfor-

mance liquid chromatography (HPLC).

An industrial process that links these uses together is the manufacture of therapeutic peptides, such as insulin and GLP-1 agonists (blockbuster weight loss and diabetes drugs). Significant quantities of TFA are used to synthesize and purify therapeutic peptides. Large volumes of aqueous effluent containing low concentrations of TFA can be formed from some of the processes, potentially leading to emissions to the local WWTP, unless the aqueous waste stream is treated to remove TFA. In this paper, the use of TFA for peptide synthesis is described, the quantities of TFA used are estimated, and treatment options for aqueous waste containing low concentrations of TFA are discussed.

2. Therapeutic Peptides

The production of therapeutic peptides has increased over the last thirty years, as shown in **Figure 1**. The number of FDA-approved peptides escalated in the 1990s and has been growing steadily since then (Al Musaimi, 2024; Lau & Dunn, 2018; Lombardi et al., 2025; Muttenthaler et al., 2021; Rossino et al., 2023; Wang et al., 2022; Xiao et al., 2025).

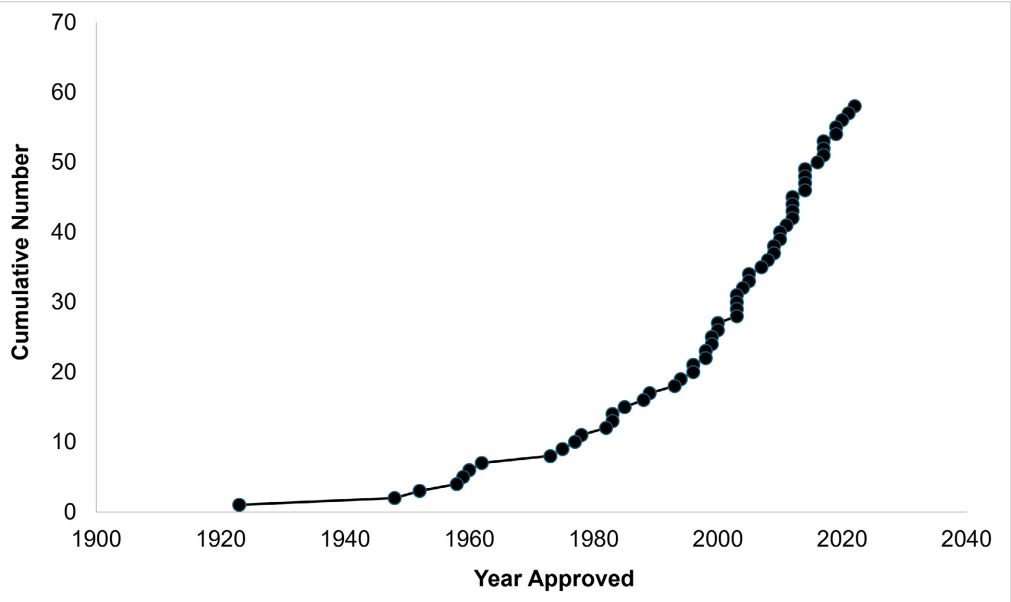


Figure 1. Growth in number of FDA-approved peptide pharmaceuticals.

Insulin and GLP-1 agonists are presently the most recognizable examples of this class of pharmaceuticals, though it also includes well-established antibiotics. Insulin is a peptide manufactured via recombinant DNA in *Escherichia coli*, requiring extensive purification including liquid chromatography. Recent examples of GLP-1 agonists are the weight loss and diabetes drugs semaglutide and tirzepatide. These two compounds accounted for ca. $50 billion in sales in 2024 (Eli Lilly and Company, 2025; Novo Nordisk, 2025). Semaglutide goes off patent in some key countries beginning in spring 2026 and there are expectations that generic versions

will quickly be produced and commercialized globally (Pratap, 2025). Sales of tirzepatide alone are expected to grow 5 - 10-fold from 2024 to 2030. Other synthetic peptides are also likely to increase in production in the coming years. In addition, there is also a growing illicit market for peptides not approved by the FDA for human use that is largely unregulated and employs solid phase peptide synthesis (SPPS), a synthetic methodology heavily dependent on TFA, *vide infra* (Esposito et al., 2012; Pflaum & Ručman, 2005; Sun, 2026).

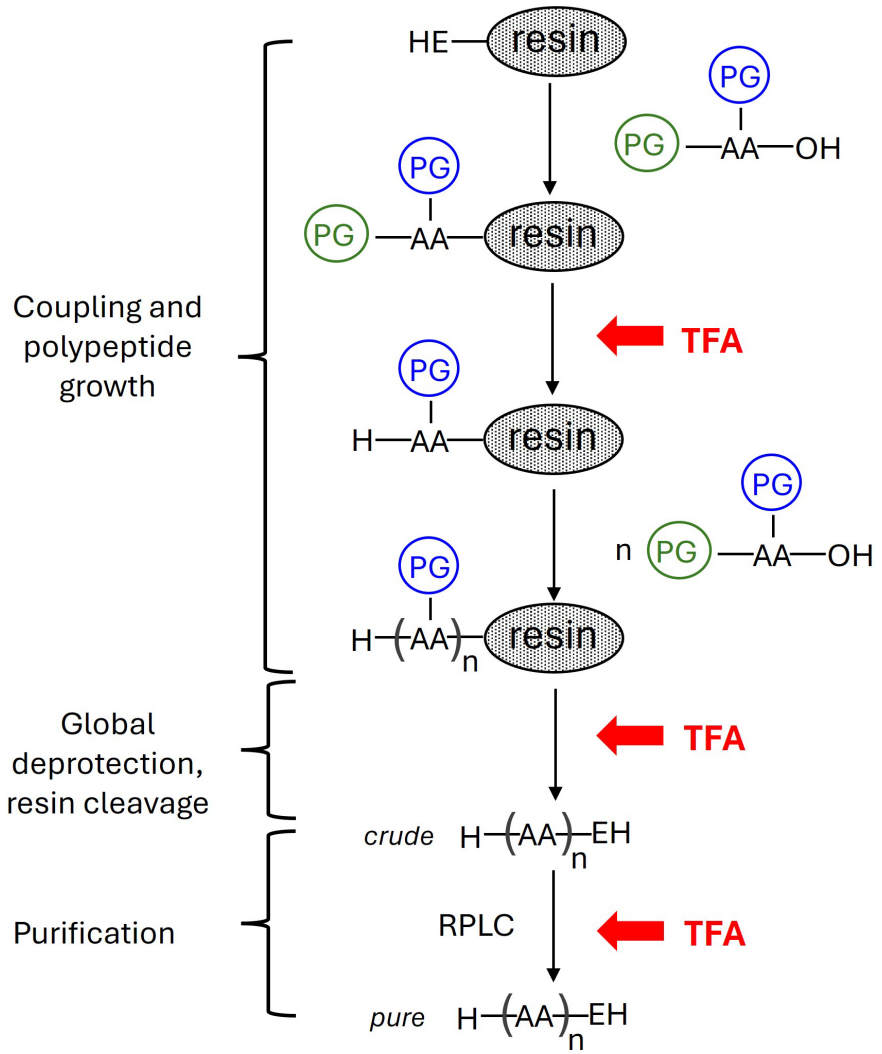
3. Why TFA Is Used in Therapeutic Peptide Synthesis and Purification

Large amounts of TFA are commonly used to synthesize and purify therapeutic peptides. The properties of TFA, including its acidity, volatility, deprotection reaction selectivity, chemical compatibility, and ion-pairing capability, make it a highly effective reagent in peptide synthesis and purification (Behrendt et al., 2016; Chakraborty & Berger, 2005; Fields & Noble, 1990; King et al., 1990; Lundt et al., 1978). In Boc (tert-butoxycarbonyl) solid-phase peptide synthesis (Boc-SPPS), the acidity of TFA and its deprotection mechanism allow repeated use in each amino acid coupling cycle for efficient Nα-Boc removal without cleavage of side-chain protecting groups or the resin linker (Lundt et al., 1978). In Fmoc (9-fluorenylmethoxycarbonyl) solid phase peptide synthesis (Fmoc-SPPS), TFA possesses sufficient acid strength to protonate and cleave acid-labile protecting groups and linker functionalities without causing extensive peptide backbone hydrolysis, enabling simultaneous cleavage of the peptide from the resin and global side-chain deprotection (Behrendt et al., 2016; Fields & Noble, 1990; King et al., 1990). The adoption of TFA-labile linkers in the late 1970s allowed TFA to replace anhydrous hydrogen fluoride used in Boc chemistry, eliminating the hazards associated with HF while preserving high cleavage efficiency and broad functional-group compatibility, a transition widely recognized as enabling the automation, scalability, and industrialization of peptide synthesis. The volatility of TFA further facilitates downstream purification after protecting group and resin cleavage, as it can be readily removed by evaporation along with other organic solvents and scavengers (Behrendt et al., 2016; Fields & Noble, 1990). In peptide purification by reversed-phase HPLC, TFA provides the appropriate acidity to lower the mobile-phase pH, suppress residual silanol interactions on the stationary phase, and act as an ion-pairing reagent; its hydrophobic yet mobile conjugate base, arising from the CF_3 group, transiently neutralizes positively charged residues on peptides. This increases effective hydrophobicity, leading to improved retention, peak shape, and resolution on reverse-phase chromatography (Chakraborty & Berger, 2005; Conlon, 2007).

There are on-going research activities to find a TFA replacement in SPPS. Although alternative acids such as formic acid have been explored for this purpose, their success has been limited by reduced chromatographic performance or incomplete compatibility with established peptide synthesis and purification work-

flows (Krokhin & Spicer, 2009; McCalley, 2002). Recently, the combination of methyl sulfonic acid in formic acid was tested successfully for deprotection and resin cleavage on multiple peptides, although an additional process step is required to manage a side reaction (Fidha et al., 2025). Alternatively, a combination of Brønsted (HCl or acetic acid) and Lewis acids ($FeCl_3$) also showed performance promise in peptide deprotection and resin cleavage. However, an additional process step is required for metal removal from the product (Pawlas et al., 2024). As a result, TFA remains the preferred and, in many cases, functionally indispensable reagent in peptide process chemistry and purification.

4. Therapeutic Peptide Synthesis Using TFA



Note: Caption. General scheme for solid phase peptide synthesis. Key steps that typically rely on TFA are indicated; see text for detailed explanation. Protecting groups required for peptide bond formation are depicted in green. Protecting groups for amino acid side chains (when needed) are shown in blue. E = NH, OH.

Figure 2. Solid phase peptide synthesis (Bachem AG, 2019) and purification (steps using TFA are indicated by red arrows).

Therapeutic peptides are manufactured by three principal methods, some involving various combinations of the three: solid phase peptide synthesis (SPPS), fermentation, and traditional organic synthesis (Chandrudu et al., 2013; Pennington et al., 2021). SPPS dominates and encompasses approximately 60% of all FDA-approved peptides. Organic synthesis alone is the least utilized and is reserved for low molecular weight peptides.

SPPS dates to the initial work by Merrifield in the 1960s and is shown schematically in **Figure 2**. The process involves the initial attachment of an appropriately protected amino acid to a functionalized resin, followed by sequential linkage of additional protected amino acids via peptide bond-forming reactions. The final peptide sequence is then cleaved from the resin and purified. TFA plays a prominent role in nearly every step of this process (NBInno, 2025): removal of protecting groups (temporary protecting groups required for amino acid coupling and peptide chain growth, and those needed for protection of reactive side groups), resin cleavage, and reversed-phase liquid chromatography (RPLC) purification. RPLC using TFA in the mobile phase is often used to purify peptides regardless of the preparation method. These steps are discussed in detail in the following section in the context of the synthesis of tirzepatide and insulin purification to illustrate TFA usage in these processes.

TFA is used in the following three key steps in the SPPS process and peptide purification. Publicly available information (publications and patents) provides some information on the TFA volumes used in these procedures, though not all steps are typically disclosed or disclosed in full detail. Therefore, while a complete, quantitative assessment of TFA utilization cannot be made, there is sufficient information to estimate conservative lower-limits scenarios. Protocols for SPPS cleavage steps are available from several resin and reagent vendors (Bachem AG, 2019; BenchChem Technical Support Team, 2026; Sigma-Aldrich, n.d.).

1) Resin Cleavage

Typically, the cleavage "cocktails" used to cleave the product peptide from the solid resin range from dilute TFA in dichloromethane (0.5% - 5% v/v, "soft cleavage") to those where TFA serves as both reactant and solvent (85% - 95% TFA, "hard cleavage").

2) Protecting Group Cleavage

The cocktails for cleavage of protecting groups vary with the protecting group or groups used. Those that are acid-labile are typically cleaved with the "hard" cleavage mixtures used for resin cleavage and largely consist of TFA in combination with scavengers and cosolvents. Two types of protecting groups are employed. Temporary protecting groups are used to block the amine end of the amino acid during peptide coupling and chain growth. These must be removed with each amino acid added to grow the peptide chain. Additional protecting groups are used to protect or block reactive side groups on individual amino acids. These latter protecting groups are left in place until the full peptide sequence is complete, then all are removed in a single step.

3) Purification

Nearly all (if not all) therapeutic peptides, regardless of manufacturing method, undergo multiple chromatographic purification steps requiring large quantities of solvent. Reversed-phase liquid chromatography (RPLC) using an acetonitrile (ACN)/TFA and water/TFA gradient mobile phase is commonly used for final purification. Sometimes methanol is used instead of ACN/TFA, and concentrations of TFA may range from 0.05% to 0.25%, with 0.1% to 0.2% being typical. For example, human insulin prepared by recombinant DNA technology is purified by a combination of chromatographic sequences, including one employing RPLC and ACN/H_2O mobile phase containing TFA. Although the mobile phase TFA concentrations are low, the large elution volumes required to separate and purify individual peptides to pharmaceutically acceptable purity levels result in large TFA usage. As discussed below, the literature suggests a conservative estimate of 6 - 12 kg TFA is required to purify 1 kg of human insulin by RPLC.

5. Tirzepatide and Semaglutide Syntheses

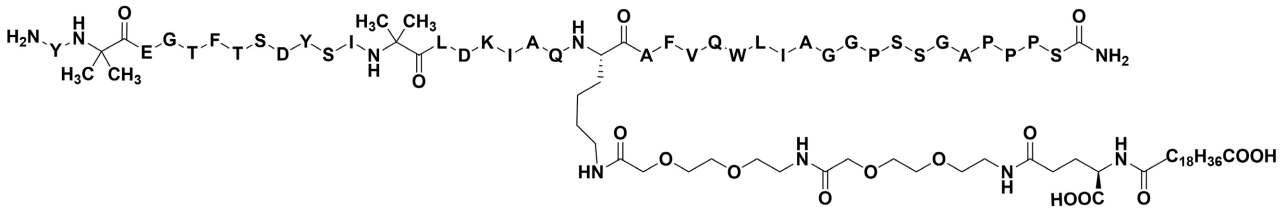


Figure 3. Tirzepatide.

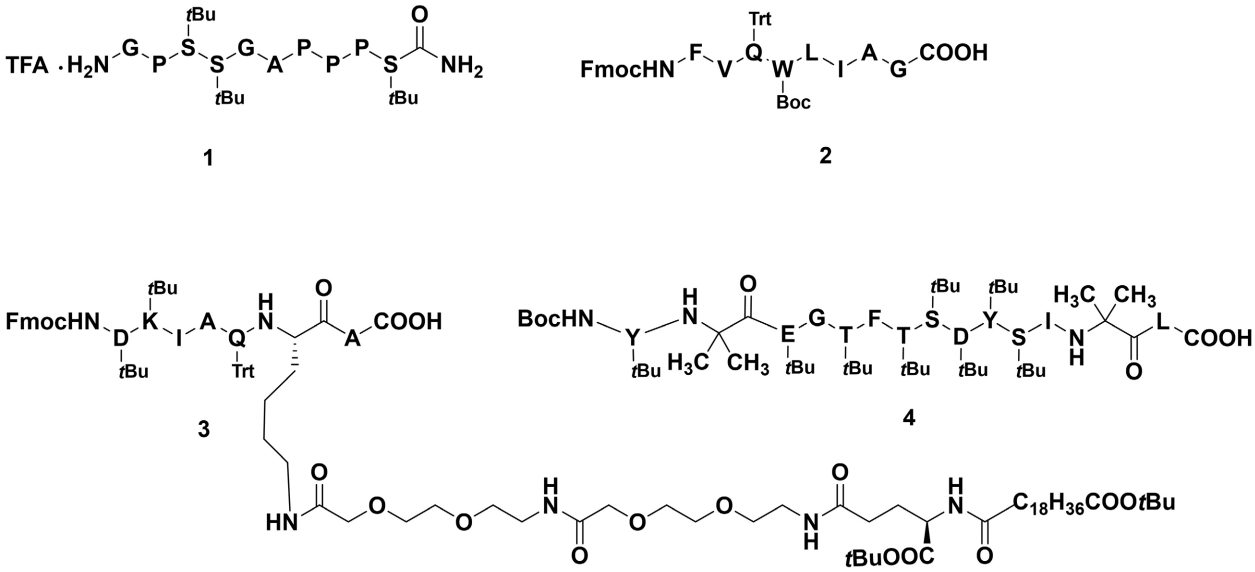


Figure 4. The four peptide fragments independently synthesized and sequentially coupled to produce tirzepatide (Frederick et al., 2021).

The structure of tirzepatide (Frederick et al., 2021) is shown in **Figure 3**. This peptide is comprised of 39 amino acids (both proteinogenic and non-proteinogenic),

functionalized near mid-strand with a long chain (C_{20}) diacid that modifies uptake and metabolism. It is prepared by sequential combination of the four smaller peptides (1 - 4) shown in **Figure 4**. The protecting groups tBu (tert-butyl), Trt (triphenylmethyl), and Boc on the functionalized amino acid segments are carried into subsequent reactions and removed in the final step.

The preparations of two of these peptide fragments have been reported while the other two have not yet been disclosed. The two that have been reported utilize SPPS and protection/deprotection steps involving TFA. It is reasonable to assume that the other two peptide fragments are prepared by similar means and thus also require TFA. Reversed-phase chromatographic purification is used throughout this complex process to purify the intermediate peptide fragments.

The synthesis of fragment 1 is described in WO 2024/077149 Example 16 (Coates et al., 2024). It is a complex synthesis, preceded by undisclosed preparation of necessary intermediates also based on SPPS. The final step involves Fmoc (9-fluorenylmethoxycarbonyl) deprotection with TFA and production of fragment 1 as the TFA salt, which is carried into the final steps to prepare tirzepatide. This synthesis uses 1.5 kg TFA per kg isolated fragment 1 (Kopach et al., 2025).

The synthesis of fragment 3 is described in U.S. patent application 2025/0188019 A1 Example 25 (Kopach et al., 2025) and involves both a "soft" cleavage step and reverse phase purification with 0.2% TFA. The cleavage step alone employed ca. 0.7 kg TFA per kg fragment 3. No details regarding the RPLC procedure are provided from which to calculate TFA utilization for purification.

The final steps in the multi-kilogram scale GMP (Good Manufacturing Practices) synthesis of tirzepatide have been reported in detail (Frederick et al., 2021), wherein these four fragments are coupled in the liquid phase. The sequence involves first coupling of fragments 1 and 2, coupling of the resulting intermediate with 3, and then final coupling of this second intermediate with fragment 4 to give the penultimate intermediate. This intermediate is the protected tirzepatide that is then subjected to treatment with excess TFA for global removal of protecting groups to provide the desired tirzepatide product. As indicated in the Experimental Section of reference (Frederick et al., 2021), the total TFA employed for the global deprotection step is ~25 kg TFA per kg tirzepatide (216.3/8.71 kg/kg).

Precise accounting for the total TFA used in the synthesis of tirzepatide is not possible, but a conservative lower limit scenario of 60 - 95 kg TFA/kg tirzepatide is supported based on the data available. This value accounts only for the final global deprotection ("hard" cleavage) step (25 kg/kg) in the published GMP procedure, cumulative "soft" cleavage steps to prepare fragments 2, 3, 4, and 5 and their respective RPLC purifications (estimated 29 - 53 kg/kg, 5 kg/kg for four fragment SPPS based on fragment 2 and 4 synthesis data; 24 - 48 kg/kg if RPLC purification is used for these fragments), and a single RPLC of the final tirzepatide (6 - 12 kg/kg; see the discussion on the insulin purification). Amounts less than this do not appear reasonable, and the quantity would increase significantly if the

peptide coupling chemistry selected to add each amino acid to grow the peptide chain requires TFA and is properly accounted for.

At the estimated production of 750 kg of tirzepatide in 2024, this analysis suggests that at least 45 - 71 metric tonnes TFA were used for this single peptide. With the tirzepatide market expected to grow 5 - 10-fold by 2030, 225 - 710 metric tonnes annually could be needed to meet that demand in a few years.

Semaglutide is a 31-amino-acid peptide (Knudsen & Lau, 2019; Lau et al., 2006, 2015). It can be manufactured either through a hybrid process involving recombinant production of the peptide backbone followed by site-specific chemical modification (European Medicines Agency, 2026; Knudsen & Lau, 2019; Knudsen et al., 2000) or entirely by solid-phase peptide synthesis (SPPS) (Chen et al., 2020; Lau et al., 2006, 2015). Reversed-phase liquid chromatography (RPLC) is employed for purification of peptide fragments and the final product in both manufacturing routes (Chen et al., 2020; Knudsen et al., 2000; Lau et al., 2006, 2015) with TFA at 0.1 vol% in mobile phase. In the SPPS route, the final resin cleavage and global deprotection step employs an 82.5 - 90 v% trifluoroacetic acid (TFA) solution, leading to TFA consumption of approximately 35 - 52 kg per kilogram of final semaglutide produced in this single step alone (Chen et al., 2020). Calculation of the total TFA usage across the full semaglutide synthesis is not possible with the data available. However, if the same TFA consumption determined for tirzepatide is assumed to apply to FDA-approved therapeutic peptides prepared by SPPS, then a minimum of 350 - 550 metric tonnes of TFA is employed annually based on an estimated total production volume of ca. 5800 kg (**Table 1**) in 2024.

6. Insulin Purification via Preparative RPLC

TFA consumption in the manufacturing of the remaining peptides in **Table 1** (i.e., those made by fermentation and/or direct synthesis) is difficult to quantify. In these cases, TFA is often used for final purification via preparative scale RPLC. Recombinant human insulin is reportedly purified by RPLC using TFA in the mobile phase. Examination of the literature suggests that this purification step consumes approximately 6 - 12 kg TFA/kg insulin (Amersham Biosciences, 2026; Kanezaki et al., 2018; Kroef et al., 1989; Mackin & Choquette, 2003; Mant et al., 2007; Sørensen et al., 2021). This estimation is based on published values for column volumes, bed volumes and densities, bed productivity (grams insulin/kg stationary phase), column washing steps, and TFA mobile phase concentration, all on a per-cycle basis. The 6 - 12 kg TFA/kg insulin estimation assumes 0.15% TFA in the mobile phase.

This analysis suggests that TFA utilization is 200 - 420 metric tonnes annually based on 35 metric tonnes of insulin produced in 2024 (Kjeldsen et al., 2024). Not all of the non-SPPS peptides shown in **Table 1** use RPLC in their purification. This is especially true of the older, albeit high-volume, peptides such as bacitracin, vancomycin, and cyclosporine. Combining annual insulin volumes with those of other

peptides manufactured via fermentation or synthesis and using RPLC purification gives a total annual volume of ca. 41,000 kg. Total TFA used in their purification is, therefore, *ca.* 250 - 500 metric tonnes.

Table 1. FDA-approved peptide pharmaceuticals and estimated production*.

Year of FDA Approval	Name	Manufacturing Method	2024 Volume, kg
2022	Tirzepatide	SPPS	750
2021	Dasiglucagon	SPPS	<1
2020	Setmelanotide	SPPS	1
2019	Afamelanotide	SPPS	<1
2019	Bremelanotide	SPPS	<1
2017	Semaglutide	Recombinant plus chemical modification	2000
2017	Macimorelin	Small molecule synthesis	3
2017	Plecanatide	SPPS	450
2016	Lixisenatide	SPPS	1
2014	Dalbavancin	Fermentation/synth modification	300
2014	Oritavancin	Fermentation/synth modification	10
2014	Albiglutide	Recombinant	0
2014	Dulaglutide	Recombinant	90
2012	Teduglutide	Recombinant	5
2012	Pasireotide	SPPS	10
2012	Linaclotide	SPPS	150
2012	Carfilzomib	SPPS	75
2011	Icatibant	SPPS	1
2010	Liraglutide	Recombinant	300
2010	Tesamorelin	SPPS	5
2009	Telavancin	Fermentation/synth modification	10
2009	Ecallantide	Recombinant	1
2008	Degarelix	SPPS	25
2007	Lanreotide	SPPS	25
2005	Pramlintide	SPPS	5
2005	Exenatide	SPPS	150
2004	Ziconotide	SPPS	<1
2003	Daptomycin	Fermentation	70
2003	Desirudin	Recombinant	1
2003	Bortezomib	Small molecule synthesis	20
2003	Enfuvirtide	SPPS	4000
2000	Triptorelin	SPPS	150

Continued

2000	Bivalirudin	SPPS	<1
1999	Cetrorelix	SPPS	1
1999	Ganirelix	SPPS	5
1998	Glucagon	Recombinant	2000
1998	Lepirudin	Recombinant	0
1996	Glatiramer acetate	Small molecule synthesis	2000
1996	Oxytocin	SPPS	5
1994	Nafarelin	SPPS	1
1993	Buserelin	SPPS	2
1989	Goserelin	SPPS	3
1988	Octreotide	SPPS	30
1985	Leuprolide	SPPS	8
1983	Cyclosporine	Fermentation/synth modification	60,000
1983	Vasopressin	SPPS	1
1982	Insulin (human)	Recombinant	35,000
1978	Cosyntropin	SPPS	<1
1977	Desmopressin	SPPS	20
1975	Calcitonin-salmon	SPPS (replaced extraction)	<1
1973	Somatostatin	SPPS (replaced extraction)	<1
1962	Colistin (polymyxin E)	Fermentation	800
1960	Lypressin	SPPS	<1
1959	Polymyxin B	Fermentation	600
1958	Vancomycin	Fermentation	4000
1952	Corticotropin (ACTH)	Extraction	3
1948	Bacitracin	Fermentation	30,000
1923	Insulin (animal)	Extraction	20

Note: *Manufacturers do not report production volumes and thus estimated values are presented. The estimates are derived from the following publicly available information: market size ($), estimated cost per dose ($/dose), dosage per patient (mg/dose), frequency of administration (times per year), and the number of patients treated per year; volume calculations are global for 2024. For example: Based on the reported 2024 sales disclosures and approved dosing regimens, global semaglutide active pharmaceutical ingredient (API) demand in 2024 was likely on the order of 1 - 3 metric tonnes, with a mid-range estimate of 2 tonnes. The reported 2024 sales of GLP-1 diabetes products of DKK 149.1 billion and obesity-care products of DKK 65.1 billion, indicate several million patient-years of semaglutide exposure worldwide (Novo Nordisk, 2025). Injectable semaglutide products use relatively little API per patient (2 - 2.4 mg/week), whereas oral semaglutide uses 7 - 14 mg/day and therefore contributes significantly to total API consumption (U.S. Food and Drug Administration [FDA], 2025). Combining estimated patient-years derived from 2024 sales with labeled dose ranges yields approximately 160 - 405 kg for injectable semaglutide, and 800 - 2650 kg for the oral product, giving a total semaglutide requirement of roughly 1000 - 3000 kg globally in 2024, with a central estimate near 2000 kg. A similar analysis with available data (Eli Lilly and Company, 2025) provides the estimated tirzepatide volume. The volume for human insulin is provided by Kjeldsen et al. (2024). These three peptides account for the bulk (75% - 80%) of TFA utilization in therapeutic peptide manufacturing.

In combination, then, a conservative and very approximate estimate of total TFA

used as a reagent and solvent in the FDA-approved therapeutic peptide industry alone was 600 - 1050 metric tonnes in 2024. Removing tirzepatide from this total, owing to its recent FDA approval, reduces this total slightly to 555 - 979 metric tonnes per year. Using this latter value gives an estimated 11,100 - 19,600 metric tonnes of accumulated TFA usage over the two decades ending in 2024. For comparison, this amount is equivalent to 33% to 58% of the reported global production of TFA in 2023 (Lindley, 2025) and is likely to increase substantially in the coming years (IndexBox, 2026). The fraction of this total that is recovered/reused, sent to waste treatment, and emitted to the environment is not calculable as the necessary data is not currently available.

As already noted, semaglutide goes off patent in some key countries beginning in spring 2026 and there are expectations that generic versions will quickly be produced and commercialised globally (Pratap, 2025). Generic versions may be synthesized, possibly exclusively, via solid-phase peptide synthesis (SPPS), as much of the recombinant manufacturing route is held as proprietary trade-secret and challenging to reproduce. A growing semaglutide generic market, with manufacture by SPPS, would result in increased TFA utilization per kg of semaglutide, due to the additional TFA-dependent steps required by SPPS compared to the recombinant manufacturing route. In addition, there is also a growing illicit market for peptides not approved by the FDA for human use, largely unregulated, synthesized by solid phase peptide synthesis (SPPS) (Choi, 2026; Esposito et al., 2012; O'Brien, 2026; Pflaum & Ručman, 2005; Sun, 2026) and whose volumes are not accounted for here.

Combined with the expected growth in tirzepatide, this points to very large growth in synthetic peptides in the coming years and increasing use and potential emissions of TFA.

7. Considerations regarding the Treatment of Waste Streams Containing TFA

For therapeutic peptides, TFA is a reagent, not incorporated in the final products, and serves multiple purposes in their manufacture. Thus, TFA is typically a waste contained in several different effluent streams. The peptide synthesis overview described is just one example of the industrial uses of TFA, where its use can generate various waste streams containing TFA. Aqueous waste streams containing low concentrations of TFA present challenges in treatment if there is a requirement to avoid TFA discharge to the environment. For example, the large volumes of waste from reversed-phase liquid chromatography (RPLC) purification are aqueous, containing low concentrations of TFA (4000 - 8000 litres per kg peptide consisting of ca. 60% water, 40% acetonitrile, and 0.15% TFA) (Amersham Biosciences, 2026; Kanezaki et al., 2018; Kroef et al., 1989; Mackin & Choquette, 2003; Mant et al., 2007; Sørensen et al., 2021). An effluent stream of water, acetonitrile, and TFA cannot be separated by conventional distillation, as water/acetonitrile forms an azeotrope (b.pt 77˚C) (Lide, 2003) and water/TFA forms a high-boiling azeotrope

(b.pt. 105˚C) (Mahajan et al., 2008). Recovery of acetonitrile from water may be achieved using, for example, extractive distillation (Sander et al., 2025). But TFA would remain in the aqueous phase. Similarly, TFA remains in the aqueous phase if methanol is distilled from a TFA/methanol/water waste stream. TFA can be separated from water using reactive distillation in a batch reactor (Devale et al., 2023), which might be suitable for small-scale processes.

Another consideration in distillation approaches is the pH of the waste stream; given the ca. 0.2 pKa value for TFA, it is the trifluoroacetate ion that is of concern in streams with pH greater than 2 - 3, not the free acid. The removal of TFA from aqueous waste streams has been evaluated (Scheurer et al., 2017) and its removal is not achieved using conventional methods such as activated carbon. The authors concluded that ion exchange or reverse osmosis may be applied to remove TFA, finding that reverse osmosis shows a much better efficiency compared to ion exchange. A photochemical reduction process has also been reported for the treatment of TFA-containing wastewater. This technology can be applied to contaminated groundwater and surface waters (e.g., lakes) and can also be integrated into industrial production lines to treat wastewater prior to discharge (Turner, 2026). Complex waste treatment processes may be required to remove other substances in the waste stream and ensure that reverse osmosis, if used, is effective for the removal of TFA.

Each industrial waste stream may require a bespoke treatment system. Aqueous waste streams from therapeutic peptide synthesis could contain solvents and other organic components. While reverse osmosis is effective in removing TFA, this creates a concentrated waste stream of TFA and other pollutants, which then requires further treatment. In a fluorochemicals complex (LANXESS AG, 2026), a three-stage process is used to remove PFAS (per- and polyfluoroalkyl substances), including TFA. In the first step, reverse osmosis produces a PFAS-depleted permeate and a small-volume, PFAS-enriched concentrate. In the second step, activated carbon filters bind the long-chain PFAS from the concentrate, and in the third step, a cascade of three resin-containing vessels removes the short-chain PFAS with two and three carbon atoms (including TFA). Overall, the process is reported to remove more than 99.9% of all fluorinated organic compounds adsorbed. After use, the resins are incinerated at high temperatures to destroy the PFAS. The presence of other ions and contaminants will likely be factors in the efficacy and cost of industrial waste stream purification. The use of reverse osmosis and nanofiltration in wastewater treatment for the semiconductor industry to remove PFAS, including TFA streams, has recently been described, including the particular challenges for the complex waste streams generated. Targeting the whole spectrum of PFAS chain lengths requires advanced concentration technologies. The concentrate from reverse osmosis is commonly incinerated but other emerging treatment options are mentioned (Shulman, 2025). Garavagno et al. (2024) discuss treatment methods for removal of TFA from water, but a recurring issue is that the efficiency of these processes is insufficient to serve as a viable solution for TFA accumulation

in large water bodies (i.e., in the environment).

If not adequately treated, TFA-bearing aqueous waste may be released directly or with WWTP effluent. Routine monitoring of WWTP effluent should also include TFA, as it is ubiquitous in surface waters and more comprehensive data for its occurrence will improve the basis for source mitigation strategies. Although TFA is not normally quantified by the LC-MS/MS methods commonly used for PFAS detection (Scholl et al., 2025), several TFA-specific analytical methods have been developed, and these are applicable to environmental monitoring. The simplest and most rapid method for direct measurement of TFA in surface waters and WWTP effluents involves methyl ester derivatization and headspace GC with electron capture detection or GC-MS in selected ion monitoring (SIM) mode (Wujcik et al., 1999; Zehavi & Seiber, 1996). These methods can achieve a detection limit better than 10 ng/L and can also be applied to plants and soils (Cahill et al., 1999). Better sensitivity, accuracy, and precision of TFA analyses in environmental waters can be accomplished using addition of isotope-labeled standards, preconcentration onto solid-phase extractants, liquid-liquid extraction, and various LC-MS/MS or SFC-MS/MS instrumental approaches with negative-ion electrospray ionization (e.g., Janda et al., 2019; Björnsdotter et al., 2021; Rodriguez et al., 2026).

8. Conclusion

TFA is an integral reagent for the synthesis and purification of therapeutic peptides such as insulin and GLP-1 agonists. The important properties of TFA include acidity, volatility, deprotection reaction selectivity, chemical compatibility, and ion-pairing capability, and the combination of these properties is difficult to reproduce effectively with other reagents. Depending on the particular peptide, it is estimated that between 60 to 95 kg of TFA may be required for the synthesis and purification of each kg of peptide. The recent and forecasted rapid growth in the manufacture of these drugs is expected to require increasing use and manufacture of TFA (IndexBox, 2026). At the estimated production of 750 kg of tirzepatide in 2024, the scenario adopted suggests that at least 45 - 71 metric tonnes TFA could have been used for this single peptide. If the same TFA consumption determined for tirzepatide applies to the 2024 production volume of FDA-approved therapeutic peptides, then a minimum of 350 - 550 metric tonnes of TFA was employed in their manufacturing processes. The tirzepatide market alone is expected to grow 5 - 10-fold by 2030, suggesting a considerable increase in the use of TFA.

TFA containing waste streams, if discharged to the environment from therapeutic peptide manufacture and other similar processes, are point source emissions to surface water. Other TFA sources have different deposition or generation patterns. The HFCs that generate TFA on degradation in the atmosphere result in global deposition of TFA due to the long atmospheric lifetimes of HFCs (Hart et al., 2026). The HFOs and HCFOs that generate TFA on degradation result in regional deposition of TFA due to the short lifetime of the HFOs and HCFOs. Sev-

eral papers have modelled TFA deposition maps and TFA concentrations in precipitation from emissions of HFO-1234yf (Garavagno et al., 2024; Henne et al., 2012; Holland et al., 2021; Luecken et al., 2010; Wang et al., 2018). A global modelling study of TFA deposition from HFOs and HCFOs estimated that variable amounts of TFA deposition from individual HFOs are found over land (with a range of 34% to 50%) and the ocean (50% to 66%). For HFO-1234yf, 55% of generated TFA is deposited over the oceans, with increased percentages for the other main HFOs and HCFOs, linked to their longer atmospheric lifetimes (Khan et al., 2026). Pesticides that degrade to give TFA can be the dominant source of TFA to environmental aqueous phases (rivers, lakes and groundwater) over cropland (Henne et al., 2025). The expected growth in the use of TFA for therapeutic peptide manufacture and other industrial uses of TFA suggests that these potential point source discharges of TFA-containing effluent may require further evaluation, characterization, and modeling.

Author Contributions

Peng is responsible for overall conceptualization, writing, content, and data compilation; Lindley is responsible for conceptualization, writing, content and data compilation; Sturchio is responsible for analytical method writing, manuscript editing; Hatzinger is responsible for abstract and environmental tox writing, manuscript editing.

Conflicts of Interest

Peng: Employee of the Chemours Company; Lindley: A science consultant to EFCTC (European Fluorocarbons Technical Committee). Some of the substances EFCTC members produce degrade to TFA, which is the subject of this paper; Sturchio: Employee of University of Delaware; Hatzinger: Employee of APTIM.

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